The Scientific Foundation For The Dietary Guidelines For Americans

Appendices

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Appendix 1. Chemical Additives and Food Packaging Contaminants in the US Food Supply

Overview

Highly processed foods and beverages often contain multiple industrial manufactured chemical additives and are commonly packaged in materials that can introduce contaminants. **Table A1** provides a non-exhaustive list of major chemical classes and representative examples of chemical food additives and food packaging contaminants. Inclusion in **Table A1** indicates presence or use in the food supply and does not imply adverse effects. The "GRAS status" column in **Table A1** reflects whether a substance is "generally recognized as safe" (or otherwise permitted) for its intended use under existing U.S. regulatory pathways. This designation is not equivalent to proof of long-term safety for chronic, combined exposures across the full life course, especially at modern intake levels.

Current Evidence Base and Its Limits

Because these chemicals are widespread and often co-occur in many packaged foods, an important question is what types of human evidence exist to evaluate potential long-term health effects. Most of the evidence linking exposures of these chemicals to adverse health outcomes, including cardiometabolic disorders, cancer and dementia, is based on observational studies.[1-10] Short-term clinical trials provide additional evidence linking specific chemical additives to disease biomarker endpoints (e.g., emulsifier intake and cardiometabolic markers);[11,12] however, significant gaps remain in our understanding of the long-term effects of dietary exposure to these chemicals and chronic diseases, especially when consumed in mixtures that are typically present in highly processed foods. For example, risks related to the combined intake of emulsifiers, sweeteners, and other additives remain unknown.

Exposure Measurement is a Major Evidence Limitation

Human data on the amount of exposure through foods is typically based on rough estimates using food frequency questionnaires rather than quantitative analytical measurements with validation of intake through biomarker assessments. Biomonitoring is further limited because established and validated biomarkers in blood or urine do not exist for many additive classes (including several emulsifiers, preservatives, and other compounds), making it difficult to quantify exposure, identify major sources, and study dose-response relationships.

High-Impact Research Priorities

To strengthen the evidence base for future dietary guidance, research is needed in the following areas: (1) direct quantitation of additives and packaging contaminants in commonly consumed US foods, particularly highly processed foods with complex ingredient lists and foods packaged in plastic; (2) improved quantification of human exposure using biomarker confirmation where feasible; (3) development of exposure biomarkers or biomarker signatures for additives that are not readily measured in blood or urine; and (4) long-term randomized studies that test whether meaningful exposure reduction (versus habitual intake) improves prespecified metabolic or clinical outcomes.

Table A1. Chemical Additives and Food Packaging Contaminants

Chemical Additive	Sources	GRAS Status
GRAS revoked		
Partially hydrogenated vegetable oils (trans fatty acids)	Industrial partially hydrogenated vegetable oils used in margarines, shortenings, baked goods and fried foods	Revoked (2018)
Brominated Vegetable Oil (BVO)	Used in citrus-flavored sodas and sports drinks to keep flavor oils evenly distributed	Revoked (2024)
Antimicrobials / Preservatives		
Propyl Paraben (Propyl p-hydroxybenzoate)	Used in baked goods, syrups, and beverages to inhibit molds	Affirmed (1977)
Sodium Benzoate	Common in acidic foods/drinks (sodas, jams) to inhibit microbes	Affirmed (1973)
Potassium Sorbate	Widely used in cheeses, wines, baked goods to prevent molds/yeast	Affirmed (1982)
Calcium Propionate	Added to breads, baked goods, and cheese to inhibit mold growth	Affirmed (1984)
Preservatives		
Sodium Nitrite (and Sodium Nitrate)	Cured meats (bacon, ham, hot dogs) and some fish products for color and botulism protection	Grandfathered (pre-1958)
Sulfites (e.g., Sulfur Dioxide, Sodium Bisulfite)	Used to preserve color and freshness in dried fruits, wine, and shrimp (prevents browning, microbial growth)	Affirmed (1959, 1977)
Flavor enhancers		
Monosodium glutamate (MSG)	Used to impart umami flavor in soups, snacks, seasonings, frozen dinners and restaurant foods.	CFR approved (1996)
Disodium inosinate & disodium guanylate	Snack foods, soups, ramen noodles, seasoning blends.	CFR approved (1977)
Artificial sweeteners		
Aspartame	Low-calorie sweetener in diet sodas, sugar-free foods, tabletop sweeteners (e.g., “Equal”)	Affirmed (1981, 1983)
Saccharin (and its salts)	Tabletop sweeteners (e.g., “Sweet’N Low”), diet sodas, pharmaceuticals (coatings)	Grandfathered (pre-1958)
Sucralose	“Splenda” – used in diet beverages, sugar-free desserts, baked goods, tabletop packets	Affirmed (1998)
Acesulfame potassium (Ace-K)	Diet sodas, baked goods, frozen desserts, tabletop sweeteners.	Affirmed (1988, 2003)
Xylitol	Sugar-free gum, mints, toothpaste, oral care products; naturally in fruits and vegetables.	Affirmed (1963)
Erythritol	Low-calorie sweetener in sugar-free candies, baked goods and beverages.	Affirmed (2001)
Sorbitol	Sugar-free candies, chewing gum, and “sugar-free” baked goods.	Affirmed (1977)
Maltitol	Sugar-free chocolates, candies, baked goods.	Self-affirmed (late 1970s/early 1980's
Lactitol	Reduced-calorie ice cream, chocolate, baked goods, candies.	Affirmed (1993)
Mannitol	Chewing gum, candies, “dusting” powder on gum or pharmaceuticals.	Affirmed (1977)
Neotame	Baked goods, beverages, gum; rarely used because extremely sweet.	Affirmed (2002)
Advantame	High-intensity sweetener used in baked goods, chewing gum and beverages; rarely used because extremely sweet.	CFR approved (2014)

Chemical Additive	Sources	GRAS Status
Emulsifiers & Thickeners		
Cellulose gum (carboxymethylcellulose)	Salad dressings, sauces, ice cream, grated cheese, yogurt, cream cheese, gluten-free baked goods.	Affirmed (1977)
Guar gum	Salad dressings, yogurt, sauces, plant milks, ice cream, canned soups.	Affirmed (1974)
Xanthan gum	Salad dressing, sauces, gluten-free flours, canned soups, ice cream, plant milks.	CFR approved (1990)
Maltodextrin	Sauces, cereals, chips, baked goods, yogurt, sodas, sports drinks.	CFR approved (1983)
Soy lecithin	Salad dressings, sauces, ice cream, yogurt, margarine, baked goods, chocolate.	CFR approved (1983)
Polysorbate 80 (Tween 80)	Used in ice cream, yogurt, salad dressings, desserts for smooth texture and mixing oils/fats with water	Grandfathered (pre-1958)
Polysorbate 20	Ice cream, salad dressings, baked goods, sauces, chewing gum.	CFR approved (1977)
Carboxymethyl Cellulose (CMC) *(Cellulose Gum)*	Used in baked goods, beverages, ice creams for texture and stability (prevents ingredient separation)	Affirmed (1979)
Carrageenan	Used in dairy products, plant-based milks, and meats to improve texture and water retention	Affirmed (1973)
Food dyes		
FD&C Red No. 3 *(Erythrosine)*	Cherries (cocktail/maraschino), candies, baked goods, snack gels	Affirmed (1969)
FD&C Red No. 40 *(Allura Red)*	Beverages, candies, snacks, cereals, desserts (one of the most common red dyes)	Affirmed (1971)
FD&C Yellow No. 5 *(Tartrazine)*	Beverages, candies, cereals, dessert powders, pickles, etc.	Affirmed (1969)
FD&C Yellow No. 6 *(Sunset Yellow FCF)*	Bakery goods, candies, beverages, snack foods (provides orange shade)	Affirmed (1986)
FD&C Blue No. 1 *(Brilliant Blue)*	Beverages, confections, frostings, ice pops, etc. (often combined with Yellow for greens)	Affirmed (1982)
Other chemical additives		
Azodicarbonamide (ADA)	Added to wheat flour and bread dough to improve texture and whiten flour	Affirmed (1962)
Potassium Bromate	Used in bread flour to promote rise and texture (stronger dough)	CFR approved (1977)
Antioxidants / preservatives		
Butylated Hydroxyanisole (BHA)	Added to fats/oils (snack foods, cereals) to prevent rancidity	Affirmed (1977)
Butylated Hydroxytoluene (BHT)	Used in cereals, snacks, and shortening to extend shelf-life	Affirmed (1973)
Tertiary Butylhydroquinone (TBHQ)	Stabilizes vegetable oils and fried foods (e.g., chips) against oxidation	Affirmed (1977)
Propyl Gallate	Often used with BHA/BHT in oils, meats, etc. to prevent spoilage	Affirmed (1977)
Food packaging contaminants		
Microplastics	Detected in salt, seafood, sugar, beer, bottled water, honey, milk, tea and other foods via contaminated packaging or environment.	N/A
Phthalates (plasticizers)	Food packaging, processing equipment, adhesives, lubricants, vinyl gloves; leach into fatty foods, dairy, meat, and fast-food.	N/A
PFAS (per- and polyfluoroalkyl substances)	Grease-resistant paper and paperboard packaging, non-stick cookware, contaminated seafood and crops near contaminated areas.	No longer sold for food contact use (2024).

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Appendix 2. Research Priorities

Overview

This appendix outlines priority research questions that, if addressed, would substantially strengthen the scientific foundation for future editions of the Dietary Guidelines for Americans. The priorities focus on dietary exposures that are highly prevalent, have plausible causal links to major health outcomes, and remain characterized by important uncertainty despite decades of research. They emphasize study designs that can reduce that uncertainty in a timely and policy relevant way.

In developing these priorities, we applied principles consistent with contemporary evidence grading systems such as GRADE, which recognize that randomized controlled trials provide the most direct evidence for causal effects of dietary interventions on health outcomes. Many existing recommendations rest on non-randomized evidence that is vulnerable to residual confounding, selection bias, and measurement error. The studies described in this appendix are intended to address these limitations by prioritizing experimental designs, clinically meaningful outcomes, and transparent, reproducible methods.

The research topics are organized by major areas of ongoing debate and uncertainty, including highly processed foods, dietary fats, protein quantity and sources, and cross cutting issues such as eating patterns and implementation strategies. Within each area, we provide examples of focused trials that are both feasible and capable of shifting the certainty of evidence for or against specific dietary recommendations. Where large, long-term trials with hard clinical endpoints are not feasible, we prioritize intermediate outcomes that have well established links to disease risk and can be measured reliably.

A recurring theme across the proposed studies is the need to prioritize clinical outcomes whenever possible, including incident disease, symptom burden, and functional status, rather than relying solely on isolated surrogate biomarkers. Another cross-cutting goal is to design research that reflects the realities of how Americans eat—using foods, cooking methods, and dietary patterns that are common in the U.S.

These priorities are not intended to be exhaustive or prescriptive. Rather, they are examples of targeted studies that could resolve key uncertainties, reduce reliance on indirect inferences, and provide a more stable, experimentally grounded basis for dietary guidance. Federal agencies, research funders, and investigators can use this appendix as a starting point for planning coordinated research efforts that will improve the rigor, transparency, and relevance of the evidence base for future Dietary Guidelines.

Highly Processed Foods

There is a pressing need for: (1) harmonized definitions of processed foods, and a more accurate classification system that includes added sugars, refined oils, and refined starches under the umbrella of highly processed foods; (2) larger, longer randomized trials testing the effects of controlled alterations in different categories of processed foods, ingredients and specific chemical additives on biochemical, toxicological and clinical endpoints, including cardiometabolic and neurological diseases. Emerging evidence indicates that chemicals derived from food packaging materials can accumulate in human tissues including atherosclerotic lesions, reproductive tissues, and brains. An emerging but limited body of evidence has linked accumulation of these compounds to adverse

health consequences. RCTs are therefore needed to determine whether consumption of minimally processed foods and diets can reduce levels of food packaging materials in human blood and tissues.

Highly Processed Foods RCT

Research Question

In adults with overweight or obesity, does reducing intake of highly processed foods and replacing them with minimally processed foods (without prescriptive energy restriction) improve body weight, body composition, or cardiometabolic outcomes over 1-2 years compared with continuing a diet in which highly processed foods provide a substantial share of energy?

Rationale

Highly processed packaged foods and ready-to-eat meals contribute a large proportion of energy intake in the United States. These products typically combine refined starches, added sugars, refined fats and oils, sodium, and various additives in formulations that are shelf-stable and highly palatable. Observational studies consistently link higher intakes of highly processed foods with greater risk of weight gain, obesity, cardiovascular disease, and type 2 diabetes, but these associations are difficult to interpret because of residual confounding by health seeking behaviors. Short-term trials suggest that diets rich in highly processed foods may promote higher energy intake and weight gain compared with minimally processed diets, but these studies are small and brief. A longer-duration randomized trial is needed to test whether reducing highly processed foods and replacing them with minimally processed foods leads to sustained improvements in body weight, body composition, and cardiometabolic risk.

Sample RCT Design

Two year, two-arm RCT in adults with overweight or obesity who report obtaining at least half of their baseline energy intake from highly processed foods (such as packaged snack foods, sugary beverages, refined ready-to-eat breakfast cereals, instant noodles, frozen entrees, fast food, and bakery items). Participants would be randomized to: (1) a minimally processed pattern in which highly processed foods are limited to a small fraction of total energy (for example, 10-15%), with replacement by minimally processed foods such as vegetables, fruits, beans, lentils, intact or minimally processed whole grains, nuts, seeds, eggs, dairy, and unprocessed or minimally processed meats and seafood; or (2) a comparison pattern that maintains a higher share of energy from highly processed foods approximating current intake. Both groups would receive similar behavioral support and, where feasible, partial food provision or vouchers to improve adherence. Diets would be ad libitum with no prescribed calorie targets, allowing the effect of processing on spontaneous energy intake and weight to be observed. This RCT should be adequately powered to capture clinical endpoints that are relevant to Americans. Outcomes could include change in body weight and body fat, incident type 2 diabetes, waist circumference, fasting glucose, insulin or HbA1c, blood lipids and lipoproteins, blood pressure, markers of liver fat, subjective satiety and cravings.

Relevance to Americans

This trial would directly test whether lowering intake of highly processed foods and replacing them with minimally processed foods leads to sustained improvements in weight, body composition, and metabolic health. Because highly processed foods are ubiquitous and heavily marketed, results would provide critical evidence to inform whether and how dietary guidance should explicitly address the degree of processing.

Dietary Fats

High-quality RCTs are urgently needed to clarify which dietary fats are most compatible with long-term health.

Dietary Fats RCT #1

Research Question

Which type of dairy is best for metabolic health of American children, whole-fat, low-fat, or fat-free?

Rationale

Public-health policies encouraging children to avoid saturated fat—especially by restricting full-fat dairy—may have displaced nutrient-dense foods with highly-processed, refined-carbohydrate snacks and sweetened low-fat products. This could contribute to higher rates of insulin resistance, obesity, and type 2 diabetes in youth. Thus, we need definitive RCT data to determine whether whole-fat dairy intake will improve the metabolic health of American children.

Sample RCT Design

Adequately powered, multi-year trial isolating milk fat as a controlled variable by comparing metabolic and clinical effects of 2-3 servings per day of whole-fat versus low-fat versus fat-free milk. To enhance adherence and maximize control, all milk will be provided.

Relevance for Americans

Finally provides definitive answer to which type of milk is best for American children (and as an extension the effects of saturated fat).

Dietary Fats RCT #2

Research Question

Can replacement of high-linoleic acid soybean oil with high-oleic soybean oil decrease lipid and lipoprotein peroxidation and improve the cardiometabolic health of Americans?

Rationale

Linoleic acid intakes have increased in the US to levels that are not possible without the addition of highly concentrated, extracted liquid vegetable oils. Linoleic acid is highly vulnerable to peroxidation when heated (cooking, food processing), which generates toxic peroxides and aldehydes. Thus, using linoleic acid-rich oils for cooking and in packaged and processed foods is potentially harmful, but we do know for sure. The major source of linoleic acid in US diets is soybean oil. However, peroxidation-resistant, high-oleic versions of soybean oil that match olive oil are available in the US.

Sample RCT Design

Adequately powered 3-4 year, two arm RCT in older adults (men and women) with subclinical atherosclerosis or pre-diabetes comparing metabolic and clinical endpoints following provision of either high linoleic acid soybean oil or high oleic soybean oil. Outcomes include a full suite of biochemical and metabolic factors related to lipid peroxidation (e.g. oxidized low-density lipoprotein (LDL)-cholesterol, oxidized very low-density lipoprotein (VLDL), hexanaldehyde) and clinical endpoints including incident cardiovascular events, incident type 2 diabetes, and all-cause mortality.

Dietary Fats RCT #3

Research Question

Does consuming fried foods prepared in more peroxidation resistant oils, compared with highly peroxidation prone oils, reduce circulating oxidized lipoproteins and other markers of lipid peroxidation in adults?

Rationale

Americans consume large amounts of fried and thermally stressed foods prepared with a wide variety of added fats and oils. Heating linoleic acid rich and other polyunsaturated oils during cooking and frying generates lipid hydroperoxides, aldehydes, and other oxidized species that can be transferred into foods and absorbed into circulation. However, different culinary oils vary substantially in their peroxidation resistance, and it is not known whether choosing more stable oils for cooking meaningfully reduces circulating oxidized lipoproteins or improves metabolic markers in humans. A combined experimental and feeding trial is needed to link oil-specific peroxidation profiles under realistic cooking conditions to short-term changes in biomarkers of lipid peroxidation and metabolic health.

Sample RCT Design

Two phase program. Phase 1: expose 6-8 commonly used fats and oils to standardized frying/sauté conditions that mimic food-service practice (fixed temperature, time, and repeated-use cycles) and rank oils by peroxidation resistance based on formation of lipid hydroperoxides, aldehydes, and related oxidized lipids. Phase 2: 4-8 week, two arm randomized feeding trial in adults comparing daily intake of a standardized serving of foods fried in either (1) a low oxidation oil (top performer from Phase 1) or (2) a high oxidation oil (bottom performer from Phase 1), with guidance to avoid other fried foods and maintain stable weight. Measured outcomes include fasting plasma oxidized LDL, oxidized lipoprotein fractions, detailed oxidized lipid species in plasma, and standard metabolic and inflammatory markers.

Relevance to Americans

Phase 1 will identify which commonly used oils produce the lowest and highest loads of oxidation products under standardized cooking conditions. Phase 2 can demonstrate whether substituting more stable oils meaningfully lowers oxidized lipids in circulation without requiring large changes to overall diet, providing near-term evidence to support practical recommendations for choosing oils that reduce exposure to oxidized lipids during high-heat cooking.

Dietary Fats RCT #4

Research Question

In adults with metabolic syndrome or nonalcoholic fatty liver disease, does the long-term lowering linoleic acid intake from current typical U.S. intakes to amounts consistent with traditional diets improve liver fat, blood triglycerides, lipoprotein oxidation, insulin sensitivity and other cardiometabolic outcomes compared with a diet that maintains current linoleic acid intake?

Rationale

Over several decades, dietary guidance and food reformulation have shifted the U.S. food supply toward higher linoleic acid intake, largely through increased use of linoleic acid rich vegetable oils in processed and prepared foods. As a result, many Americans now consume linoleic acid at levels well

above historical intakes and virtually all Americans have historically high levels of linoleic acid in adipose and other tissues. Much of the evidence supporting high linoleic acid intake comes from either 1) observational studies that use the percentage of linoleic acid in plasma fatty acids as an exposure marker, which is vulnerable to confounding by metabolic health and changes in lipid pools and 2) trials using surrogate markers, particularly serum low density lipoprotein cholesterol. However, randomized controlled trials specifically increasing linoleic acid (while replacing saturated fat) have not shown clinical benefits. In contrast, experimental and clinical data, including randomized controlled trials that combine increased n-3 intake with linoleic acid lowering, suggest that linoleic acid influences inflammatory/pain pathways, lipid mediators, and clinical symptoms such as pain, but its net effect on cardiometabolic and liver outcomes at current high intake levels remains uncertain. A randomized trial that specifically lowers linoleic acid from typical U.S. intakes of ~8% to 2% of energy, while maintaining overall diet quality, is needed to clarify its impact on liver fat, triglycerides, insulin resistance, and related outcomes. Because the half-life of linoleic acid in adipose tissue is estimated to be almost two years, long-term reduction to historically normative dietary levels is needed to understand the effects of dietary linoleic acid.

Sample RCT Design

Three-year, two arm RCT in adults with overweight or obesity and either metabolic syndrome or nonalcoholic fatty liver disease. Participants would be randomized to: (1) a lower linoleic acid diet that reduces linoleic acid intake to approximately 2% of total energy by replacing linoleic acid rich vegetable oils with oils low in linoleic acid and higher in monounsaturated fats, or (2) a comparison diet that maintains linoleic acid at levels typical of current intakes (~8% of total energy), using oils and foods common in the U.S. food supply. Both groups would follow overall dietary patterns consistent with current recommendations for diet quality (including vegetables, fruits, whole grains, and appropriate energy intake), and both would receive comparable behavioral support. Linoleic acid and other fatty acids could be monitored with repeated measurements of red blood cell and plasma fatty acid composition to confirm separation between groups. Primary outcomes could include change in liver fat, fasting triglycerides, apolipoproteins, cholesterol levels, lipoprotein peroxidation markers, measures of insulin sensitivity, changes in body weight and adiposity, blood pressure, inflammatory markers, and profiles of linoleic acid and arachidonic acid derived lipid mediators. Prespecified exploratory outcomes could include symptom measures such as headache or musculoskeletal pain.

Relevance to Americans

This trial would directly test whether the long-term lowering of linoleic acid from current high intake levels, within a high-quality diet, improves liver fat, triglycerides, lipoprotein peroxidation, and insulin sensitivity in adults at high cardiometabolic risk. Because current dietary patterns and prior recommendations have led to widespread, chronically high linoleic acid intakes and accumulation in Americans, results would provide critical experimental evidence to inform whether maintaining, increasing, or lowering linoleic acid should be a priority in future dietary guidance.

Protein

Protein plays a central role in maintaining muscle mass, strength, metabolic health, and physical function across adulthood, yet current recommendations for adults are based largely on short-term studies rather than long-term clinical outcomes. Many midlife and older adults consume protein at or modestly above the RDA, but below levels hypothesized to be optimal for preserving lean mass and preventing functional decline, and a growing share of protein comes from powders, shakes, and other processed products rather than whole foods. Key uncertainties include whether increasing protein

intake above typical levels improves long-term muscle and functional outcomes, how higher protein intakes affect cardiometabolic risk and safety (for example, kidney health), and whether the source and processing of protein (whole foods versus isolates and shakes) meaningfully influence weight regulation, body composition, and metabolic markers at a given total protein intake. The priority trials in this section are designed to address these questions using realistic dietary patterns, clinically relevant endpoints, and explicit monitoring for potential adverse effects.

Protein RCT #1

Research Question

In midlife adults, does consuming a higher protein diet (for example, about 1.6 g/kg/day) compared with a diet reflecting current average protein intakes (for example, about 1.0-1.2 g/kg/day) improve muscle mass, physical function, and cardiometabolic health over several years?

Rationale

Protein is essential for maintaining muscle mass, strength, and metabolic health, yet most adult protein recommendations are based on short-term nitrogen balance studies rather than long-term clinical outcomes. Across the menopausal transition and into older adulthood, Americans commonly experience loss of muscle mass and strength, weight gain, and worsening metabolic risk. Observational studies and small, short-duration trials suggest that protein intakes above current average levels may help preserve lean mass and function and improve cardiometabolic markers, but no adequately powered, long-duration trial has directly compared a realistic higher protein target with a pattern reflecting current average intakes, using real foods and clinically meaningful endpoints. A randomized trial is needed to test whether increasing protein intake from typical levels to about 1.6 g/kg/day improves long-term muscle, functional, and metabolic outcomes and to evaluate safety at these higher intakes.

Sample RCT Design

Three to five year, two arm RCT in adults aged approximately 45-70 years, enriched for women in the menopausal transition and early post-menopause and including men of similar age. Participants would be randomized to: (1) a higher protein diet providing about 1.6 g/kg/day of protein, or (2) a comparison diet providing protein at levels similar to current average intakes (for example, about 1.0-1.2 g/kg/day), with total energy matched between groups to avoid systematic weight loss or gain. Protein in both groups would come primarily from minimally processed foods (e.g., meat, seafood, poultry, eggs, dairy, beans, lentils, and nuts), with limited use of protein powders or bars. Both groups would receive comparable behavioral support to achieve their assigned protein targets within overall diet patterns consistent with current recommendations. Outcomes could include change in appendicular lean mass and standardized measures of physical function (for example, gait speed, chair rise performance, and grip strength), changes in bone mineral density, body weight and adiposity, fasting glucose, insulin and HbA1c, blood lipids, blood pressure, incident prediabetes and type 2 diabetes, and falls or fractures where feasible. Safety monitoring would include kidney function (serum creatinine, estimated glomerular filtration rate [GFR], and albuminuria), liver enzymes, and other prespecified adverse events, overseen by an independent data and safety monitoring board.

Relevance to Americans

This trial would directly test whether increasing protein intake above current average levels, using real foods, improves muscle mass, functional status, and cardiometabolic health in midlife adults without causing harm. Results would inform whether typical protein intakes in this age group should be

increased and would give clinicians and policy makers a stronger basis for advising adults on practical protein targets to prevent frailty, disability, and metabolic disease as they age.

Protein RCT #2

Research Question

Does consuming a diet in which a substantial share of protein comes from isolates and shakes, compared with a diet emphasizing whole-food protein sources, differentially affect body weight, body composition, satiety, and cardiometabolic outcomes in adults with overweight or obesity?

Rationale

Many Americans obtain protein from powders and ready-to-drink shakes based on whey, casein, soy, pea, and other isolates. These products are convenient and heavily marketed for weight management, sports performance, and healthy aging, yet most protein recommendations are grounded in studies using mixed or minimally processed foods. Protein isolates differ from whole-food sources in matrix structure, digestion rate, and typical co-ingredients (sweeteners, refined starches, added fats), and may produce different patterns of amino-acid appearance, satiety, and metabolic responses even at equivalent protein doses. Small, short-term studies suggest that whole-food protein can promote greater satiety and more favorable postprandial glucose and insulin responses than shakes, but long-duration trials comparing whole-food protein with protein isolates at matched total protein intake and energy are limited. A randomized trial is needed to test whether reliance on protein isolates, versus whole-food protein, affects weight, body composition, and cardiometabolic risk in free-living adults.

Sample RCT Design

Twelve-month, two arm RCT in adults with overweight or obesity, targeting the same total protein intake in both groups (for example, about 1.6g/kg/day) and similar total energy intake. Participants would be randomized to: (1) an isolate-based protein pattern, in which at least half of daily protein is provided by protein isolates (powders and ready-to-drink shakes from dairy and plant sources) incorporated into meals and snacks, or (2) a whole-food protein pattern, in which at least 90 percent of daily protein comes from minimally processed foods such as meat, seafood, poultry, dairy, eggs, beans, lentils, and nuts, with minimal use of isolates. Both groups would receive comparable behavioral support and menu guidance, and overall diet quality (e.g., vegetables, fruits, whole grains, added sugars, sodium) would be aligned with current recommendations. Outcomes could include changes in body weight and body composition (lean and fat mass), fasting glucose, insulin or HbA1c, blood lipids, blood pressure, subjective satiety and energy intake patterns, kidney function (serum creatinine, estimated GFR, and albuminuria), liver enzymes and liver fat where feasible, and gastrointestinal symptoms.

Relevance to Americans

This trial would directly address whether meeting protein needs with powders and shakes is comparable to using whole-food protein sources for weight, body composition, and metabolic health, at the same total protein intake. Because protein isolates are widely used for convenience, sports, and weight management, results would provide practical evidence to guide clinicians, consumers, and policy makers on the appropriate role of these products in everyday diets.

Appendix 3. Is Linoleic Acid in Blood an Adequate Biomarker for Dietary Intake?

Background

As reviewed in the **DGA scientific report and Appendix 4.6**, randomized controlled trials failed to demonstrate anticipated benefits from replacing saturated fat with linoleic acid-rich oils. Nevertheless, the belief that dietary linoleic acid-rich oils are beneficial has been sustained in part by findings from non-randomized studies showing that low levels of linoleic acid in plasma—when expressed as a percentage of total fatty acids—are associated with slightly higher risk of cardiometabolic diseases and premature death.[1] The relative amount of linoleic acid in blood is assumed to be an adequate biomarker that can be used as a proxy for dietary intake. However, since the relative amount of linoleic in the blood is affected by factors other than diet, it may not be a valid biomarker for dietary linoleic acid intake.

First, linoleic acid is highly enriched within cholesteryl esters [2-5] and much less abundant in triglycerides, which consist mostly of saturated and monounsaturated fatty acids (see **Fig. 5.6 main report**). This means that high blood triglycerides, a cardinal feature of the metabolic syndrome and an established risk factor for multiple chronic diseases and premature death [6-8] could lower the *percentage* of linoleic acid in blood, thus potentially skewing relationships between blood linoleic acid and chronic diseases in observational studies.

Second, although humans readily make saturated and monounsaturated fatty acids from carbohydrates and alcohol, we lack the ability for *de novo* synthesis of linoleic acid. As a result, low-quality carbohydrate diets, heavy alcohol drinking,[9,10] and excess caloric intake could potentially dilute linoleic acid in all blood lipid pools by stimulating *de novo* synthesis of saturated and monounsaturated fatty acid (but not linoleic acid).[11-14] It follows that when linoleic acid is expressed as a percentage of total fatty acids, high blood triglycerides, metabolic distress, heavy drinking, and excess caloric intake could artificially dilute the amount of linoleic acid in blood. Because high blood triglycerides, insulin resistance, liver dysfunction, and heavy drinking are all established risk factors for chronic disease and premature death,[6-8,15-21] these metabolic sources of linoleic acid dilution could potentially distort observational associations between linoleic acid and chronic diseases and death.

To illustrate these concepts, analyses of publicly available National Health and Nutrition Examination Survey (NHANES) data is shown below.

Methods

Data were obtained from the 2011-2012 and 2013-2014 cycles of the NHANES. These cycles were selected because they represent the most recent releases containing plasma fatty acid data. Adults aged ≥20 years were included if they did not have a diagnosis of cardiovascular disease, cancer, or diabetes. **Plasma fatty acid** data were used to calculate both absolute concentrations (μmol/L) and relative composition (percentage of total fatty acids). When calculating the percent composition of total fatty acids, data were required for all "major fatty acids," defined as those comprising more than 1% of total fatty acids. Participants missing data for any major fatty acid were excluded from these calculations, resulting in a 6.5% reduction in participants with fatty acid data. Fasting triglycerides, glucose, and insulin were measured by standard laboratory protocols. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as fasting insulin (μU/mL) × fasting glucose (mg/dL) / 405. Liver function markers included γ-glutamyl transferase (GGT) and aspartate aminotransferase (AST).

Dietary intake was assessed using the average of the two 24-hour dietary recalls. Percent of energy from macronutrients was computed using the Atwater system, converting grams of nutrients to kilocalories and dividing by the total energy intake variable. **Alcohol consumption** was assessed using NHANES Alcohol Use Questionnaire variables. Heavy drinking (ALQ151: "Was there ever a time or times in your life when you drank 4/5 or more drinks almost every day?") was supplemented with variables identifying participants who consumed less than 12 drinks over the prior year (ALQ101) and/or during their life (ALQ110).

All analyses were conducted on the combined dataset from both survey cycles. In accordance with NHANES analytic guidelines, survey weights were selected based on the variable with the smallest number of observations to ensure appropriate representation. Analyses were conducted using Stata version 19.5, employing survey commands to account for the complex sampling design. Pearson correlations were estimated using Stata's structural equation modeling framework for complex survey data,[22] and t-tests were performed using survey-adjusted regression models.[23]

Continuous variables with skewed distributions—fatty acid concentrations, triglycerides, insulin-resistance measures, liver enzymes, and dietary intakes—were natural-log transformed. In regression models, fatty acids were entered either as concentrations (log-transformed) or as percent of total (untransformed), with covariate adjustment for age, sex, and body mass index (BMI). Statistical significance was defined as two-sided $p<0.05$ with survey-adjusted standard errors.

Results

The clinical characteristics of the NHANES population described above are provided in **Table A2**.

Table A2. Clinical characteristics by quintile of plasma linoleic acid (percent of total fatty acids)

	Quintile 1 Mean (SE)	Quintile 2 Mean (SE)	Quintile 3 Mean (SE)	Quintile 4 Mean (SE)	Quintile 5 Mean (SE)	p for trend
Triglycerides						
Fasting (mg/mL)	156.2 (5.4)	113.8 (3.1)	95.2 (2.1)	81.6 (2.6)	70.3 (1.6)	<0.001
Non-fasting (mg/mL)	162.4 (6.3)	119.9 (3.5)	99.2 (2.0)	83.2 (2.4)	70.9 (1.5)	<0.001
Insulin resistance						
HOMA-IR	2.7 (0.1)	2.7 (0.1)	2.3 (0.1)	2.2 (0.1)	2.0 (0.0)	<0.001
OGTT (mg/mL)	116.5 (2.9)	109.2 (2.2)	104.2 (1.8)	99.2 (1.7)	94.0 (1.3)	<0.001
Liver damage						
AST (U/L)	26.6 (0.5)	23.6 (0.3)	22.5 (0.4)	22.4 (0.3)	21.7 (0.3)	<0.001
GGT (U/L)	28.4 (1.1)	20.9 (0.8)	18.2 (0.5)	17.4 (0.4)	16.0 (0.5)	<0.001
Dietary linoleic acid (% energy)	5.9 (0.2)	6.7 (0.2)	6.9 (0.1)	7.5 (0.1)	7.8 (0.1)	<0.001

Values are weighted means with standard errors (SE) derived using NHANES survey design variables. Quintiles are based on linoleic acid expressed as percent of total fatty acids. P-values reflect tests for linear trend across quintiles using the median value of each quintile. Analyses include adults ≥20 years and exclude participants with major chronic disease. Estimates combine data from the 2011-2012 and 2013-2014 NHANES cycles.

The results of unadjusted and adjusted cross-sectional analysis among this 'disease-free' US population are provided in **Table A3 and A4,** respectively. Absolute concentrations of linoleic acid, and absolute and relative concentrations of oleic (major monounsaturated fatty acid) and palmitic acid (major saturated fatty acid) are all strongly, positively associated with blood triglyceride levels (**Table A3 and A4**). In contrast, linoleic acid is unique because—when expressed as a percentage of total

fatty acids—it is strongly, inversely associated with blood triglycerides (**Table A3** and **Fig A1**). Figure A1 graphically depicts that the magnitude and direction of the relationship between plasma linoleic acid and triglycerides is dependent on the decision of whether to express linoleic acid as an absolute concentration or as a percentage of total fatty acids.

Since dietary linoleic acid has no effect on triglycerides in controlled trials,[24,25] the inverse correlation between linoleic acid as a percentage of total fatty acids and blood triglycerides cannot be construed as a cause-and-effect relationship. The percentage of linoleic acid in total plasma fatty acids is also strongly, inversely associated with multiple other biomarkers of metabolic distress and poor overall health that have no clear biological link to dietary linoleic acid, including pre-existing insulin resistance, subclinical liver disease, and heavy drinking (**Table A3 and A4**).

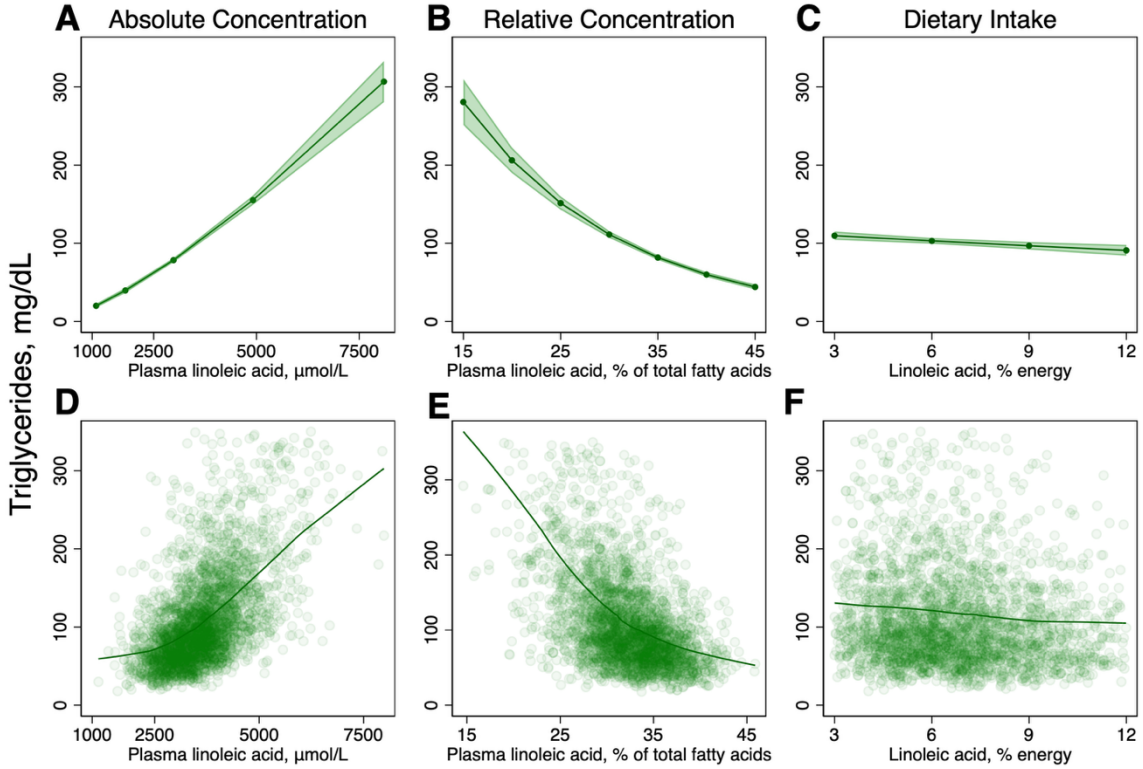

Figure A1. Associations of fasting triglycerides with linoleic acid expressed as plasma absolute concentration, plasma relative concentration, and estimated dietary intake. Panels A-C show survey-weighted adjusted mean triglyceride concentrations (mg/dL) from linear regression models adjusted for age, sex, and body mass index among adults ≥20 years without major chronic disease and not taking statins. Panel A displays triglycerides across the distribution of plasma linoleic acid expressed as absolute concentration (µmol/L); Panel B shows plasma linoleic acid expressed as percentage of total plasma fatty acids; Panel C shows usual dietary linoleic acid intake as percentage of total energy. Shaded bands indicate 95% confidence intervals. Panels D-F show the corresponding unadjusted relationships between triglycerides and each exposure using scatter plots with locally weighted regression (LOWESS) smooths. When plasma linoleic acid is expressed as an absolute concentration (µmol/L), higher levels are strongly and positively associated with fasting triglycerides (A). In contrast, when linoleic acid is expressed as a percentage of total plasma fatty acids, the association reverses, showing a strong inverse relationship with triglycerides (B). Estimated dietary linoleic acid intake (% of total energy) shows only a slight inverse association with triglyceride levels (C). Sample sizes range from 2,916 to 3,132.

Table A3. Correlations between plasma fatty acids with cardiometabolic, hepatic, and dietary variables among 'disease-free' US adults.

	Linoleic Acid Plasma concentration		Oleic Acid Plasma concentration		Palmitic Acid Plasma concentration	
	Absolute (Log µmol/L)	Relative (% of total FA)	Absolute (Log µmol/L)	Relative (% of total FA)	Absolute (Log µmol/L)	Relative (% of total FA)
Triglycerides						
Fasting (log mg/dL)	**0.60****	**-0.52****	**0.88****	**0.66****	**0.84****	**0.49****
Non-fasting (log mg/dL)	**0.59****	**-0.53****	**0.87****	**0.66****	**0.84****	**0.51****
Insulin resistance						
OGTT (log mg/dL)	**0.16****	**-0.23****	**0.26****	**0.16****	**0.28****	**0.21****
HOMA-IR (log)	**0.21****	**-0.15****	**0.27****	**0.16****	**0.29****	**0.21****
Liver damage						
GGT (log U/L)	**0.09***	**-0.35****	**0.30****	**0.23****	**0.32****	**0.30****
AST (log U/L)	0.05	**-0.23****	**0.21****	**0.19****	**0.21****	**0.20****
Heavy drinking						
≥4 alcoholic drinks/day	-0.01	**-1.95****	**0.10****	**0.78****	**0.09****	**0.89****
Dietary linoleic acid (% energy)	**0.13****	**0.28****	**-0.08***	**-0.11****	**-0.11***	**-0.25****

Data are from the NHANES 2011-2014 cycles (n=2939-3581), analyzed with appropriate survey weights. The analytic sample included adults aged ≥20 years without diagnosed cancer, cardiovascular disease, or diabetes. Pearson correlation coefficients are shown for all measures except heavy drinking (≥4 drinks/day), which was estimated using survey-weighted linear regression. Fatty acid concentrations, triglycerides, insulin resistance, liver damage, and dietary variables were log-transformed prior to analysis. **p < 0.001; *p < 0.05. Abbreviations: AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; OGTT = oral glucose tolerance test; % of total FA (percent of total fatty acids).

Table A4. Covariate-adjusted regression coefficients for associations of plasma fatty acids with cardiometabolic, hepatic, and dietary variables among 'disease-free' US adults.

	Linoleic Acid Plasma concentration		Oleic Acid Plasma concentration		Palmitic Acid Plasma concentration	
	Absolute (Log µmol/L)	Relative (% of total FA)	Absolute (Log µmol/L)	Relative (% of total FA)	Absolute (Log µmol/L)	Relative (% of total FA)
Triglycerides						
Fasting (log mg/dL)	0.28**	-3.96**	0.59**	3.19**	0.50**	2.00**
Non-fasting (log mg/dL)	0.27**	-4.00**	0.57**	3.15**	0.48**	2.03**
Insulin resistance						
HOMA-IR (log)	0.09**	-0.55*	0.13**	0.52**	0.13**	0.57**
OGTT (log mg/dL)	0.09**	-2.43**	0.22**	1.01**	0.23**	1.51**
Liver damage						
GGT (log U/L)	0.03*	-2.28**	0.15**	0.70**	0.15**	1.03**
AST (log U/L)	0.03	-2.78**	0.18**	1.10**	0.18**	1.28**
Heavy drinking						
≥4 alcoholic drinks/day	-0.02	-1.73**	0.07*	0.38	0.08*	0.77**
Dietary linoleic acid (% of energy)	0.01**	0.59**	-0.01*	-0.13**	-0.02*	-0.25**

Data are from the NHANES 2011-2014 cycles (n=2939-3581), analyzed with appropriate survey weights. Analyses included adults aged ≥20 years without diagnosed cancer, cardiovascular disease, or diabetes. All models were **adjusted for age, sex, and BMI**, using appropriate NHANES survey weights. Fatty acid concentrations, triglycerides, insulin resistance, liver enzyme, and dietary variables were log-transformed prior to analysis. Shaded cells indicate statistically significant associations: red for positive and blue for negative relationships. Asterisks denote significance levels: **p<0.001; *p<0.05. Abbreviations: AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; OGTT = oral glucose tolerance test; % of total FA (percent of total fatty acids).

Interpretation

In nationally representative U.S. adults free of diagnosed cardiovascular disease, cancer, or diabetes, lower plasma linoleic acid expressed as a percentage of total fatty acids is inversely associated with triglycerides and other markers of metabolic distress (HOMA-IR, OGTT, GGT, AST), and heavy drinking. By contrast, when linoleic acid is expressed as a concentration it is positively associated with triglycerides and other markers of metabolic distress (HOMA-IR, OGTT, GGT); these patterns persist after adjustment for age, sex, and BMI.

Strengths and Limitations

Strengths include the inclusion of a large, nationally representative US sample from the two most recent NHANES cycles with fatty acid data, standardized laboratory measures, use of complex survey methods and appropriate weighting, and systematic evaluation of both concentration and percent composition across several metabolic domains. Another strength is the use of detailed data for alcohol use, allowing us to identify the strong relationship between heavy drinking and the percentage of linoleic acid in plasma fatty acids. Notably, previous observational studies examining associations between linoleic acid and alcohol did not identify heavy drinkers. This analysis required complete data for all major plasma fatty acids to compute percent composition and therefore exclude ~6.5% of participants. Multivariable models were adjusted only for age, sex, and BMI. Future studies

are needed to determine whether the reported associated are confounded by use of medications, dietary supplements, or other factors.

Summary and Conclusion

These associations are consistent with the concept that low linoleic acid—when expressed as a percentage of total fatty acids—may be a proxy for hypertriglyceridemia, subclinical metabolic distress, heavy drinking, and poor overall health status, even in individuals that do not have established cardiovascular disease, cancer, or diabetes. When these complex metabolic factors are not properly addressed in observational analyses, associations between linoleic acid and disease may appear more favorable than they truly are, reflecting reverse causation, effect modification, and/or residual confounding by underlying health status rather than direct dietary effects. Therefore, high quality randomized controlled trials (such as those outlined in **Appendix 2**) are needed to truly understand the effects of linoleic acid intake on health and disease.

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Appendix 4.1. Highly Processed Foods & Health

IMPACT OF HIGHLY PROCESSED FOODS ON MULTIPLE HEALTH OUTCOMES

Umbrella Review of Prior Meta-Analysis

Michael Goran, PhD
Professor and Vice Chair for Research
Department of Pediatrics, Keck School of Medicine
Children's Hospital of Los Angeles
University of Southern California

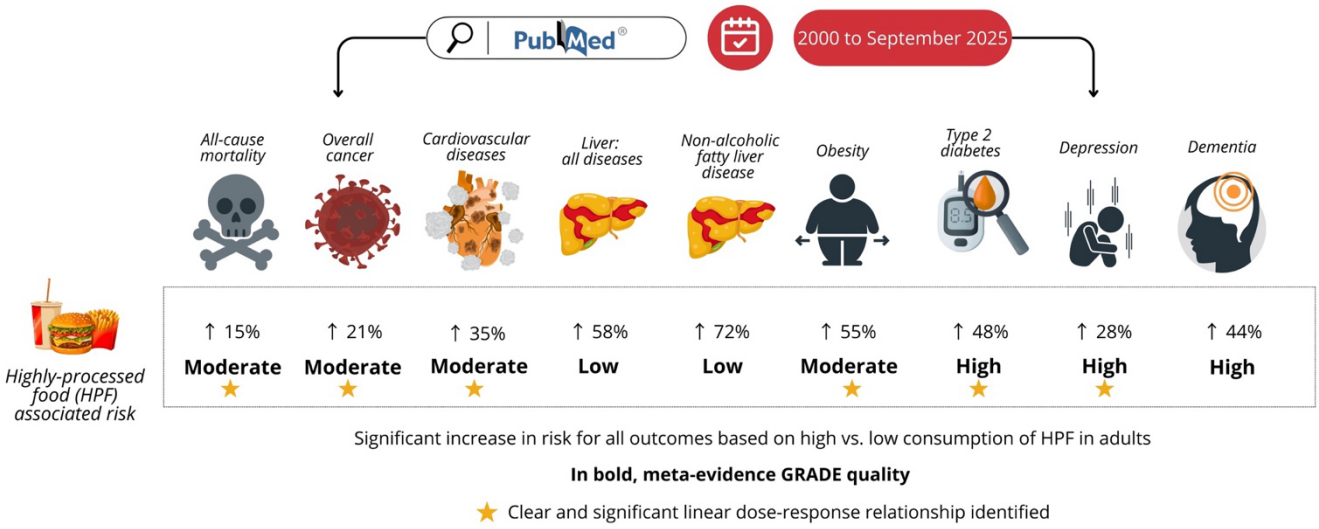
Abstract

Objective: The purpose of this study was to conduct an updated umbrella review of prior meta-analysis that examined the links between highly processed food (HPF) consumption and chronic disease outcomes.

Methods: An umbrella review of meta-analyses published through September 2025 evaluating links between consumption of HPFs and major health outcomes was conducted. We used a broad definition of HPF that included terms such as “junk food”, “ultra-processed food” and “industrial food”. Data were extracted on relative risks, dose–response relationships, heterogeneity, and sensitivity analyses, and the GRADE framework was applied to assess certainty of evidence. Outcomes included all-cause mortality, cancer, cardiovascular disease (CVD), liver disease, obesity, type 2 diabetes (T2D), dementia, and depression. A standardized approach was used to identify a lead meta-analysis for each outcome.

Results: Twenty-seven meta-analyses met the inclusion criteria (all observational studies), and eight lead meta-analyses were identified across outcomes. Analysis of high versus low HPF consumption revealed high-certainty evidence for increased risk of T2D (RR 1.48, 95% CI 1.36–1.61), dementia (RR 1.44, 95% CI 1.09–1.90), and depression (RR 1.28, 95% CI 1.19–1.38). Moderate-certainty evidence was found for all-cause mortality (RR 1.15, 95% CI 1.09–1.22), cancer (RR 1.12, 95% CI 1.06–1.19), CVD (RR 1.35, 95% CI 1.18–1.54), and obesity (RR 1.55, 95% CI 1.36–1.77), and low-certainty evidence for liver disease (RR 1.58, 95% CI 1.34–1.86). Most associations showed dose–response relationships, where a 10% higher proportion of calories from HPFs was associated with a 14% higher risk of T2D, 13% higher risk of cancer, 10% higher mortality risk, and 7% higher obesity risk, while each additional serving per day of HPF increased CVD risk by 4%. No study demonstrated any protective effect of HPF consumption.

Conclusions: Consumption of HPF is consistently and adversely associated with a broad range of chronic disease outcomes, with multiple dose-response gradients and moderate-to-high certainty for several major conditions. These findings support urgent, precautionary action at the clinical, population, and policy levels to identify and reduce the more harmful processed foods and replace them with less processed and minimally processed foods and home-prepared meals. In parallel, further research is needed to refine the definition and classification of HPFs and to elucidate the mechanisms underlying their health effects, including potential differential effects across subtypes of HPFs.



Introduction

The global burden of chronic disease continues to rise [1], driven in part by rapid shifts in dietary patterns, including a notable increase in global production and consumption of highly-processed and ultra-processed foods [2]. In recent decades, there has been a growing interest in the role of food processing in explaining the links between nutrition and health [3]. Consideration of food processing takes into account aspects of food that expand beyond specific nutrient content, and includes products high in refined starches, added sugars, sodium, preservatives, sweeteners, flavorings, emulsifiers, and other additives, and low in fiber and micronutrients [3,4]. In addition, aspects of industrial-grade food processing are hypothesized to alter the structure and function of foods in ways that may adversely affect health [5,6]. This can occur, for example, through the breakdown of natural food matrices to increase energy density and glycemic load, stripping protective compounds during refining, and incorporating chemical additives that may disrupt gut microbiota and/or promote inflammation [7–9]. These changes can accelerate overconsumption, impair satiety signaling, and expose consumers to substances not normally present in minimally processed foods, thereby compounding the risk of chronic disease [5,7,9,10].

While there is currently no consensus definition for highly-processed or ultra-processed foods, a joint USDA-FDA effort to establish a uniform definition is underway [11]. For the purposes of this report, highly processed foods (HPF) are defined as any food, beverage, or engineered food-like item that is made primarily from substances extracted from food (eg refined sugars, grains, starches or oils) and/or containing industrially manufactured chemical additives. The most used definition in the research domain is the Nova classification of food processing [3], which has been used to identify ultra-processed foods (UPF). In the US, for example, the percentage of calories consumed as UPF in adults rose from 53.5% in 2001-2002 to 57% in 2017-2018 [12]. In children and teenagers, analysis of population-based data show even higher levels of consumption

increasing from 61.4% of calories in 1999 to 67.0% in 2018 [13]. The widespread presence of processed foods extends beyond supermarkets into critical food environments, such as schools, hospitals, and workplaces, where they are often cheaper and more accessible than minimally processed alternatives. Emerging evidence links high consumption of processed foods to a wide range of health outcomes, including obesity [10], cardiometabolic diseases like type 2 diabetes and cardiovascular disease [14,15], liver disease [16], several cancers [14], all-cause mortality [15], and, more recently, cognitive development in children [17]. Importantly, no study to date has demonstrated any health benefits associated with processed food consumption, underscoring the asymmetry of risk versus benefit.

Conventional dietary guidelines, such as the most recent from the 2020 – 2025 USDA Dietary Guidelines, have historically emphasized single nutrients (e.g., limiting saturated fat, added sugar, or sodium) rather than considering the overall degree of food processing [18]. No previous USDA Dietary Guidelines have addressed the impact of processed foods on population health. While the nutrient-focused approach has led to important advances in reducing nutrient deficiencies and diet-related risk factors, it may no longer fully capture the realities of modern food environments where individuals typically consume diets dominated by packaged, ready-to-eat, and convenience products. Importantly, people choose to eat foods, not isolated nutrients, and the health risks associated with processed foods appear to extend beyond their nutrient profiles, implicating food matrices, additives, and industrial processing methods.

Two umbrella reviews on the topic of processed foods and adverse health outcomes were published in 2024 [14,15]. Both studies concluded that higher levels of processed food consumption (especially in the UPF category based on the Nova classification) was associated with increased risk of obesity, type 2 diabetes, cardiovascular disease, cancer, and all-cause mortality. While this was an important step in consolidating evidence, important gaps remain, for example, establishing dose–response relationships, and evaluating outcomes that have been less frequently studied, such as liver disease and cognitive health. Moreover, the field is expanding at a rapid pace, with numerous large-scale cohort studies and meta-analyses published in the months since the aforementioned reviews, which had a literature cutoff of March 2023 [15] and June 2023 [14].

The present umbrella review aims to provide the most comprehensive and up-to-date evaluation of the evidence linking processed food consumption with a wide range of health outcomes to inform the evolution of dietary guidelines, food policies, and overall public health. By integrating emerging evidence across multiple outcomes, this review has the potential to clarify the broader health implications of processed food consumption and support a shift from nutrient-centric recommendations toward food- and processing-based approaches that better reflect real-world eating patterns and public health needs. In addition, by identifying consistent gaps and methodological challenges, this work aimed to identify priorities for future research (e.g. mechanistic

studies on processing-related harms, intervention trials, policy evaluations) to ensure the translation of scientific advances into meaningful improvements in public health.

Methods

Literature Search

One researcher conducted a systematic literature search in PubMed (MEDLINE) up to September 2025 for potential meta-analyses of prospective cohort and randomized controlled trials related to processed food consumption and health outcomes published since January 1, 2000. The complete search strategy is provided in **Appendix A**. Two other researchers reviewed the search criteria to ensure agreement on the literature search. Any discrepancies were noted on internal team documents in red for tracking, and disagreements were resolved by consensus.

Selection of meta-analyses

Studies with the following criteria were considered eligible for inclusion in the present umbrella review: 1) previously published meta-analyses of prospective cohort and randomized controlled trials related to consumption of processed foods and health outcomes in the general population for children, adolescents, and adults; 2) assessed dietary intakes by a standard dietary assessment tool or tools (e.g., food frequency questionnaire, 24-hour dietary recall, and dietary records and had a well-defined classification for processed food consumption, either by Nova or defined food groups or classified in some cases as “junk food” for example; 3) a reported clinical outcome or surrogate; 4) assessed the incidence of chronic disease, with a focus on type 2 diabetes, obesity, cardiovascular disease, non-alcoholic fatty liver disease, cancer, mental health disorders, and all-cause mortality; 5) published in English; 6) included at least one study from the United States. Meta-analyses that lacked generalizability (e.g., had specific geographic constraints), were published in a language other than English, or had no meta-analysis were excluded. The following studies were also excluded: 1) Meta-analyses that examined other outcomes outside of our pre-defined scope; 2) Any narrative, systematic, or scoping reviews, as well as any umbrella reviews that did not conduct a new meta-analysis; 3) Studies that exclusively enrolled participants with a disease or chronic condition at baseline.

Screening of Search Results

One researcher screened all records against the eligibility criteria. A second and third researcher each verified a 50% random sample to ensure consistency. Disagreements were resolved by discussion or adjudication. Selected articles could further be discarded at the data extraction level (full text screening).

Data extraction

One researcher led the data extraction. The following data was extracted using a Google Sheets template: citation details, last search date, databases search for the analyses, number of RCTs (and/or total studies), eligibility criteria, total sample size for

outcome of interest, number of cases, countries included in meta-analysis, intervention/exposure, diet assessment, comparator, outcome(s) reported, pooled effects and model, lower CI, upper CI, heterogeneity, dose response, dose response linearity, GRADE, risk of bias and method, and conflicts. After the initial data extraction two other researchers divided the selected meta-analyses by row for a second verification of the data extraction. Any discrepancies were noted in red, and disagreements were resolved by consensus.

Assessment of methodological quality

The methodological quality of each of the identified meta-analyses was assessed using a simplified approach adapted from the ROBIS tool [19]. This tool considers four domains on a categorical scale of: High, Moderate, Poor: 1) clarity and pre-specification of eligibility criteria; 2) adequacy of the search strategy (i.e., multiple databases, transparent methods); 3) accuracy of data collection and presence of risk of bias assessment for included studies, and; 4) appropriateness of synthesis methods and reporting of findings. The quality appraisal was assessed into one final overall grade (High, Moderate, Low), and a qualitative note was provided for the quality grade justification. This assessment was conducted on all meta-analyses regardless of the reported estimate or GRADE score and was further validated by two other researchers.

Selection of Lead Articles

For each outcome, we identified a lead article to report. To facilitate this, we organized the evidence table by outcomes, including both clinical endpoints and surrogate outcomes. We prioritized meta-analysis with clinical endpoints, those rated as high quality, recency and ideally including dose-response analysis. By following these criteria, the selection of lead articles was clear, and no ties occurred in the selection process. This allowed for a straightforward ranking of meta-analyses for each outcome as a clinical endpoint.

Grading the Evidence

The certainty of evidence was assessed using the GRADE tool [20]. This tool grades the evidence as high, moderate, low, or very low quality. Studies are initially assessed on the level of confidence, where RCTs indicate high confidence and observational studies low confidence. Studies are then upgraded based on a large effect size, dose-response relationship, and the direction of plausible effect. Downgrading criteria included risk of bias, inconsistency, indirectness, and publication bias. Among the selected lead meta-analyses, some reported GRADE scores, while others did not. For those that did not provide a GRADE score, a GRADE adjustment was performed, providing an initial high/low grade based on the study type (RCT or observational), and then recorded the upgrades (a large effect size, dose-response relationship, and the direction of plausible effect) and downgrades (risk of bias, inconsistency, indirectness, and publication bias). The final grade was determined by one researcher and verified by two others. Discrepancies in ratings were noted, and disagreements were resolved by consensus.

Evidence to Decision

We next translated all of the evidence into **Strong (**benefits clearly outweigh harms/burdens for most people at Moderate/High certainty) or **Conditional (**benefits likely outweigh harms, but certainty is lower or trade-offs vary) recommendations using a GRADE-consistent evidence to decision process that considers: (1) certainty of evidence; (2) balance of desirable vs undesirable effects; (3) outcome importance; and (4) feasibility.

Results

Studies Identified and Their Characteristics

Figure 1 presents the results of the literature search and selection process. We identified and screened 53 articles from the original search and excluded 25 of these for various reasons, as indicated in **Figure 1**. One additional article was excluded during the extraction process (did not contain any studies conducted in the US), resulting in 27 meta-analyses that were reviewed for a total of eight outcomes. The outcomes that were examined were all-cause mortality (five meta-analyses), cancer (five meta-analyses, including one for all-cancers, one for breast cancer, one for colorectal cancer, one for liver cancer and one for lung cancer), cardiovascular disease (eight meta-analyses, including cardiovascular mortality in three studies, dyslipidemia in one, all cardiovascular events in one, heart disease mortality in one and hypertension in three), liver disease (four meta-analyses, including liver fibrosis in one study, all adverse liver outcomes in one study and NAFLD in two studies), obesity (four meta-analyses, including three for obesity and one for abdominal obesity), type 2 diabetes (four meta-analyses), dementia (two meta-analyses, including one for cognitive impairment and one for all-cause dementia) and depression/anxiety (five meta-analyses, including one for anxiety, three for depression and one for depression/anxiety). A summary of all the meta-analyses, their key characteristics, and the results of the major outcomes examined in each of them is shown in **Appendix Table 1**. Note that many of the meta-analyses included analysis of multiple outcomes, and each outcome is included as a separate sub-row. The quality appraisal for each meta-analysis review of each outcome is shown in **Appendix Table 2.**

Summary of Findings

Figure 1 shows a Forest Plot that summarizes all the relative risks that were extracted from each meta-analysis for each outcome examined. Consistent and significant adverse effects of HPF on all examined outcomes were identified, except for lung cancer. **Table 1** summarizes the lead review for each of the 8 outcomes examined, described in detail below for each outcome.

For **all-cause mortality,** we identified four meta-analyses, published between 2021 and 2025, and all of them reported a significant and consistent effect with RR ranging from 1.15 to 1.25. The lead meta-analysis was published in 2025, was also the largest and most comprehensive (15 studies reviewed), and identified a RR of 1.15 [21]. A dose-

response meta-analysis was included for 12 studies and revealed a significantly positive linear association (p<0.001), indicating that a 10% increase in the proportion of HPF was associated with a 10% higher risk of all-cause mortality. The association of HPF with all-cause mortality received a GRADE certainty rating of Moderate.

For **cancer**, we identified five meta-analyses that examined cancer outcomes related to all cancers (except melanoma and skin), as well as specific cancers for breast, liver, colorectal, and lung. All showed significant effects of HPF except for lung, with RR ranging from 1.10 (for colorectal cancer) to 1.35 (for liver cancer). In the lead meta-analysis, the outcome related to all cancers was selected because of its general relevance and showed a RR of 1.12 no significant heterogeneity, and a dose response [22]. This indicates that a 10% higher HPF was associated with a 13% risk of any cancer. However, the study for breast cancer was the most recent and largest study, with a larger RR (1.25). The association of HPF with cancer (all cancers) received a GRADE certainty rating of Moderate.

For **CVD,** we identified eight studies that assessed the impact of HPF on various outcomes, including CVD mortality (n=2), CVD events (n=1), heart disease (n=1), dyslipidemia (n=1), and hypertension (n=3). All studies showed consistent effects with RR ranging from 1.23 (hypertension) to 1.66 (heart disease). We selected a 2023 paper [23], which was rated as Moderate GRADE certainty and identified a significant, linear dose-response analysis ($P_{non\text{-}linearity}$ = 0.095). This study showed that high consumption of HPF had a RR of 1.35 for any CV event, and each serving per day of HPF increased the risk of a CV event by 4%. This finding was found to be robust across different diet assessment tools, average BMI, follow-up years, geographical region or adjustment for diabetes or hypertension. One factor found to be significant was age, with a higher risk for studies with an average age of $\geq$ 50y (RR: 1.40; CI 1.17 – 1.67 versus RR of 1.24; CI: 1.06 – 1.44 for less than 50y; p=0.044). In addition, there were 3 meta-analysis that showed consistent associations of HPF with greater risk of hypertension (RR ranged from 1.23 to 1.32).

For **liver outcomes**, we selected the 2025 paper by Guo et al. because it examined multiple liver outcomes from 17 studies [16] and received a Moderate-to-High rating in our Quality Appraisal. This study found that high levels of HPF increased RR of any adverse liver outcome by 1.34 times, with the highest effect seen for non-alcoholic fatty liver disease (NAFLD), with a RR of 1.72. This study did not perform a dose-response, which partly accounted for a GRADE certainty rating of Low.

For **obesity**, four studies found a consistent RR of 1.26 to 1.55. We selected the study by Moradi et al [24], even though it was not the most recent (from 2023), because this study was specific to obesity as an outcome and included risk estimates for overweight and abdominal obesity, and conducted dose-response analysis. The highest level of HPF intake was associated with a 1.55 relative risk of obesity, 1.36 risk of overweight, and 1.41 risk of abdominal obesity. From a dose-response perspective, a 10% increase in HPF was associated with a 7% higher risk of obesity, 6% higher risk of overweight,

and 5% higher risk of abdominal obesity (significant dose response effects <0.001; not significant for non-linearity). The association of HPF with obesity received a GRADE certainty rating of Moderate.

For **type 2 diabetes**, four meta-analyses reported a RR ranging from 1.24 to 1.48. We selected the most recent review, which was rated as High in our quality appraisal, and also conducted a dose-response [25]. In this paper, the relative risk of type 2 diabetes was 1.48, and a 10% higher HPF was associated with a 14% higher risk of type 2 diabetes (no evidence of non-linearity). Interestingly, in a smaller subset of studies in this meta-analysis, the investigators were able to calculate that each serving per day of HPF was associated with a 4% higher risk of type 2 diabetes. Notably, this meta-analysis also showed that the significant effect of HPF on type 2 diabetes remained significant after adjusting for obesity (based on body mass index).

For **mental health outcomes**, we extracted data from seven meta-analyses that included four on depression (RR ranged from 1.15 – 1.62), one on dementia (all cause; RR: 1.44), one on cognitive impairment (RR: 1.17), and one on anxiety (RR: 1.24). Since the outcomes here were broad, we selected one paper for depression [26] and one for all-cause dementia [27]. Both papers received a certainty GRADE of High based on the authors' own analysis. For the depression outcome, HPF increased risk by 1.28 times, and in a dose-response analysis, a 10% higher HPF was associated with an 11% higher risk of depression ($p<0.001$ for dose response; no evidence for non-linearity). This finding was robust to different definitions of HPF. For all-cause dementia, HPF increased the outcome risk by 1.44 times. In a dose-response analysis, the relationship was found to be linear with no significant effect apparent in moderate consumers. Importantly, these findings for dementia remained significant after adjusting for obesity (based on body mass index), cardiovascular disease, and socioeconomic status. However, the effect for dementia (all cause) was not significant after adjusting for type 2 diabetes.

Evidence to Decision

Criterion	Description
Problem & importance	Classification of foods in terms of processing status is a relatively new phenomenon and the proportion of daily calories that are consumed as highly or ultra-processed is increasing in the population. Numerous studies are now exploring the association between processed food and health and disease outcomes. No previous US dietary guideline has considered the degree of food processing and their effects on health in their recommendations despite a growing and consistent evidence base of health effects.
Certainty of evidence (per outcome)	Our analysis revealed a consistent and dose-response pattern of increased risk across all outcomes examined. In the final analysis we identified a **HIGH** certainty of evidence for risk of HPF on dementia, depression and type 2 diabetes; a **MODERATE** certainty of evidence for all-cause mortality, cancer (all forms of cancer combined), cardiovascular disease and obesity; and a **LOW** certainty of evidence for liver disease outcomes.
Benefits vs harms	Anticipated effects of a reduction in processed food consumption are strong and broad across multiple health outcomes with zero anticipated health risks. Dose response analysis were consistently significant and linear indicating that any level of reduction will be associated with benefit with zero risk to health. There could be additional perceived burden in terms of likely higher cost and more inconvenience and time needed for preparing food rather than relying on processed food products.
Implementation considerations/ feasibility	Concerns with implementation might be that consuming foods that are less processed are more expensive and will take more time to prepare and be less convenient. This will likely need to be coupled with education as well as a mechanism such as front of package labeling to indicate the more harmful types of processed foods. In addition, there could be concerns that there is no broadly accepted definition of what defines the more harmful types of processed foods beyond the existing Nova framework. However, our analysis revealed significant adverse effects even when different definition approaches were used. Further work is needed to develop more specific definitions of the more harmful types of processed foods, but this should not delay going forward with a strong recommendation given that the benefits of this far outweigh the risk of not recommending given the continued increases in health burden associated with processed foods in the population.
Preliminary Recommendation Statement	The evidence supports a **strong recommendation** for reduction in the consumption of highly processed foods for broad risk reduction for all-cause mortality, cancer, cardiovascular disease, liver disease, obesity, type 2 diabetes, dementia and depression.

Preliminary Statement of Findings

This umbrella review found consistent adverse associations between HPF consumption and major chronic disease outcomes. Higher HPF intake was linked to increased risk of all-cause mortality, cancer, cardiovascular events, liver disease, obesity, type 2 diabetes, dementia, and depression, with all outcomes (except liver disease) supported by dose–response evidence. Certainty of evidence ranged from Low for liver outcomes to High for type 2 diabetes, dementia, and depression. Dose-response findings indicate that a 10% reduction in the proportion of calories consumed as HPF would reduce the risk of type 2 diabetes by 14%, any cancer by 13%, all-cause mortality by 10%, and obesity by 7%. Furthermore, studies showed that a reduction in just one daily serving of HPF would reduce risk of any cardiovascular event or type 2 diabetes by 4%. These are significant health gains based on relatively small dietary changes. Taken together, the evidence base now supports moderate-to-high certainty of harm for chronic disease and mental health outcomes, with consistent dose–response gradients and no evidence of benefit. Collectively, these findings provide a strong rationale for a recommendation to reduce HPF intake at the population and policy level and to replace HPF with minimally processed, nutrient-dense foods.

Discussion

Our umbrella review provides evidence that higher consumption of HPF is consistently associated with adverse health outcomes spanning cardiometabolic disease (obesity, type 2 diabetes, cardiovascular disease, liver disease), all-cause mortality, selected cancers, as well as cognitive and mental-health outcomes. The evidence base was limited to observational studies, with preference in our analysis given to prospective cohort studies and clinical endpoints rather than surrogates. Notably, all the meta-analyses we reviewed were published after 2020, even though our search included the years 2000 to 2025. This indicates that concerns related to the health effects of HPF and the knowledge base has increased significantly just in the last 5 years. Despite the reliance on prospective cohort studies, the effects observed were consistent across meta-analyses. Additionally, outcomes indicated a significant linear dose-response, and in most of the meta-analyses this persisted after multivariable adjustment, with the same directions of effect across age groups, sexes, and geographies. Notably, we found no evidence of health benefit from higher HPF intake in any outcome throughout any meta-analysis. While residual confounding and exposure misclassification are possible (e.g., heterogeneity in dietary assessment and definition of HPF foods), sensitivity analyses were performed across the board to examine and eliminate this possibility. Collectively, the weight, coherence, and breadth of evidence support the strong recommendation of a population-level reduction in HPF consumption.

Interpretation of Findings Relative to Recent Umbrella Reviews on This Topic

This new umbrella review builds on and updates two recent umbrella reviews on HPFs and health outcomes [14,15]. Both prior umbrella reviews also concluded that HPF consumption was consistently associated with increased risk of multiple adverse

outcomes, including obesity, type 2 diabetes, cardiovascular disease, cancer, and all-cause mortality. However, both reviews had literature cutoffs in early to mid-2023, and the pace of new publications in this field has been rapid, with numerous large-scale cohort studies and meta-analyses appearing since that time. Notably, one-third of the meta-analyses included in this umbrella review had literature cut-offs after June 2023, and four out of nine of our lead meta-analyses to support the specific outcomes were published in 2024 or 2025.

Across outcomes, our updated synthesis generally found stronger and more consistent associations than either of the prior umbrella reviews, as summarized in **Table 2**. For example, both prior reports reported very low–certainty evidence linking HPFs with obesity, whereas our review identified more recent meta-analyses showing higher relative risks (RR 1.55, 95% CI 1.36–1.77) with moderate certainty of evidence. For type 2 diabetes, earlier reviews reported RRs of 1.23–1.40 with very low certainty, whereas our updated review, drawing on new studies published in 2025, identified an even stronger association (RR 1.48, 95% CI 1.36–1.61) and upgraded the certainty to high. Similarly, for NAFLD, where studies are generally lacking, our updated estimate showed a very high level of risk (RR 1.72, 95% CI 1.36 – 2.17) compared to prior studies and upgrades the certainty from very low to Low. In addition, our review uniquely incorporated outcomes not assessed in prior umbrellas, such as dementia (RR 1.44, 95% CI 1.09–1.90, high certainty), supported by new large-scale prospective studies published after 2023. Another important advance is the availability of new dose–response data. Our synthesis includes recently published dose–response meta-analyses, demonstrating graded risk increases for outcomes such as all-cause mortality, cardiovascular disease, obesity, and type 2 diabetes. This not only strengthens causal inference but also provides critical information for setting potential thresholds or targets for HPF reduction at the policy level.

Limitations

Several limitations of this review warrant consideration. These reflect both methodological constraints of our review process and broader challenges in the evidence base. One major limitation is that we did not identify any meta-analyses focused specifically on childhood outcomes or applying a life course perspective. This represents a critical gap, since childhood is a particularly vulnerable period for exposure to highly processed diets and the establishment of long-term dietary patterns, and recent studies show higher levels of HPF consumption in children/teens compared to adults [12,13]. One meta-analysis published just as we were conducting our search examined HPFs and cognitive development in children and adolescents [17], but at the time was not referenced in PubMed. That study synthesized 35 studies (n=84,062) and found that higher HPF intake in children was associated with poorer cognitive performance across attention, executive function, language, and visuospatial ability, though not for memory or processing speed. In addition, one of the prior umbrella reviews that examined a broader set of outcomes [15] identified an association between HPF and wheezing in children and adolescents from four meta-analyses (OR: 1.42;

CI: 1.34 – 1.49; Low GRADE). These findings underscore the importance of expanding future work on HPFs and health outcomes to include pediatric and developmental outcomes.

Another limitation relates to uncertainties regarding definition and identification of the more harmful types of processed foods. Most epidemiological studies have relied on the Nova classification system, which categorizes foods into four groups based on processing, with UPFs (Nova Group 4) representing the highest degree of industrial formulation [3]. While Nova has provided a valuable common framework, it is not without limitations. Criticisms of Nova include subjective classification, variability across food cultures, and challenges in operationalizing UPF measurement in dietary surveys. While Nova provides a practical framework, it is subject to misclassification, particularly for foods that fall near category boundaries (e.g., fortified breads, flavored yogurts, or plant-based alternatives). Also, Nova does not designate refined cooking ingredients such as refined starches, added sugars or extracted oils as ultra-processed, and therefore may underestimate the percentage of highly processed items. Nevertheless, the Nova definition remains the most widely applied tool in observational and interventional studies. Notably, not all studies in our review used Nova exclusively, yet the direction of associations was broadly consistent across different definitions used.

One of the main criticisms of the Nova classification is that the Nova 4 category may group together foods of varying health risk [28]. For example, relatively less concerning items such as supermarket breads with added sugars or snacks sweetened with a natural sweetener may be classified alongside products that are more clearly detrimental, such as sugar-sweetened beverages, packaged desserts, or highly processed ready-to-eat meals. This raises the concern that Nova 4 may “over-classify” certain foods as ultra-processed. However, this potential misclassification would strengthen rather than weaken our conclusions: if some foods within Nova 4 are in fact less harmful, their inclusion would dilute the overall risk estimates. The fact that prior studies observe significant and consistent adverse associations using the current Nova 4 grouping suggests that the true effect of processed foods may be even stronger if classification could more precisely distinguish the most harmful products. In this sense, our estimates are likely conservative, reinforcing the robustness of the observed associations. Still, the absence of a universally accepted and operationalized definition of processed foods complicates cross-study comparisons and policy translation, while we wait for the outcome of the USDA-FDA effort to establish a more uniform definition[11]. Greater methodological consensus is needed to ensure robust and reproducible evidence.

Related to the definition of processed foods are general limitations regarding dietary assessment. The primary dietary assessment tools in the included studies were food-frequency questionnaires (FFQs) and 24-hour recalls. While widely used in epidemiological research, these instruments were not designed to specifically capture food processing categories, and they rely heavily on participant recall and self-report. This introduces the potential for misreporting, recall bias, and limited resolution in

distinguishing processing levels. For example, participants may correctly report “bread” or “cereal” consumption, but questionnaires may not reliably differentiate between minimally processed and ultra-processed variants. Newer tools, including barcode scanning, digital food diaries, and linkage with retail purchase data, could help overcome these limitations and provide more accurate assessment of exposure to different types of processed foods in future studies.

Finally**,** the evidence base is almost entirely observational, with no large or extended RCTs available to directly test the health effects of HPFs compared with minimally processed diets. However, there are a small number of experimental studies that provide proof-of-concept support for causal effects. The most compelling is the randomized, inpatient crossover trial by Hall et al [10], which demonstrated that adults consuming an ultra-processed diet ad libitum consumed ~500 kcal/day more and gained weight compared with the same participants on a minimally processed diet matched for calories, sugar, fat, sodium, and fiber, and other studies have found similar effects [29,30]. Other human feeding studies have shown that specific ingredients within HPF can alter gut microbiota composition and markers of intestinal inflammation [31], while artificial sweeteners, which are very common in HPF, have been linked to impaired glycemic responses [32] and microbiome disruption [33]. Taken together, these experimental studies reinforce the plausibility of the epidemiological findings while also underscoring the scarcity of intervention research, highlighting a critical gap for future investigation.

In sum, these limitations emphasize the need for more pediatric- and life course–focused analyses, refinement of definitions for processed foods, improved dietary assessment methods, and carefully designed intervention studies. However, even when considering these methodological constraints, the evidence consistently points in the same direction, high levels of processed food consumption is harmful to health, with no evidence of benefit. Thus, while methodological refinements and new studies will strengthen the field, the current body of evidence already provides a strong rationale for precautionary action to reduce processed food consumption.

Research Gaps

A critical priority for advancing the field is to strengthen causal inference and identify biological mechanisms by which processed foods harm health. RCTs, natural-experimental designs, and short-term feeding studies are needed to isolate the effects of different processed foods and their specific components. Particular attention should be given to ingredients such as emulsifiers, sweeteners, and other additives, as well as matrix-related effects, including loss of intact food structure or hyperpalatability. These mechanistic studies should span both children and adults and include emerging endpoints such as microbiome-mediated pathways, blood glucose regulation, and neurocognitive outcomes. The goal is to identify the specific components and categories of processed foods that are most harmful. For example, epidemiological analysis suggests that the more harmful categories of processed foods could include

sugar and non-sugar sweetened beverages, ready-to-eat meals, ultra-processed dairy products and ultra-processed oils, sauces, condiments, refined breads [34,35].

While observational evidence is consistent and compelling, it cannot fully establish causality due to residual confounding and measurement error. However, it is important to point out that designing and executing adequately powered RCTs would likely require 5–10 years and investments on the order of hundreds of millions of dollars. Moreover, trials designed to reach clinical endpoints, such as chronic disease incidence, are likely not feasible and would rely on surrogate or pre-clinical outcomes. In the meantime, the prevalence of obesity, diabetes, non-alcoholic fatty liver disease, and other chronic conditions continues to rise. Waiting for perfect evidence risks worsening the burden of preventable disease and delaying urgently needed public health responses. Given the trade-offs between scientific certainty and timely action, it is critical to act on the best available evidence to protect population health while continuing to strengthen the evidence base.

Longitudinal studies and interventions with a life-course focus are also a major research priority. Early-life exposures during pregnancy, infancy, and childhood may shape lifelong risk trajectories for obesity, metabolic disease, and cognitive or mental health outcomes. Yet, to date, most evidence comes from adult populations. Future research should therefore prioritize birth cohorts, pediatric studies, and intergenerational designs that can capture developmental windows of vulnerability. Where possible, existing datasets should be re-analyzed, and new cohort and intervention studies should incorporate dietary measures that allow for stronger classification of processed foods. These approaches will help determine how early and sustained exposure to processed foods influences health across the life course.

An important question in interpreting the associations between processed food consumption and health outcomes is whether these effects are mediated entirely through obesity. Most large cohort studies account for this possibility by adjusting for baseline body mass index (BMI) and other markers of adiposity, yet the associations with chronic disease and mortality often remain significant even after such adjustment. For example, in the NutriNet-Santé cohort in France [36], higher consumption of processed food was associated with increased all-cause mortality and cardiovascular events, but these relationships persisted after adjustment for BMI. Similarly, analyses from the UK Biobank [37], and the NIH-AARP Diet and Health Study [38] found that while hazard ratios for all-cause mortality were attenuated when BMI was included in the models, they remained statistically significant, suggesting that adiposity explains only part of the observed effect. Thus, while obesity is an important downstream outcome of processed food consumption, it is unlikely to be the sole mediator, and policy and clinical strategies should recognize that reducing processed food consumption may confer health benefits independent of body weight.

Finally, it is essential to evaluate whether policies and structural interventions to reduce processed food consumption will have the same degree of effectiveness across

populations. Studies should examine the impacts of front-of-pack labeling, targeted advertising, taxes on processed foods or their components, procurement reforms in schools and public institutions, and stronger nutritional standards in school meal programs and other federal programs that support nutrition. Importantly, these evaluations must consider how policies affect populations across the socioeconomic spectrum, as processed foods are often more accessible and affordable in disadvantaged communities. Policy evaluation research can therefore play a critical role in addressing health inequities while providing an evidence base for governments seeking to implement effective strategies to curb consumption levels of processed food.

Implications for Dietary Recommendations

No prior USDA Dietary Guidelines have addressed the issue of processed foods and their impact on health outcomes. The prior 2025 Dietary Guidelines Advisory Committee (DGAC) did commission a systematic review on the association between UPF (Nova 4) and health outcomes, but that was limited to growth, body composition, and obesity risk, and was also limited to include cohorts with >1,000 participants [39]. That review concluded that there was evidence in children, adolescents, and adults but was limited in strength, showing suggestive associations between higher UPF consumption and greater adiposity or risk of overweight/obesity, while evidence for infancy, pregnancy, and postpartum periods was deemed insufficient ("grade not assignable"). Importantly, the DGAC highlighted methodological heterogeneity, particularly in the definition and measurement of UPFs, as a major limitation to drawing firmer conclusions. In contrast, our umbrella review demonstrates broad and consistent associations across multiple chronic disease endpoints**,** including type 2 diabetes, cardiovascular disease, liver disease, dementia, depression, and all-cause mortality, with several outcomes graded as high-certainty. These results extend well beyond obesity, showing adverse effects, not only body weight but also metabolic, cognitive, and mental health outcomes. Furthermore, by incorporating nearly two additional years of evidence beyond the DGAC's literature cutoff (January 2024), our findings provide a more comprehensive and timely synthesis. Taken together, this strengthens the case that reducing processed food consumption should be a central public health priority, supported by dietary guidelines and policy actions.

The primary recommendation emerging from this body of evidence is to reduce consumption of processed foods, especially the more highly processed foods (pending future FDA/USDA definitions) and replace them with minimally processed, home-prepared meals that emphasize whole foods, vegetables, fruits, legumes, nuts, whole grains, and simple protein sources. Clinical guidance should prioritize food-based counseling over nutrient-specific targets, with an emphasis on practical substitutions such as replacing sugar-sweetened beverages with water, packaged pastries with whole-grain breads, or boxed breakfast products with oats, eggs, and fruit. Even though no specific meta-analyses focused solely on children, in pediatric populations, attention should be given to school food environments, family-style meals, and strategies for lunch packing, emphasizing that gradual and sustainable reductions are both

achievable and beneficial. At the institutional and policy level, there is a need for stronger operational definitions of different categories of processed foods to guide procurement standards, front-of-pack labeling, and marketing restrictions, as well as investments in culinary education, scratch-cooking capacity, and healthier school meal standards to reduce reliance on processed foods in critical public settings.

Reformulation of processed foods has often been advanced as a public health strategy, particularly for reducing added sugars, sodium, or unhealthy fats. While such approaches can yield incremental improvements, as illustrated by the elimination of industrial trans fats and population-level salt reduction initiatives, they are fundamentally limited by their nutrient-specific focus, leaving intact the broader characteristics of processed foods such as additives, altered food matrices, and hyper-palatability that drive overconsumption and adverse health outcomes. In some cases, reformulation has been counterproductive, for example, when added sugars are replaced with artificial sweeteners of uncertain or potentially harmful effects. Substitution analyses highlight that the greatest benefits likely arise when processed foods are replaced with whole or minimally processed foods, more modest improvements when replaced with meals prepared from basic culinary ingredients, and the weakest or most uncertain benefits when replaced with reformulated processed foods. Collectively, this evidence underscores that reformulation should be considered a complementary but secondary strategy, while the primary focus of dietary guidance and policy should be a structural shift away from HPFs and toward minimally processed, nutrient-dense foods and traditional dietary patterns.

Conclusion

In conclusion, the findings of this umbrella review contribute to a rapidly expanding body of evidence showing robust and consistent adverse associations between HPF consumption and a broad range of chronic health outcomes, often in a dose–response fashion. Across studies, no health benefits of HPFs have been identified, while the evidence indicates that significant improvements in health are likely to result from replacing HPFs with minimally processed, nutrient-dense foods and home-prepared meals**.** Although further methodological refinements, definitions and intervention studies are warranted, the current evidence base provides a strong rationale for immediate action at the individual, population, institutional, and policy levels. Emphasis should be placed on children and youth, among whom consumption of HPF is highest, and longitudinal evidence remains most limited. Future dietary guidelines and public health strategies should move beyond nutrient-focused approaches to directly address the role of food processing and the broader food environment, with the overarching goal of reducing consumption of HPF, and promoting healthier, whole-food dietary patterns to lessen the burden of preventable disease. In parallel, further research is needed to refine the definition and classification of processed foods and to elucidate the mechanisms underlying their health effects, including potential differential effects across subtypes.

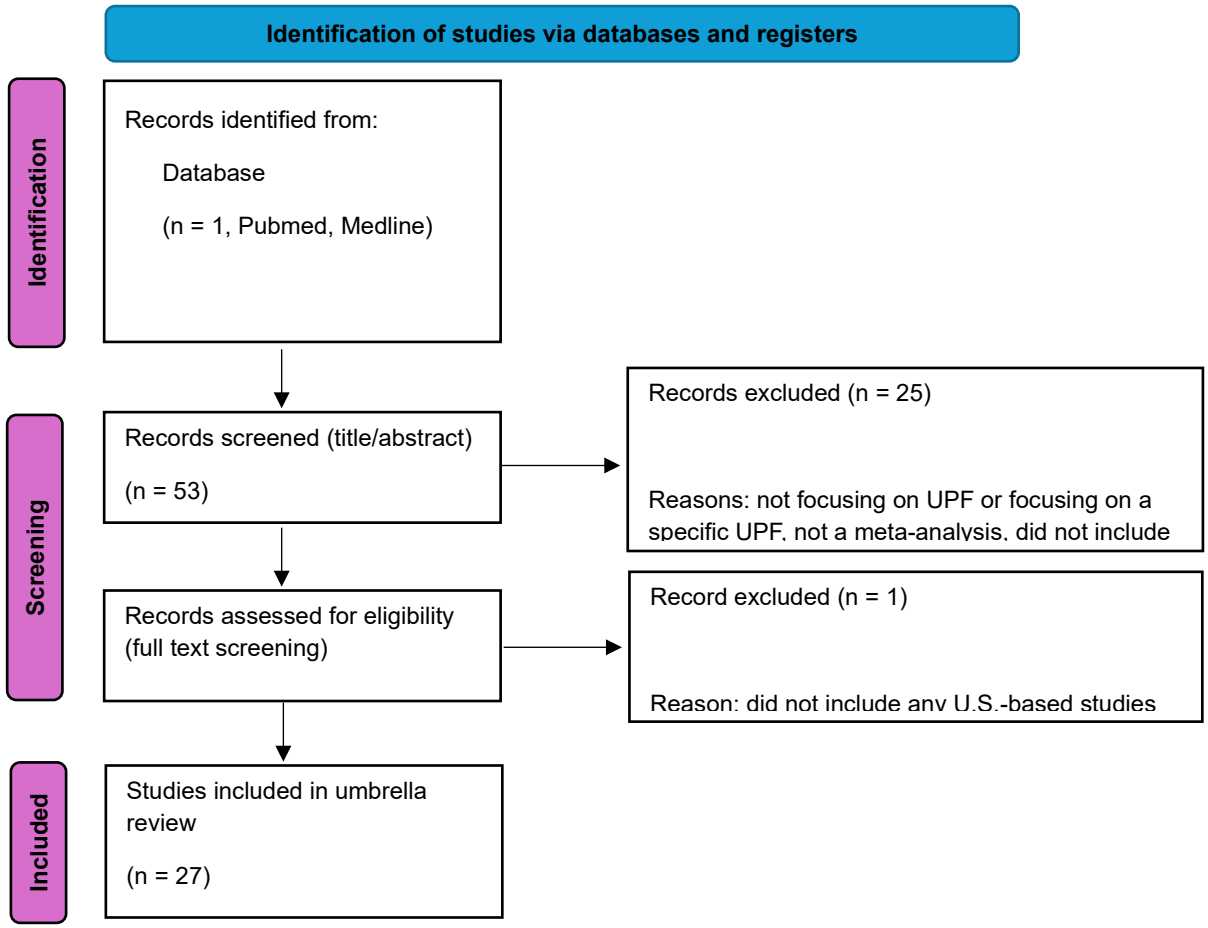


Figure 1. Flow Diagram Showing Selection of Papers at Various Stages of the Process

Figure 2. Forest Plot Showing the Relative Risk in Each of the Meta-Analysis vs Outcomes of Interest Across All Studies Reviewed (Circled risk scores identify the lead review for each outcome examined)

TABLE 1: Summary of Findings for Each Outcome Based On Lead Reviews

Outcome	Lead Meta Analysis (citation)	Effect Estimate/CI and I^2	Dose Response Analysis	GRADE Certainty	Rationale for Downgrade/Upgrade
All-cause mortality	Liang et al; 2025	RR: 1.15 CI: 1.09 - 1.22 I^2 = 83%	10% increase in the proportion of HPF associated with 10% higher risk of all-cause mortality (p<0.001)	MODERATE	Upgrade: Dose response; Direction of plausible effect Downgrade: Inconsistency
Cancer (all cause)	Isaksen and Dankel; 2023	RR: 1.12 CI: 1.06 - 1.19 I^2 = 33%	10% higher HPF associated with 13% risk of any cancer	MODERATE	Upgrade: Dose response; Direction of plausible effect Downgrade: Risk of Bias
Cardiovascular events	Yuan et al; 2023	RR: 1.35 CI: 1.18 - 1.54 I^2 = 62.1%	Each daily serving of HPF increased risk of CV event by 4%	MODERATE	Upgrade: Dose response; Direction of plausible effect Downgrade: Inconsistency
Liver/All Adverse Liver/NAFLD	Guo et al; 2025	RR: 1.58 CI: 1.34 - 1.86 I^2 = 89.9% RR: 1.72 CI: 1.36 - 2.17 I^2 = 93.3%	Not Studied	LOW LOW	Upgrade: Direction of plausible effect; Large effect Downgrade: Inconsistency; Imprecision
Obesity	Moradi et al; 2023	RR: 1.55 CI: 1.36 - 1.77 I^2 = 54.8%	10% higher HPF associated with 7% higher risk of obesity, 6% higher risk of overweight and 5% higher risk of abdominal obesity	MODERATE	Upgrade: Dose response; Large effect; Direction of plausible effect Downgrade: Inconsistency; Risk of Bias
Type 2 Diabetes	Kim et al; 2025	RR: 1.48 CI: 1.36 - 1.61 I^2 = 73.3%	10% higher HPF associated with 14% higher risk of Type 2 Diabetes. In addition, each serving per day of HPF was associated with a 4% higher risk of type 2 diabetes	HIGH	Upgrade: Dose response; Direction of plausible effect; Large effect Downgrade: Inconsistency
Dementia (all-cause)	Henney et al; 2024	RR: 1.44 CI: 1.09 - 1.90 I^2 = 97%	Highest versus lowest intake of HPF was significant but no significant effect of moderate versus lowest.	HIGH	Reported as HIGH using GRADE in the lead meta-analysis
Depression	Mazioomi et al; 2023	RR: 1.28 CI: 1.19 - 1.38 I^2 = 61.8%	10% higher HPF associated with 11% higher risk of depression	HIGH	Reported as HIGH using GRADE in the lead meta-analysis

TABLE 2: Comparison of This Umbrella review with 2 Prior Umbrella Reviews

Outcome	RR and GRADE in Dai et al [15]	RR and GRADE in Lane et al [14]	RR and GRADE in current study
Meta-analysis included; Search end period	33; March 2023 Included any health outcome)	14; June 2023	27; September 2025 Limited to chronic disease outcomes, cancer and mental health
All-cause mortality	1.21 (1.12 – 1.31) VERY LOW	1.21 (1.15 – 1.27) LOW	1.15 (1.09 – 1.22) [from study published 2025] MODERATE
Cancer (all causes)	1.06 (0.99 – 1.14) VERY LOW	1.12 (1.06 – 1.19) VERY LOW	1.12 (1.06 – 1.19) [from study published 2023] MODERATE
Cardiovascular events	1.11 (1.07 – 1.16) VERY LOW	1.35 (1.18 – 1.54) VERY LOW	1.35 (1.18 – 1.54) [from study published 2023] MODERATE
Dementia (all cause)	Not studied	Not Studied	1.44 (1.09 – 1.90) HIGH [from study published 2024]
Depression	1.40 (1.26 – 1.55) VERY LOW	1.22 (1.16 – 1.28) LOW	1.28 (1.19 – 1.38) HIGH [from study published 2023]
Liver (NAFLD)	1.30 (0.98 – 1.74) VERY LOW	1.23 (1.03 – 1.46) VERY LOW	1.72 (1.36 – 2.17) [from study published 2025] LOW
Obesity	1.26 (1.18 – 1.36) VERY LOW	1.55 (1.36 – 1.77) LOW	1.55 (1.36 – 1.77) MODERATE [from study published 2023]
Type 2 Diabetes	1.23 (1.13 – 1.33) VERY LOW	1.40 (1.23 – 1.59) VERY LOW	1.48 (1.36 – 1.61) HIGH [from study published 2025]

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Appendix A. Search strings.

("ultra-processed food"[Title/Abstract] OR "ultra processed food"[Title/Abstract] OR "ultra-processed foods"[Title/Abstract] OR "ultra processed foods"[Title/Abstract] OR "highly processed food"[Title/Abstract] OR "highly processed foods"[Title/Abstract] OR "highly-processed food"[Title/Abstract] OR "highly-processed foods"[Title/Abstract] OR "UPF"[Title/Abstract] OR "UPFs"[Title/Abstract] OR "NOVA classification"[Title/Abstract] OR "processed food"[Title/Abstract] OR "processed foods"[Title/Abstract] OR "industrial food"[Title/Abstract] OR "industrial foods"[Title/Abstract] OR "junk food"[Title/Abstract] OR "junk foods"[Title/Abstract] OR "junk food"[MeSH Terms] OR "refined food"[Title/Abstract] OR "refined foods"[Title/Abstract])
AND
(obesity[Title/Abstract] OR overweight[Title/Abstract] OR "obesity"[MeSH Terms] OR "type 2 diabetes"[Title/Abstract] OR "diabetes mellitus, type 2"[MeSH Terms] OR "cardiovascular disease"[Title/Abstract] OR "cardiovascular diseases"[MeSH Terms] OR "myocardial infarction"[Title/Abstract] OR stroke[Title/Abstract] OR "heart attack"[Title/Abstract] OR "cardiovascular event*"[Title/Abstract] OR "non-alcoholic fatty liver disease"[Title/Abstract] OR NAFLD[Title/Abstract] OR "Fatty Liver"[MeSH Terms] OR Alzheimer*[Title/Abstract] OR "Alzheimer Disease"[MeSH Terms] OR dementia[Title/Abstract] OR "cognitive function"[Title/Abstract] OR cognition[Title/Abstract] OR memory[Title/Abstract] OR learning[Title/Abstract] OR "cognition disorders"[MeSH Terms] OR depression[Title/Abstract] OR anxiety[Title/Abstract] OR "major depressive disorder"[Title/Abstract] OR "anxiety disorders"[Title/Abstract] OR "dental caries"[Title/Abstract] OR "dental caries"[MeSH Terms] OR "tooth decay"[Title/Abstract] OR HbA1c[Title/Abstract] OR "glycated hemoglobin"[Title/Abstract] OR "hemoglobin A1c"[Title/Abstract] OR BMI[Title/Abstract] OR "body mass index"[Title/Abstract] OR "body weight"[Title/Abstract] OR "blood pressure"[Title/Abstract] OR hypertension[Title/Abstract] OR cancer[Title/Abstract] OR "neoplasms"[MeSH Terms] OR mortality[Title/Abstract] OR "mortality"[MeSH Terms])
AND ("meta-analysis"[pt] OR "meta-analysis"[Title])
AND Humans[MeSH]
AND ("2000/01/01"[Date - Publication] : "3000"[Date - Publication]

APPENDIX TABLE 1: Evidence Table

Citation	*Last search*	*Databases*	*No. RCTs/Studies*	*Eligibility criteria*	*Outcomes reported*	*RR and CI*	*Heterog. (I^2)*	*Dose Response*	*Certainty (GRADE)*	*Risk of Bias method*
Askari et al (2020)	November 2019	Scopus, PubMed and Web of Science	10 studies (9 cross-sectional and 1 cohort)	Observational; examined UPF and obesity; English	Obesity	1.26 (1.13, 1.41)	92.7%	No dose-response given	Not specified	Begg's funnel
Delpino et al (2022)	April 2021	PubMed, LILACS, Scielo, Scopus, Embase, Web of Science	18 cohorts	Cohort studies with UPF as main exposure	Type 2 diabetes	1.31 (1.21, 1.42)	60.0%	Linear dose-response	Moderate to High	Newcastle-Ottawa Scale and Eggers
Ejtahed et al (2024)	July 2023	PubMed, Web of Science, Scopus, Cochrane, Google Scholar and Embase	6 studies	Cross-sectional	Mental Health/Depression	1.161 (1.039, 1.283)	66%	No dose response given	Not specified	Newcastle-Ottawa Scale
Guo et al (2025)	October 17, 2024	PubMed, Cochrane, Embase, Web of Science	4 cohorts	Observational studies examining UPF and liver outcomes	Cancer/ Liver	1.35 (1.030, 1.76)	65.0%	No dose-response given	Not specified	Newcastle-Ottawa Scale
	October 17, 2024	PubMed, Cochrane, Embase, Web of Science	3 cohorts	Observational studies examining UPF and liver outcomes	Liver Fibrosis	1.31 (1.080, 1.59)	1.1%	No dose-response given	Not specified	Newcastle-Ottawa Scale
	October 17, 2024	PubMed, Cochrane, Embase, Web of Science	17 studies (11 cohorts, 3 case-control and 3 cross-section)	Observational studies examining UPF and liver outcomes	Liver: All Adverse Outcomes	1.58 (1.34, 1.86)	89.9%	No dose-response given	Not specified	Newcastle-Ottawa Scale
	October 17, 2024	PubMed, Cochrane, Embase, Web of Science	14 cohorts	Observational studies examining UPF and liver outcomes	Liver/NAFLD	1.72 (1.36, 2.17)	93.3%	No dose-response given	Not specified	Newcastle-Ottawa Scale
Henney et al (2023)	December 2022	Ovid, Web of Science	9 cohorts	Observational study in adults; UPF defined by NOVA, 18+	Liver/NAFLD	1.42 (1.16, 1.75)	89.0%	No dose-response given	High (8)	Newcastle-Ottawa Scale

Citation	Last search	Databases	No. RCTs/Studies	Eligibility criteria	Outcomes reported	RR and CI	Heterog. (I^2)	Dose Response	Certainty (GRADE)	Risk of Bias method
Henney et al (2024)	December 2022	Ovid, Medline and Web of Science	10 studies (8 longitudinal, 1 case-control, 1 cross-sectional)	Observational study with UPF measured by Nova or enough dietary data to assign NOVA, and validated dementia outcome	Mental Health/Dementia (all cause)	1.44 (1.090, 1.90)	97.0%		High	Newcastle-Ottawa Scale
Isaksen and Dankel (2023)	January 2023	PubMed, Embase	11 cohorts	Observational studies in adults, UPF defined by NOVA	Cancer (all except melanoma and skin)	1.121 (1.060, 1.19)	33 (ns)	No dose-response given	Not specified	NIH Quality Assessment
Karimi et al (2025)	May 1, 2025	PubMed, Web of Science, Scopus	17 studies (6 cohorts and 11 case-control)	Observational studies on UPF/fast food and breast cancer outcome	Cancer/Breast	1.25 (1.090, 1.43)	79.0%	No dose-response given	Not specified	Newcastle-Ottawa Scale
Kim et al (2025)	January 2024	PubMed, Embase, Web of Science	12 cohorts	Prospective cohort with NOVA defined UPF	Type 2 Diabetes	1.48 (1.36, 1.61)	73.3%	Linear dose-response; 10% increase in UPF = 14% higher risk of T2D (or in a smaller sub-set 1 serving/day increase = 4% higher risk)	Not specified	Eggers test and funnel plots
Lane et al (2022)	March 2022	Medline, Embase, Scopus	15 cross-sectional and 2 longitudinal cohorts	English, any age, observational and had to use NOVA for UPF	Mental Health/Depression/anxiety	1.53 (1.43, 1.63)	8.9%	No dose-response given	Not specified	Not stated
Liang et al (2025)	July 2, 2024	PubMed, Embase, Cochrane	15 cohorts	Prospective cohort with NOVA defined UPF; excluded if analysis limited to specific foods	All-cause mortality	1.15 (1.090, 1.220)	83.0%	Linear dose-response; 10% increase in UPF = 10% higher risk of mortality	Not specified	Newcastle-Ottawa Scale; Beggs test
Malmir et al (2023)	December 2022	PubMed, Web of Science, Cochrane, Embase	9 cohorts	Observational studies in children	Mental Health (Anxiety)	1.24 (1.35, 1.50)	80.7%	No dose-response given	Not specified	Newcastle-Ottawa Scale

Citation	Last search	Databases	No. RCTs/Studies	Eligibility criteria	Outcomes reported	RR and CI	Heterog. (I^2)	Dose Response	Certainty (GRADE)	Risk of Bias method
	December 2022	PubMed, Web of Science, Cochrane, Embase	9 cohorts	Observational studies in children	Mental Health/Depression	1.62 (1.030, 1.95)	99.4%	No dose-response given	Not specified	Newcastle-Ottawa Scale
Mazloomi et al (2023)	December 2021	Web of Science, PubMed, Scopus	26 cohorts	Observational studies in adults	Mental Health/Depression	1.28 (1.19, 1.38)	61.8%	Linear dose-response; 10% increase in UPF proportion = 11% higher risk of depression	High	Newcastle-Ottawa Scale
Meine et al (2024)	June 27, 2023	PubMed, Embase, Cochrane	4 cohorts	Observational cohort studies in adults with UPF measures by NOVA and cancer outcomes	Cancer/Colorectal	1.11 (1.030, 1.21)	31.0%	No dose-response given	Not specified	NIH Quality Assessment
Mekonnen et al (2025)	January 2024	PubMed, Embase, PsycInfo, Scopus, ProQuest, Web of Science, Cochrane	6 cohorts	RCTs and cohort studies in adults with NOVA defined UPF and ICD codes for lung disease outcomes	Cancer/Lung	1.050 (0.83, 1.31)	83.7%	No dose-response given	Very low	NIH Quality Assessment
Moradi et al (2023)	December 30, 2020	PubMed, Scopus, Embase, Web of Science	10 cohorts	Observational studies in adults	Obesity	1.55 (1.36, 1.77)	54.8%	Linear dose-response; 10% increase in UPF proportion = 7% higher risk of obesity	Not specified	Newcastle-Ottawa Scale
	December 30, 2020	PubMed, Scopus, Embase, Web of Science	6 cohorts	Observational studies in adults	Obesity (Abdominal)	1.41 (1.18, 1.68)	62.2%	Linear dose-response; 10% increase in UPF proportion = 5% higher risk of obesity	Not specified	Newcastle-Ottawa Scale

Citation	Last search	Databases	No. RCTs/Studies	Eligibility criteria	Outcomes reported	RR and CI	Heterog. (I^2)	Dose Response	Certainty (GRADE)	Risk of Bias method
Pagliai et al (2021)	June 2020	Medline, Embase, Scopus, Web of Science, Google	5 cohorts	Healthy subjects 18y or older	All-cause mortality	1.25 (1.14, 1.37)	2.0%	No dose-response given	Not specified	Newcastle-Ottawa Scale
Pourmotabbed et al (2025)	June 24, 2023	Scopus, PubMed, Web of Science	17 cohorts	Observational study in adults	Mental Health:Cognitive Impairment	1.17 (1.06, 1.30)	74.1%	No dose-response given	Moderate	Newcastle-Ottawa Scale; Beggs test; Eggers test
Souza et al (2025)	November 14, 2024	Pubmed, Embase	10 cohorts	Prospective cohort study in adults	Type 2 Diabetes	1.24 (1.14, 1.34)	69.0%	Linearity not given; 10% increase in UPF	Very low	ROBINS-I tool
Suksatan et al (2021)	August 2021	Web of Science, PubMed, Scopus	7 cohorts	Adult cohort studies	All-cause mortality	1.21 (1.13, 1.30)	21.9%	Linear dose-response	Not specified	Eggers and Beggs
	August 2021	Web of Science, PubMed, Scopus	4 cohorts	Adult cohort studies	CVD mortality	1.50 (1.37, 1.63)	0.0	Linear dose-response	Not specified	Eggers and Beggs
	August 2021	Web of Science, PubMed, Scopus	2 cohorts	Adult cohort studies	CVD/Heart disease mortality	1.66 (1.50, 1.85)	0.0	Linear dose-response	Not specified	Eggers and Beggs
Taneri et al (2022)	January 2021	Medline, Embase, Web of Science, Cochrane, Google Scholar	5 cohorts	Prospective studies, adults; excl trials and cross-sectional	All-cause mortality	1.29 (1.17, 1.42)	0.0	No dose-response given	Not specified	Newcastle-Ottawa Scale
Vitale et al (2024)	April 1, 2023	PubMed, Medline, Web of Science, Scopus	25 cohorts	Observational studies in adults, English, 18+	CVD/Dyslipidemia	1.47 (1.120, 1.93)	46.0%	No dose-response given	Low	Newcastle-Ottawa Scale
	April 1, 2023	PubMed, Medline, Web of Science, Scopus	25 cohorts	Observational studies in adults, English, 18+	CVD/Hypertension	1.32 (1.190, 1.45)	21.0%	No dose-response given	Low	Newcastle-Ottawa Scale
	April 1, 2023	PubMed, Medline, Web of Science, Scopus	25 cohorts	Observational studies in adults, English, 18+	Obesity	1.32 (1.20, 1.45)	81.0%	No dose-response given	Low	Newcastle-Ottawa Scale
	April 1, 2023	PubMed, Medline, Web of Science, Scopus	25 cohorts	Observational studies in adults, English, 18+	Type 2 Diabetes	1.37 (1.20, 1.56)	52.0%	No dose-response given	Moderate	Newcastle-Ottawa Scale

Citation	Last search	Databases	No. RCTs/Studies	Eligibility criteria	Outcomes reported	RR and CI	Heterog. (I^2)	Dose Response	Certainty (GRADE)	Risk of Bias method
Wang et al (2022)	January 2022	PubMed, Embase, Cochrane	9 studies (5 cross-sectional and 4 cohorts)	Observational studies in adults, UPF defined by NOVA	CVD/Hypertension	1.23 (1.110, 1.37)	51.9%	No dose-response given	Not specified	Newcastle-Ottawa Scale
Wang et al (2024)	August 9, 2022	Cochrane, Embase, PubMed, Web of Science	9 studies (5 cross-sectional and 4 longitudinal cohorts)	Population based studies of UPF and blood pressure; people of any age, sex, race, nationality	CVD/Hypertension	1.23 (1.110, 1.37)	51.9%	No dose-response given	Low	Newcastle-Ottawa Scale
You et al (2025)	January 2023	PubMed, Embase, Web of Science	12 studies (1 cross-sectional, 11 cohort)	Observational study in adults, excluding pregnancy, RCT	CVD mortality	1.31 (1.010, 1.62)	86.2%	No dose-response given	High to moderate	Newcastle-Ottawa Scale
Yuan et al (2023)	July 21, 2022	PubMed, Embase, Web of Science	9 cohorts	Prospective cohort, adults, used NOVA definition; excluded major disease at baseline or exposure related to specific foods	All-cause mortality	1.21 (1.15, 1.27)	11.6%	Linear dose response; Each daily serving of UPF = a 2% higher risk of CVE	Not specified	Newcastle-Ottawa Scale
	July 21, 2022	PubMed, Embase, Web of Science	8 cohorts	Prospective cohort, adults, used NOVA definition; excluded major disease at baseline or exposure related to specific foods	CVD/events	1.35 (1.18, 1.54)	62.1%	Linear dose-response; Each daily serving of UPF = 4% higher risk of CVE	Not specified	Newcastle-Ottawa Scale

Note: Ultra-professed food exposure was defined either by NOVA or other dietary descriptions. Studies in blue indicate selection as lead meta-analysis. The comparator is highest versus lowest level of exposure across all meta-analyses. No meta-analyses reported conflicts, with the exception of Lane et. al (2022).

APPENDIX TABLE 2: Risk of Bias / Quality Appraisal of Reviews Table

Citation	Outcomes reported	Quality Appraisal	One-line rationale
Askari et al (2020)	Obesity	Low	Most studies were conducted outside the US.
Delpino et al (2022)	Type 2 diabetes	High	Very thorough analysis including sensitivity and sub-group analysis.
Ejtahed et al (2024)	Mental Health	Low	Downgraded due to inconsistent definitions and assessments of UPF, unclear outcome measures and analytical methods, and inclusion of only longitudinal data despite mixed study designs.
Guo et al (2025)	Cancer	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing.
	Liver Disease	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing.
	Liver Disease	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing.
	Liver Disease	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing.
Henney et al (2023)	Liver Disease	Moderate to high	Thorough study evaluated bias, sensitivity and GRADE using appropriate methodology.
Henney et al (2024)	Mental Health	Moderate to high	Comprehensive analysis meeting criteria for bias and sensitivity analysis and included Grading assessment as High.
Isaksen and Dankel (2023)	Cancer	High	Comprehensive study.
Karimi et al (2025)	Cancer	Moderate	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing.
Kim et al (2025)	Type 2 Diabetes	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing and dose response.
Lane et al (2022)	Mental Health	Low	No mention of bias assessment or GRADE.
Liang et al (2025)	All-cause mortality	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing and dose response.
Malmir et al (2023)	Mental Health	Moderate to high	Good quality study specific to depression/anxiety in children, but reported high sensitivity.

Citation	Outcomes reported	Quality Appraisal	One-line rationale
	Mental Health	Moderate	Good quality study specific to depression/anxiety in children. Downgraded because of high sensitivity, but otherwise rigorous analysis incorporating examination for bias and sensitivity
Mazloomi et al (2023)	Mental Health	High	Comprehensive and rigorous analysis using robust methods as well as dose response.
Meine et al (2024)	Cancer	High	Comprehensive and thorough analysis including for bias and sensitivity analysis;
Mekonnen et al (2025)	Cancer	Very low	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing.
Moradi et al (2023)	Obesity	Moderate to high	Comprehensive analysis meeting criteria for bias and sensitivity analysis.
	Obesity	Moderate to high	Comprehensive analysis meeting criteria for bias and sensitivity analysis.
Pagliai et al (2021)	All-cause mortality	Moderate	Although extensive analyses were conducted, MIG focused only on prospective studies, specifically five cohorts examining all-cause mortality, excluding other outcomes due to limited data from only two or three studies.
Pourmotabbed et al (2025)	Mental Health	Moderate	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing and dose response;as well as evidence grading.
Souza et al (2025)	Type 2 Diabetes	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing and dose response;as well as evidence grading.
Suksatan et al (2021)	All-cause mortality	High	Thorough and comprehensive analysis including for bias and sensitivity - the combination of the HR and dose response give this high quality.
	CVD	High	Thorough and comprehensive analysis including for bias and sensitivity - the combination of the HR and dose response give this high quality.
	CVD	Low	Downgraded to low because of the smaller sample size of cohorts for this estimate.
Taneri et al (2022)	All-cause mortality	High	Extensive analysis in 5 cohorts with NOVA defined UPF shows clear sig effect.
Vitale et al (2024)	CVD	Moderate	Mixed definitions used for UPF.

Citation	Outcomes reported	Quality Appraisal	One-line rationale
	CVD	Moderate	Mixed definitions used for UPF.
	Obesity	Moderate	Mixed definitions used for UPF.
	Type 2 Diabetes	Moderate	Mixed definitions used for UPF.
Wang et al (2022)	CVD	Moderate	Very specific study on hypertension using appropriate checks for bias and sensitivity showing sig relationship with hypertension in a mix of observational studies.
Wang et al (2024)	CVD	High	This study re-analyzed prior systematic reviews using more rigorous methods due to identified methodological flaws, but the nine included studies had varying or unreported definitions of hypertension.
You et al (2025)	CV	Moderate to high	Comprehensive study with rigorous methodology, checks for bias and sensitivity testing and dose response.
Yuan et al (2023)	All-cause mortality	High	Comprehensive and rigorous analysis using robust methods as well as dose response.
	CVD	Moderate to high	Comprehensive and rigorous analysis using robust methods as well as dose response

Note: meta-analyses in blue indicate lead reviews.

Appendix 4.2. Added Sugars, Sugar-Sweetened Beverages, Juice & Chronic Disease

ADDED SUGARS, SUGAR-SWEETENED BEVERAGES, 100% FRUIT JUICE, AND NON-SUGAR SWEETENED BEVERAGES IN RELATION TO CHRONIC DISEASE OUTCOMES IN CHILDREN AND ADULTS

An Umbrella Review

Michael Goran, PhD
Professor and Vice Chair for Research
Department of Pediatrics, Keck School of Medicine
Children's Hospital of Los Angeles
University of Southern California

Abstract

Objective: The goal of this umbrella review was to synthesize evidence on the associations of added sugars, sugar-sweetened beverages (SSBs), 100% fruit juice, and non-sugar-sweetened beverages (NSSBs) with chronic disease-related health outcomes in children and adults, aiming to provide insights for public health policy and identify future research priorities.

Methods: PubMed was systematically searched (including publications from January 2000 to September 2025) for meta-analyses of prospective cohort studies and randomized controlled trials that examined the relationships between 4 exposures (added sugars, SSB, 100% fruit juice and NSSBs) relative to 8 chronic disease outcomes (type 2 diabetes (T2D), cardiovascular diseases (CVD), obesity, cancer, non-alcoholic fatty liver disease (NAFLD), dental caries, cognitive function, and mortality). Data was extracted from each identified meta-analysis, and the methodology/bias was evaluated. Using a systematic approach, we identified lead meta-analysis for each exposure vs outcome examined and the meta-evidence quality was assessed using the GRADE framework.

Results: Fifty-four meta-analyses for added sugars/SSBs/100% fruit juice and 19 for NSSBs were included, and 27 were identified as lead studies. High consumption of added sugars was associated with 31% higher risk of NAFLD (Low-quality evidence, GRADE), but the evidence for other outcomes was non-existent or inconsistent and of low quality. SSBs were significantly associated with dental caries (57% higher risk; High), adult obesity (20%; Moderate), T2D (39%; Moderate), all-cause mortality (10%; Low), CVD (20%; Low), childhood obesity (20%; Low), depression (25%; Moderate) and cognitive disorders (17%; Very Low). Fruit juice (100%) was associated with significantly greater risk of obesity in children (High) and T2D (7%; Low). There was evidence of harm associated with the consumption of NSSBs, including for all-cause mortality (13%, Moderate), cardiovascular disease (17%, Low), T2D (8%, low), and Alzheimer's disease (42%, Moderate). Neutral associations were observed for some outcomes for both 100% fruit juice and NSSBs without evidence of any beneficial effect. Dose-response relationships for added sugars,100% juice and NSSBs were unclear, but for SSBs, there was evidence for linear effects on all-cause mortality, CVD, and T2D. Each 12 fluid ounce can of SSB per day (355 mL, 39g added sugars) was associated with 10% increased risk for all-cause mortality, 14% for CVD, and 23.4% for T2D.

Conclusion: Higher consumption of SSBs showed consistent associations with increased risk of multiple chronic diseases with evidence for dose-response for several outcomes. In contrast, evidence for added sugars was more limited, mainly indicating higher risk of NAFLD. Evidence for NSSBs and 100% fruit juice was mixed but did include some adverse cardiometabolic outcomes and no indication of benefit for any outcomes. Collectively, the current evidence base indicates that the most promising opportunity to address added sugars is through public health and policy strategies that promote reduction in consumption of SSBs. Precautions are warranted regarding added sugars, NSSBs, and 100% fruit juice until further high-quality longitudinal and mechanistic research strengthens the meta-evidence, elucidates dose–response relationships, and clarifies their long-term metabolic and life-course health implications.

Graphical Abstract

	All-cause mortality	Overall cancer	Cardiovascular diseases	Non-alcoholic fatty liver disease	Childhood obesity	Adult obesity	Type 2 diabetes	Depression	Cognitive disorders	Dental caries
Sugar sweetened beverages	★ ↑ 10% (S) **Low**	↑ 7% (NS) **Very Low**	★ ↑ 20% (S) **Low**	↑ 39% (S) **Very Low**	↑ 20% (S) **Low**	↑ 17% (S) **Moderate**	★ ↑ 39% (S) **Moderate**	↑ 25% (S) **Moderate**	↑ 17% (S) **Very Low**	↑ 57% (S) **High**
Added sugars	↑ 5% (NS) **Low**		↑ 8% (NS) **Very Low**	↑ 31% (S) **Low**			↓8% (NS) **Low**			
100% fruit juice	↑ 0% (NS) **Very Low**	↑ 9% (NS) **Low**	↓5% (NS) **Low**		↑0.03 BMI (S) **High**	↑0.07kg (NS) **Very Low**	↑ 7% (S) **Low**			
Non-sugar sweetened beverages	↑ 13% (S) **Moderate**	0% (NS) **Very Low**	↑ 17% (S) **Low**		↑0.05 BMI (NS) **Moderate**	↑ 39% (NS) **Low**	↑ 8% (S) **Low**		↑42% (S) **Moderate**	

★ Clear and significant linear dose-response relationship identified

In bold, meta-evidence GRADE quality

S: significant or NS: non-significant relationships (mainly from high vs. low consumption comparison); if not specified, the population is assumed to be adults by default

Introduction

In recent decades, the global food landscape has undergone a profound transformation, largely driven by the rapid expansion of the processed food and beverage industries [1]. Among the most notable shifts has been the widespread inclusion of added sugars (those introduced during production or preparation) into an ever-growing range of everyday products. From sweetened yogurts and cereals to sauces, snack bars, and beverages, added sugars have become almost ubiquitous in packaged foods [2].

Sugar-sweetened beverages (SSBs) are the single largest source of added sugars in the American diet [3]. These drinks are typically sweetened with sucrose, high-fructose corn syrup, or fruit juice concentrates [4]. and include, but are not limited to, regular soda, fruit drinks, sports drinks, energy drinks, sweetened waters, and coffee/tea beverages with added sugars. SSB consumption in the U.S. remains a major public health concern: between 2011 and 2014, U.S. adults consumed an average of 145 kcal per day from SSBs, making up 6.5% of total daily caloric intake [5]. Among youth, 63% consumed a SSB on any given day, with the average intake reaching 413 kcal/day from these beverages [6].

A growing body of research has linked high added sugar and SSB consumption to a broad array of chronic health issues. A recent umbrella review on sugar consumption (particularly from SSBs) provides valuable insights but is limited by its inclusion of studies only up to 2022 [7]. This underscores the need for an updated assessment, particularly as the research landscape on added sugars evolves rapidly, with advancements in study designs, variations in measurements of dietary sugar intake, shifting definitions of exposure, and the development of more sophisticated statistical approaches. In addition, much of the meta-analytic evidence influencing current dietary guidelines on SSBs has focused largely on anthropometric outcomes, obesity and type 2 diabetes, without consideration of other critical health areas, such as dental health, cardiovascular disease, cancer, mental health, and mortality [8]. A more comprehensive, updated evaluation of the health risks associated with added sugar and SSBs is needed to provide a more robust foundation for food-based public health recommendations.

Equally deserving of attention is 100% fruit juice (defined as unsweetened juice derived entirely from whole fruit with no added sugar). The 2020–2025 Dietary Guidelines for Americans include 100% fruit juice within the fruit group and consider it part of a healthy dietary pattern and considers this as part of recommended fruit intake. However the previous guidelines also specify that at least half of fruit intake come from whole fruits [3]. While 100% juice does provide fiber, vitamins and phytonutrients like whole fruit, some of these beneficial nutrients can be lost during juice extraction, and it also contributes to greater dietary sugar intake than the whole fruit alone (often referred to as “free” sugars) on a per serving basis. However, recent umbrella reviews highlight some ambivalence regarding the health impact of 100% juice [9]. Notably, this previous umbrella review, like the most recent one on dietary sugar, only includes studies up to 2022, and further highlights the need for updated guidance in this area.

Another beverage category of interest relates to alternative sweeteners. The global shift toward reducing added sugars has fueled rapid growth in the consumption of both low-calorie and non-nutritive sweeteners which include aspartame, sucralose, saccharin, acesulfame-K, stevia, monk fruit sweetener, allulose and sugar alcohols. These sweeteners are now used widely in beverages and other foods, although for this umbrella review, we will limit the focus to beverages, and will be defined as non-sugar sweetened beverages (NSSBs). The popularity of NSSBs has been driven by consumer demand for “sugar-free” or “reduced-calorie” products and by public health efforts to curb obesity, diabetes, and other diet-related chronic diseases. However, the extent to which NSSB achieve these health goals, or introduce new risks, remains a matter of ongoing scientific debate. A 2023 umbrella review revealed that NSSBs exhibit suboptimal health effects (higher risk of obesity, T2D, all-cause mortality, hypertension, and cardiovascular disease incidence) [10]. The study also emphasized the need for additional research, pointing out the limited number of randomized controlled trials, short intervention periods, and methodological challenges.

The overall objective of this umbrella review was to therefore update the meta-evidence on the health impacts of added sugars, focusing on their main source in the diet, particularly SSBs. The review also explored health risks of potential alternatives to SSBs, such as 100% fruit juice and NSSBs. Using a robust evidence-to-decision framework, the review aimed to provide clear, actionable recommendations to inform public health policies, dietary guidelines, and consumer choices, with the goal of reducing the risk of chronic diseases associated with high added sugar consumption.

Methods

Systematic Research

While all 4 exposures examined are related, two separate literature searches were conducted in this umbrella review, one focusing on added sugars, SSBs, and 100% fruit juice, and another on NSSBs. This distinction was made due to the differences between nutritive sweeteners (in SSBs and 100% fruit juice) and non-nutritive sweeteners as well as the separate bodies of literature regarding their health impacts.

One reviewer conducted a systematic literature search in PubMed (MEDLINE) from 2000 up to September 11, 2025, for potential meta-analyses of prospective cohort and randomized controlled trials (RCTs) related to added sugars, SSBs, and 100% fruit juice and clinical and health outcomes. The complete search strategy is provided **in Appendix 1.** The search terms for NSSBs are provided in **Appendix 2**. Two other reviewers reviewed the search criteria to ensure agreement on the literature search.

Selection of Meta-Analyses

Studies meeting the following criteria were deemed eligible for inclusion in this umbrella review: 1) meta-analyses of human-based observational studies and RCTs (including studies/models based on addition or reduction but not substitution) that evaluate at least one clinical or health outcome in the general population, including children, adolescents,

and adults; 2) included individual studies assessing dietary intake using standard dietary assessment methods (e.g., food frequency questionnaires, 24-hour dietary recalls, or dietary records); 3) included individual studies reporting clinical outcomes, such as type 2 diabetes (T2D), cardiovascular diseases (CVD) (e.g., myocardial infarction, stroke, coronary heart disease (CHD), hypertension), obesity or overweight, non-alcoholic fatty liver disease (NAFLD), dental caries, cognitive function (e.g., memory, learning, general cognition), Alzheimer's disease and dementia, depression and anxiety disorders, cancer, and all-cause mortality, as well as biomarkers like HbA1c, body weight, BMI, or blood pressure; 4) studies published in English; 5) including at least one individual study conducted in the U.S. Meta-analyses were excluded if they: 1) lacked generalizability (e.g., had specific geographic constraints); 2) were narrative, systematic, or scoping reviews; 3) exclusively enrolled participants with a specific existing disease or health outcome; 4) focused on exposures other than added sugars, SSBs, 100% fruit juice or NSSBs with ambiguous or unclear definitions; or 5) were published in a language other than English.

Screening

One researcher screened all PubMed records against the eligibility criteria (titles and abstracts). Two other reviewers verified a 50% random sample to ensure consistency and any disagreements were flagged for discussion and final decision. At the end of the screening process, selected articles underwent data extraction.

Data Extraction

One reviewer conducted the primary extraction across all selected articles (full text), followed by a cross-check by two other reviewers. Data was extracted using a Google Sheets template including: citation, last search date, databases, number of RCTs/studies, eligibility criteria, outcomes reported, estimates (RR, OR, HR, or MDs) and CI, heterogeneity, dose response, GRADE, and risk of bias (ROB) method. For observational studies, notably, wherever possible during data extraction, estimates comparing "high versus low levels" of consumption were prioritized (including high versus low, never/low versus moderate/high, any versus none). Upon identifying a linear dose-response relationship, when applicable, the results were subsequently transformed to ensure a uniform interpretation, with the increased risk expressed on a per-can basis (350 mL, 39g added sugars).

Assessment of Methodological Quality

Based on the ROBIN tool[11], assessment of the included meta-analyses was conducted with the following quality appraisal categories (on a categorical scale of: High, Moderate, Poor): 1) clarity and pre-specification of eligibility criteria; 2) adequacy of the search strategy (i.e., multiple databases, transparent methods); 3) accuracy of data collection and presence of risk of bias assessment for included studies, and 4) appropriateness of synthesis methods and reporting of findings. The quality appraisal was assessed into one final grade by one reviewer, and a qualitative note was provided for the quality grade justification. A cross-check by two other reviewers was conducted. This

assessment was conducted on all meta-analyses regardless of the reported estimates and GRADE score (meta-evidence quality).

Selection of Lead Reviews

For each exposure–outcome dyad, the most relevant meta-analysis was selected based on the highest score from the quality appraisal system described above. Meta-analyses rated as Low or Very-low quality were excluded from consideration unless no higher-quality alternatives were available for that specific exposure-outcome pair. When multiple high-quality meta-analyses were identified, we prioritized the most recent and comprehensive one. In terms of outcomes, we favored clinical endpoints over surrogate markers. For major clinical outcomes such as cancer, mortality, and CVD, we prioritized overall outcomes (e.g., all-cause mortality and total cancer incidence). However, when these were not available, we included specific or subclinical outcomes such as hypertension, CHD, stroke, or site-specific cancers (e.g., breast cancer).

Grading the Meta-Evidence of Lead Studies

The certainty of evidence was assessed using the GRADE tool[12]. This tool grades the evidence as high, moderate, low, or very low quality. Studies are initially assessed on the level of confidence, where RCTs indicate high confidence and observational studies low confidence. Studies are then upgraded based on a large effect size, dose-response relationship, and the direction of plausible effect. Downgrading criteria included risk of bias, inconsistency, indirectness, and publication bias.

For the lead meta-analyses that did not report a GRADE score, one reviewer conducted a GRADE adjustment. For each meta-analysis, an initial high/low grade was assigned based on the study type (based on RCTs or observational studies), and then upgrades (a large effect size, linear dose-response relationship, and direction of plausible effect) and downgrades (risk of bias, inconsistency, indirectness, and publication bias) were recorded. The final grade was determined by the first reviewer and independently checked by two other reviewers. Discrepancies in ratings were noted, and disagreements were resolved by consensus.

Evidence to Decision

For each exposure considered (added sugars, SSBs,100% fruit juice and NSSBs), we finally translated the evidence from the lead meta-analyses into *Strong* (benefits clearly outweigh harms/burdens for most people at Moderate/High certainty) or *Conditional* (benefits likely outweigh harms, but certainty is lower or tradeoffs vary) recommendations using a GRADE consistent evidence to decision process that considers: (1) certainty of evidence; (2) balance of desirable vs undesirable effects; (3) outcome importance; and (4) feasibility.

Results

Studies Identified and Their Characteristics

Figures 1 and 2 illustrate the results of the literature search and selection process. For added sugars, SSBs, and 100% fruit juice, a total of 232 articles was initially identified and screened, with 130 articles excluded for various reasons as detailed in the figure. An additional 48 articles were excluded during the extraction process, leaving 54 meta-analyses for review (53 focused at least on the effects of SSBs on health). For NSSBs, an initial number of 36 meta-analyses were screened, and 19 were included in the analysis. A summary of all selected meta-analyses, including their key characteristics and major outcomes, is provided in **Appendices 3 and 4**. Many of the meta-analyses examined multiple outcomes and exposures, so separate rows are provided to present their respective estimates. However, the general characteristics (population and included studies) apply to the entire meta-analysis (unless otherwise stated). The quality assessments for each included meta-analysis are included in **Appendix 5 and 6**.

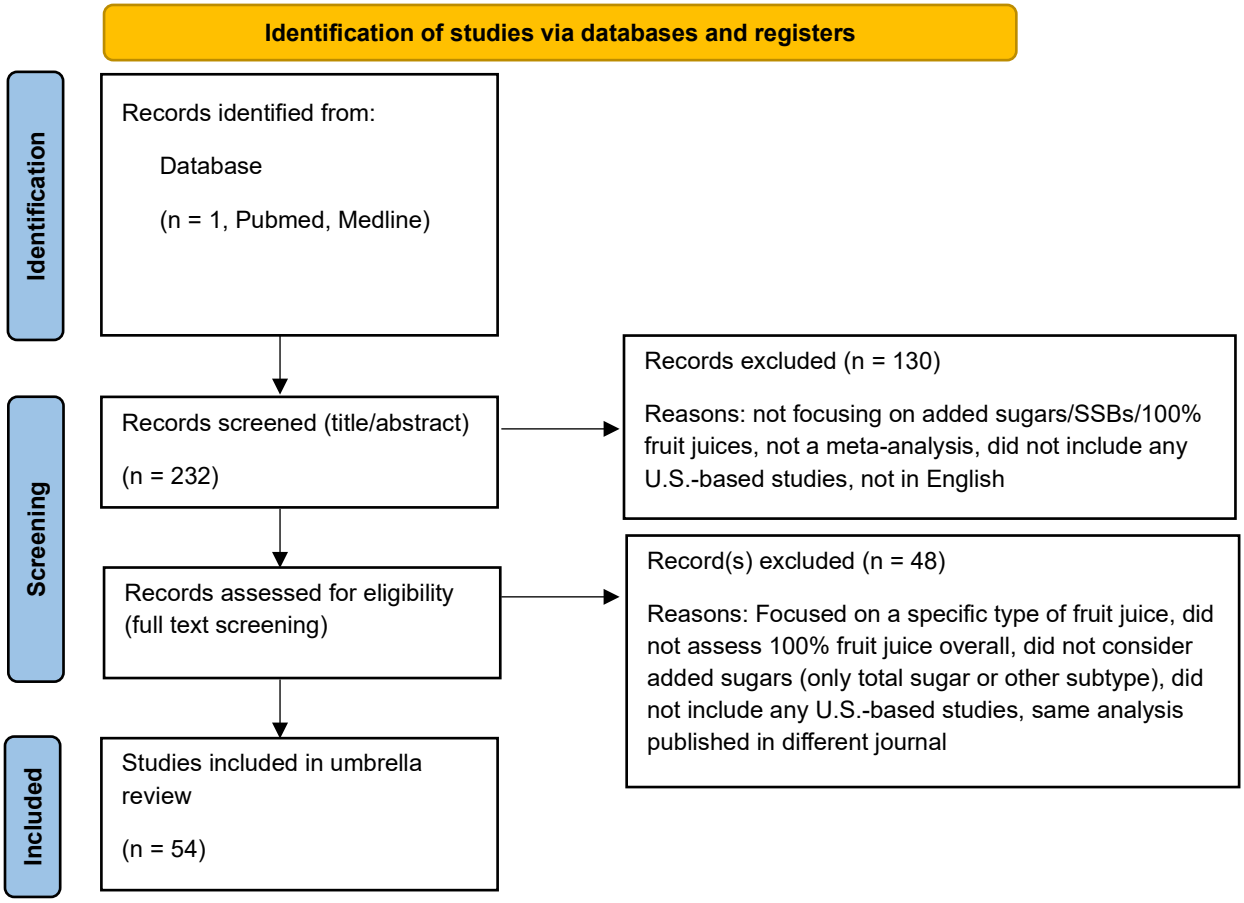

Figure 1. PRISMA Flowchart (Added sugars, Sugar Sweetened Beverages and 100% Fruit Juice)

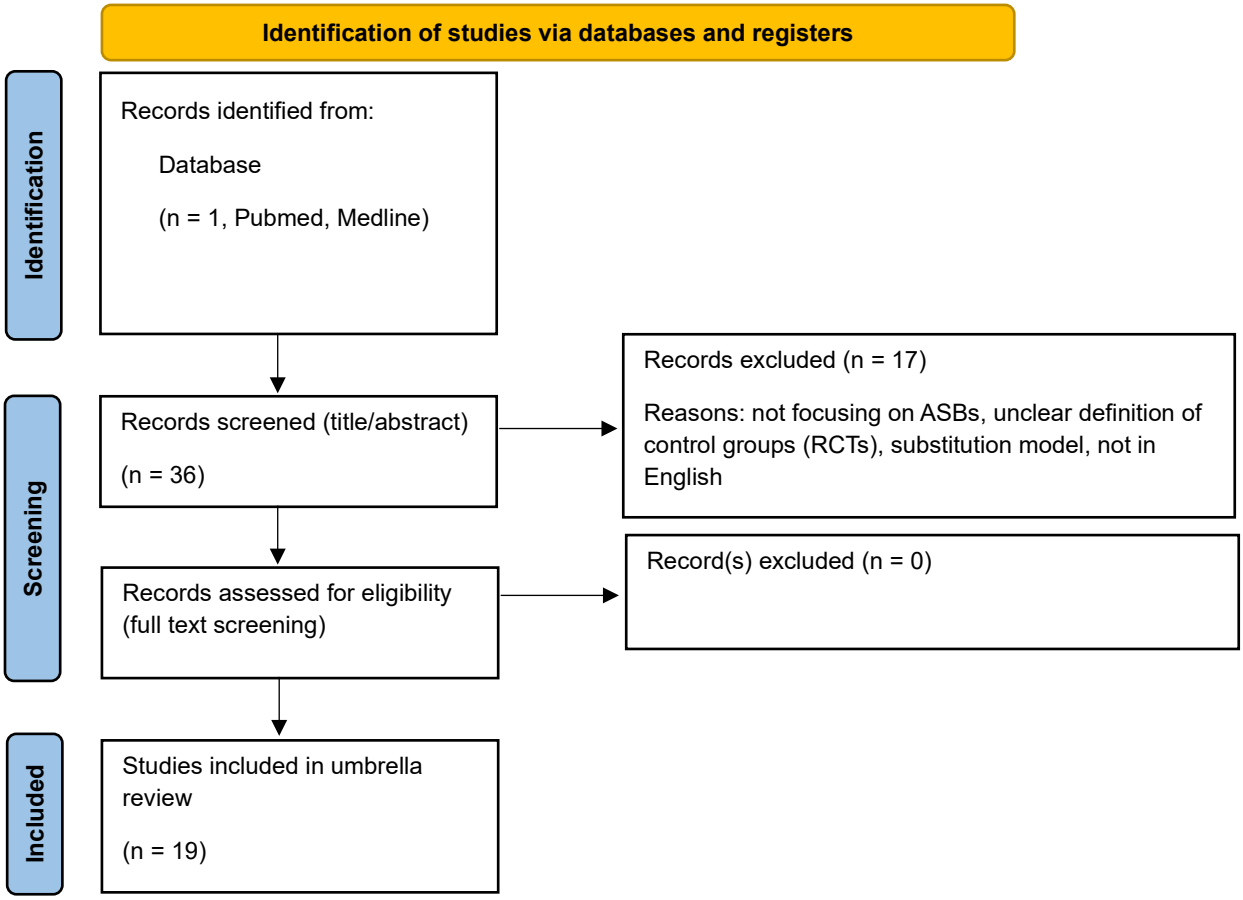

Figure 2. PRISMA Flowchart (Non-Sugar Sweetened Beverages)

Lead Meta-Analyses

Table 1 and Figures 3 and 4 provide a summary of the lead review for each exposure-outcome pair examined in this umbrella review, with a narrative description further presented below. Among the 27 lead meta-analyses identified, only three studies published before 2020 were included, and 13 of them were published in 2023-2025. All meta-analyses focused on clinical endpoints, except for three studies that specifically examined surrogate outcomes related to obesity in children and adults, associated with 100% fruit juice consumption, and children's obesity linked to NSSB consumption. All studies were observational. Five articles provided separate estimates. Most of the meta-analyses conducted a high vs. low consumption estimate (22 out of 27). Finally, all articles focused on the adult population, except for three that examined the relationship between SSBs, 100% fruit juice, and NSSBs in relation to obesity and changes in BMI in children. Conflicts of interest were declared in five of the selected lead meta-analyses.

Although the initial aim was to explore all potential exposure-outcome combinations, the analysis was ultimately limited due to data availability in the literature. The following exposures versus outcomes were ultimately the ones included based on the availability of evidence:

- Added sugars with all-cause mortality, cardiovascular disease, NAFLD, and type 2 diabetes;
- SSBs with all-cause mortality, overall cancer, cardiovascular disease, NAFLD, obesity (in both children and adults), type 2 diabetes, depression, cognitive disorders (including dementia), and dental caries;
- 100% fruit juice with all-cause mortality, overall cancer, hypertension, BMI/weight change (in both children and adults), and type 2 diabetes;
- NSSBs with all-cause mortality, overall cancer, cardiovascular diseases, obesity (in both children and adults), cognition (Alzheimer's).

Table 1. Summary of Findings.

Exposure/Outcome	Lead Meta-analysis (Citation)	Effect Estimate (RR/HR/OR/ MD, 95% CI)	Linear and Non-linear Dose response analysis	GRADE rating	Rationale for Downgrade/Upgrade
Added sugars					
Mortality	Huang C, et al., 2023	RR: 1.05 (0.97, 1.14)	No evidence for a nonlinear relation	Low	Upgrade: Direction of plausible effect, Downgrade: Inconsistency
Cardiovascular diseases	Yang B, et al., 2022	RR: 1.08 (0.86, 1.36)	Not evaluated	Very Low	Upgrade: Direction of plausible effect, Downgrade: Imprecision +++ (only one individual study included)
NAFLD	Liu W, et al., 2023	OR: 1.31 (1.17, 1.48)	Not evaluated	Low	Upgrade: Direction of plausible effect, Downgrade: Imprecision
Type 2 Diabetes	Della Corte KA, et al., 2025	RR: 0.92 (0.79, 1.07)	No evidence of a nonlinear dose-response association	Low	Reported as LOW using GRADE in the lead meta-analysis
SSBs					
Mortality	Kazemi A, et al., 2023	HR: 1.10 (1.05, 1.16)	Linear	Low	Reported as LOW using GRADE in the lead meta-analysis
Cancer	Pan B, et al. 2023	RR: 1.07 (0.95, 1.22)	Non-linear	Very low	Reported as VERY LOW using GRADE in the lead meta-analysis
Cardiovascular diseases	Sun T, et al., 2023	HR: 1.20 (1.07, 1.34)	Linear	Low	Reported as LOW using GRADE in the lead meta-analysis
NAFLD	Chen H, et al. 2019	RR: 1.39 (1.29, 1.50)	Non-linear	Very low	Upgrade: Direction of plausible effect, Downgrade: Imprecision, Inconsistency; Publication Bias
Obesity (children)	Jakobsen DD, Brader L, Bruun JM, 2023	OR:1.20 (1.09, 1.33)	Not evaluated	Low	Upgrade: Direction of plausible effect, Downgrade: Imprecision, Inconsistency
Obesity (adults)	Santos LP, et al. 2022	RR: 1.17 (1.10, 1.25)	Not evaluated	Moderate	Upgrade: Direction of plausible effect, Downgrade: None
Type 2 Diabetes	Della Corte KA, et al., 2025	RR: 1.39 (1.26, 1.55)	Linear	Moderate	Reported as MODERATE using GRADE in the lead meta-analysis
Depression	Wang Y, et al., 2022	RR: 1.25 (1.11, 1.41)	Non-linear	Moderate	Upgrade: Direction of plausible effect, Downgrade: None
Cognitive disorders (including dementia)	Liu H, et al., 2022	OR: 1.17, (1.05, 1.29)	Not evaluated	Very low	Upgrade: Direction of plausible effect, Downgrade: Inconsistency, Imprecision
Dental caries	Valenzuela MJ, et al., 2021	OR: 1.57 (1.28, 1.92)	Non-linear	High	Reported as HIGH using GRADE in the lead meta-analysis
100% fruit juice					
Mortality	Pan B, et al., 2022	HR: 1.0 (0.78, 1.29)	Not evaluated	Very low	Reported as VERY LOW using GRADE in the lead meta-analysis
Cancer	Pan B, et al., 2023	RR: 1.09 (0.98, 1.21)	Non-linearity not properly evaluated	Low	Reported as LOW using GRADE in the lead meta-analysis
Cardiovascular diseases	Liu Q, et al., 2019	RR: 0.95 (0.85, 1.07)	U shape	Low	Reported as LOW using GRADE in the lead meta-analysis
Change in BMI (children)	Nguyen M, et al., 2024	MD**: 0.03 BMI unit (0.01, 0.05)	Not evaluated	High	Reported as HIGH using GRADE in the lead meta-analysis
Change in weight (adults)	Nguyen M, et al., 2024	MD**: 0.07 kg (−0.06, 0.20)	Unclear	Very Low	Reported as VERY LOW using GRADE in the lead meta-analysis
Type 2 Diabetes	Imamura F, et al., 2016	RR**: 1.07 (1.01, 1.14)	Linear (non-linearity not tested)	Low	Reported as LOW using GRADE in the lead meta-analysis

Exposure/Outcome	Lead Meta-analysis (Citation)	Effect Estimate (RR/HR/OR/ MD, 95% CI)	Linear and Non-linear Dose response analysis	GRADE rating	Rationale for Downgrade/Upgrade
NSSBs					
Mortality	Chen Z, et al., 2024	RR: 1.13 (1.06, 1.21)	Non linear	Moderate	Reported as MODERATE using GRADE in the lead meta-analysis
Cancer	Pan B, et al., 2023	RR: 1.00 (0.87, 1.15)	Not properly tested	Very low	Reported as VERY LOW using GRADE in the lead meta-analysis
Cardiovascular diseases	Meng Y, et al., 2021	RR: 1.17 (1.06,1.29)	Non-linear	Low	Upgrade: Direction of plausible effect; Downgrade: Inconsistency
Change in BMI (children)	Espinosa A, et al., 2024	MD**: 0.05 kg/m2 (−0.03, 0.13)	Not evaluated	Moderate	Reported as MODERATE using GRADE in the lead meta-analysis
Obesity (adults)	Qin P, et al., 2020	RR : 1.39 (0.96, 2.01)	Linear (significant association when NSSBs considered as continuous)	Low	Upgrade: Direction of plausible effect, linear dose-response; Downgrade: Inconsistency, Publication bias
Type 2 Diabetes	Imamura F, et al., 2016	RR** : 1.08 (1.02, 1.15)	Linear (non-linearity not tested)	Low	Reported as LOW using GRADE in the lead meta-analysis
Cognition (Alzheimer)	Jouni N, et al., 2025	RR: 1.42 (1.14, 1.78)	Linear (non-significant association when NSSBs considered as continuous)	Moderate	Reported as MODERATE using GRADE in the lead meta-analysis

BMI: body mass index; HR: hazard ratio; MD: mean difference; NAFLD: Non-Alcoholic Fatty Liver Disease; NSSBs: non-sugar sweetened beverages; OR: odds ratio; RR: relative risks, SSBs: sugar-sweetened beverages

**For the specified relationships, the interpretation reflects the effect per unit of consumption, under the assumption of a linear relationship between exposure and outcome. It should be noted that non-linear dose-response analyses were not always systematically conducted. For all other relationships in the table, the effect is interpreted as consumer vs. non-consumer or high vs. low consumption, based on how it was defined in the original meta-analysis.

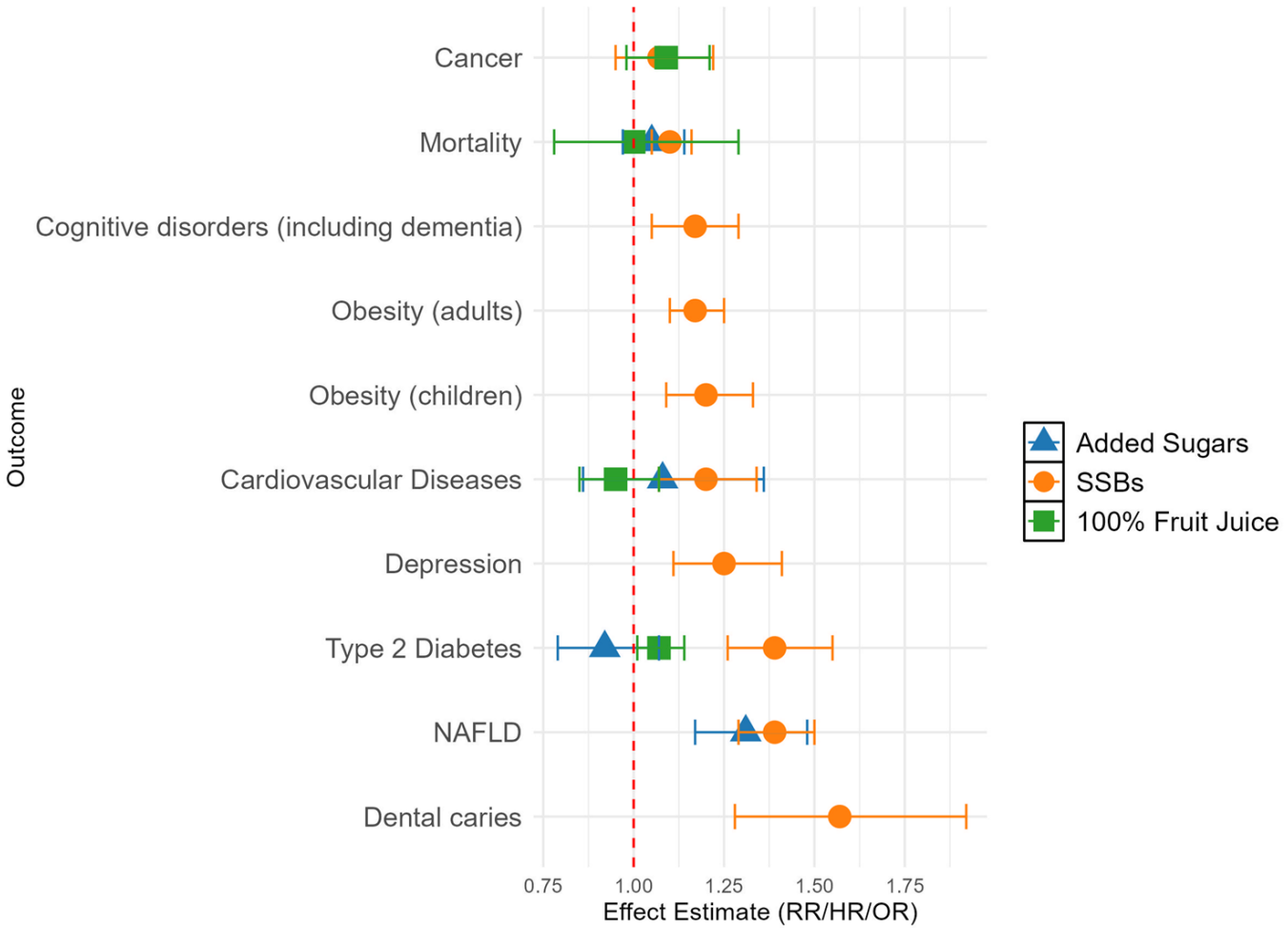

Figure 3. Effects of Added Sugars, Sugar-Sweetened Beverages and 100% Fruit Juice on various Health Outcomes. HR: hazard ratio; MD: mean difference; NAFLD: Non-Alcoholic Fatty Liver Disease; OR: odds ratio; RR: relative risks, SSBs: sugar-sweetened beverages. Two estimates were omitted: change in BMI/weight related to 100% fruit juice, as they act as surrogate variables and were not measured on the same scale as the other estimates.

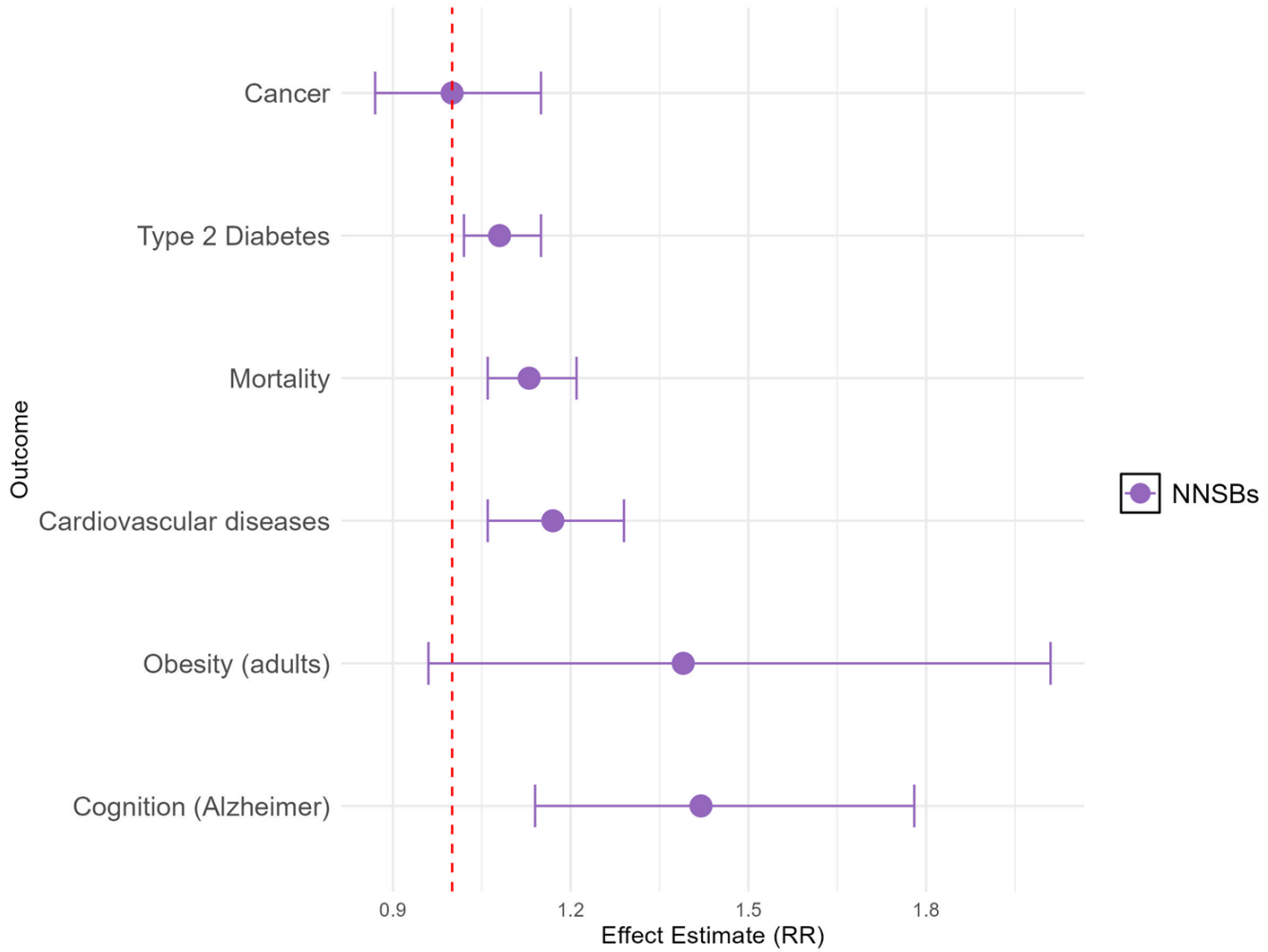

Figure 4. Effects of Non-Sugar Sweetened Beverages on Various Health Outcomes. NSSBs: Non-Sugar sweetened beverages; RR: relative risks. The Forest plot does not include change in BMI (children), since it acts as surrogate variable and was not measured on the same scale as the other estimates.

Analysis of Added Sugars

All-cause mortality: The selected lead meta-analysis, based on nine individual studies, found a summary RR for all-cause mortality of 1.05 (95% CI: 0.97–1.14) when comparing the highest and lowest intakes of added sugar, suggesting no clear or significant association between added sugar consumption and all-cause mortality[13]. No evidence of a non-linear dose-response relationship was observed (P-non-linearity = 0.182) and the summary RR for each 10% increase in added sugar intake was 1.03 (95% CI: 0.97–1.08). The evidence quality was classified as Low based on the GRADE evaluation.

CVD: The lead meta-analysis identified a RR of 1.08 (95% CI: 0.86–1.36) for the highest versus lowest consumption of added sugars, based on a single study [14]. No linear or non-linear dose-response analyses were available. The GRADE assessment indicated Very Low-quality evidence.

NAFLD: The pooled OR for high versus low consumption of added fructose, using a random-effects model across 15 studies included in the lead review, was 1.31 (95% CI: 1.17–1.48) [15]. Neither linear nor non-linear analyses were conducted in the meta-analysis. A positive association was found between the consumption of added fructose and the prevalence of NAFLD in Asia (OR = 1.32, 95% CI = 1.13–1.53) and North America (OR = 1.73, 95% CI = 1.27–2.36) but not in Europe (OR = 1.79, 95% CI = 0.82–3.92). The quality of evidence, according to the GRADE framework, was rated as Low.

T2D: Our search only revealed 1 meta-analysis on added sugars versus T2D, published in 2025. When comparing the highest and lowest categories of added sugar intake, no association with T2D risk was observed (RR: 0.92; 95% CI: 0.79–1.07; based on just 2 individual studies) [16]. No evidence of a non-linear dose-response relationship was found (P-nonlinearity = 0.180), and an increase of 20 g/day in added sugar intake was not associated with T2D risk (RR: 0.99; 95% CI: 0.96–1.01). The GRADE rating was assessed as Low.

Analysis of Sugar-Sweetened Beverages (SSBs)

All-cause mortality: The pooled analysis of nine individual studies in the lead meta-analysis revealed that higher SSB consumption was significantly associated with a 10% increased risk of all-cause mortality (HR = 1.10; 95% CI: 1.05–1.16) [17]. Additionally, ten studies were included in the linear dose-response analysis. There was a positive linear relationship between SSB intake and all-cause mortality risk (P non-linearity = 0.28). Each 250 g increase in SSB intake was associated with a 7% higher risk of all-cause mortality (HR = 1.07; 95% CI: 1.04–1.10). Thus, each can of SSB per day (350 mL, 39g added sugars) is associated with a 10% higher risk. The GRADE assessment indicated Low-quality evidence.

Cancer: Based on four studies, the lead meta-analysis found no significant association between high vs low SSB consumption and overall cancer risk (RR: 1.07, 95% CI: 0.95–

1.20) [18]. A non-linear relationship was identified. The quality of evidence, according to the GRADE framework, was rated as Very Low.

CVD: The lead meta-analysis, which included two individual analyses, showed that SSB consumption was significantly associated with an increased risk of CVD, with a HR of 1.20 (95% CI = 1.07–1.34) for the highest vs lowest intake[19]. The association was linear (no sign of non-linearity, HR of 1.10 [95% CI: 1.02–1.17] per 250 mL/d increase). Thus, for every can of SSB per day (350 mL, 39g added sugars), the risk increases by 14%. The evidence quality was classified as Low based on the GRADE evaluation.

NAFLD: Comparing higher to lower consumption groups (12 individual studies), the pooled RR of NAFLD in individuals consuming SSBs was 1.39 (95% CI: 1.29–1.50)[20]. The dose-response meta-analysis showed a non-linear relationship between SSB consumption (cups/week) and NAFLD (p for non-linear trend < 0.00001): RR = 1.10 for 1 cup/week; RR = 1.56 for 7 cups/week (1 cup/day). The GRADE assessment indicated Very Low-quality evidence.

Obesity (children): Based on 26 individual studies, the OR for higher versus lower intake of sugar-sweetened beverages in children and adolescents (ages 5–18) was 1.20 (95% CI: 1.09–1.33)[21]. Linear and non-linear dose-response analyses were not conducted. The evidence quality was classified as Low based on the GRADE evaluation.

Obesity (adults): The lead meta-analysis identified four studies comparing low/moderate to no intake of SSB found an increased 17% risk of obesity (RR = 1.17; 95% CI: 1.10–1.25)[22]. Linear and non-linear dose-response analyses were not conducted. The GRADE assessment indicated Moderate-quality evidence.

T2D: Comparing the highest to the lowest intake of SSBs, a significant association with the risk of T2D was observed in the lead meta-analysis (RR: 1.39; 95% CI: 1.26–1.55, n = 23 individual studies) [16]. Focusing specifically on sugar from SSBs, a clear linear dose-response relationship was observed, with no indication of non-linearity: a daily intake of 20 g of added sugar from SSBs was associated with the highest increase in the risk of developing T2D (RR: 1.12; 95% CI: 1.08, 1.17). Therefore, each can of SSB per day (350 mL, 39g added sugars) contributes to 23.4% rise in risk. The GRADE assessment indicated Moderate-quality evidence.

Depression: Three cohorts with four study groups were included to assess the link between SSB consumption and depression in the lead meta-analysis. The results showed that higher SSB intake (compared to lower) was associated with a higher risk of depression (RR: 1.25, 95% CI: 1.11–1.41) [23]. Subgroup analysis indicated that participants living in the US were more likely to develop depression from SSB intake. The association was non-linear. The GRADE assessment indicated Moderate-quality evidence.

Cognitive disorders (including dementia): Higher SSB intake was significantly and positively associated with cognitive disorders, including dementia (OR = 1.17, 95 % CI =

1.05–1.29, n = 13 individual studies) [24]. Linear and non-linear dose-response analyses were not conducted. The evidence quality was classified as Very-Low based on the GRADE evaluation.

Dental caries: Moderate consumers of SSBs had significantly higher odds of caries (OR: 1.57, 95% CI: 1.28–1.92, n= 16 individual studies) compared to never/low-level consumers[25]. Ten individual studies were included in the dose-response analysis, exploring both linear and non-linear associations between SSB consumption and dental caries risk. The likelihood test revealed a non-linear relationship. The evidence quality was classified as high based on the GRADE evaluation.

Analysis of 100% Fruit Juice

All-cause mortality: Using three individual studies, the lead meta-analysis did not identify a significant association between highest versus lowest consumption of 100% fruit juice (HR = 1.00; 95% CI: 0.78–1.19)[26]. No linear or non-linear dose–response analyses were conducted. The GRADE assessment indicated very Low-quality evidence.

Cancer: Using two individual studies, the lead meta-analysis did not identify a significant association of 100% juice consumption with cancer (RR = 1.09; 95% CI: 0.98–1.21) when comparing the highest to lowest consumption of 100% fruit juice[18]. Per 250 mL/day increase, the RR was 1.31 (95% CI: 1.04–1.65), but non-linearity was not tested. The evidence quality was classified as Low based on the GRADE evaluation.

Hypertension (best proxy for CVD): Using two individual studies, the lead review reported no significant association between highest versus lowest consumption of 100% fruit juice and hypertension (RR = 0.95; 95% CI: 0.85–1.07)[27]. Within a dose range of 0 to 230 mL/day, a nonlinear U-shaped dose–response relationship was observed (P for nonlinearity = 0.001). The curve suggested a protective association between 50 and 150 mL/day, with a potentially harmful association emerging above 200 mL/day. The quality of evidence, according to the GRADE framework, was rated as Low.

Change in BMI (children): Based on 23 comparisons from 16 cohorts, pooled estimates using a random-effects model showed a significant 0.03 higher BMI for each 8-oz (around 237 mL) serving of 100% fruit juice per day (95% CI: 0.01–0.05). Additionally, each extra serving per day of 100% fruit juice was associated with a 0.01 higher BMI z score (95% CI: 0.001–0.02) [28]. Non-linearity was not tested. The GRADE assessment indicated High-quality evidence.

Weight change (adults): In the lead meta-analysis of 6 observational studies, the association between 100% fruit juice consumption for each additional 8-oz (237-mL) serving of 100% fruit juice consumed per day with body weight change was examined[28]. Pooled-effect estimates using a random-effects model showed no significant association between 100% fruit juice consumption and body weight (MD = 0.07 kg (95% CI, −0.06–0.20 kg). Both linear and non-linear dose-response analyses were significant. However, the comparisons were only based on 2 cohorts, thus the dose-response analysis is not

representative of the overall association. The evidence quality was classified as Very Low based on the GRADE evaluation.

T2D: Using data from 13 individual studies and adjusting for adiposity, an increase of one serving per day of 100% fruit juice was linked to a 7% higher incidence of type 2 diabetes (95% CI: 0.8% to 14%)[29]. Additionally, no departure from linearity was found. The GRADE assessment indicated Low-quality evidence.

Analysis of Non-Sugar Sweetened Beverages (NSSBs)

All-Cause Mortality: A positive and significant association was found between high NSSB consumption (vs. low) and all-cause mortality (RR = 1.13, 95% CI: 1.06–1.21, n= 12 estimates, seven studies), with a non-linear relationship [30]. The certainty of evidence was Moderate based on the GRADE evaluation.

Overall cancer: No significant association was found between high (vs. low) NSSB consumption and overall cancer risk (RR = 1.00, 95% CI: 0.87–1.15, n= 2 studies), with Very Low evidence quality based on GRADE assessment[18]. Non-linearity was not tested.

CVD: A significant positive association was found between high (vs. low) NSSB consumption and CVD (RR = 1.17, 95% CI: 1.06–1.29, n=8 studies), with a non-linear relationship [31]. The GRADE assessment indicated Low-quality evidence.

Change in BMI (children): The lead meta-analysis found a non-significant positive difference in BMI change with each serving (12-fl oz or 355 mL) of non-sugar sweetened beverages (MD = 0.05 kg/m², 95% CI: -0.03–0.13, n= 8 studies) [32]. Non-linearity was not tested. The quality of evidence was Moderate (GRADE).

Obesity (adults): The lead meta-analysis found a non-significant increased risk of obesity with NSSB consumption (RR = 1.39, 95% CI: 0.96–2.01, n=5 studies) [33]. With no evidence of non-linearity, for each additional 250-mL/day of NSSB intake, the RR was 1.21 (95% CI 1.09–1.35). The certainty of the evidence was Low according to GRADE assessment.

T2D: A small but significant positive association (adjusting for adiposity) was found between NSSB consumption and the risk of T2D (RR = 1.08 per serving/day, 95% CI: 1.02–1.15, n=10 studies) [29]. There was no evidence of non-linearity. The quality of the evidence was Low (GRADE).

Alzheimer's Disease (AD): In the only cognition-related meta-analysis, a significant and positive association was observed between NSSB consumption (high vs. low) and AD (RR = 1.42, 95% CI: 1.14–1.78, n=2 studies) [34]. The relationship was linear though non-significant when considering NSSB as a continuous variable (RR: 1.30; 95% CI: 0.64, 2.62; per 250 mL/d). The quality of evidence was Moderate (GRADE).

Evidence to Decision Framework

Our decision-making process, outlined in **Tables 2, 3, 4, and 5**, resulted in a strong recommendation for SSBs, and conditional recommendations for NSSBs, 100% fruit juice and added sugars.

Table 2. Evidence to Decision Table: Added Sugars

Criterion	Evidence Summary
(1) Problem & importance	Added sugars are widely consumed and have been linked to several chronic diseases. With industry's increasing use of added sugars in products, an updated umbrella review is needed to clarify evidence and support public health guidance.
(2) Certainty of evidence (per outcome)	The meta-evidence was surprisingly limited for added sugars and, where available, was inconsistent. Evidence for all-cause mortality, CVD and T2D was not significant and either Low or Very Low (GRADE assessment). Significant evidence was found for NAFLD (31% risk) with Low GRADE certainty. Dose-response relationships were unclear.
(3) Benefits vs harms	Potential benefits of reducing added sugars intake exist but are not strongly supported by current meta-analytical evidence.
(4) Implementation considerations/ feasibility	Focusing on added sugar reduction may be feasible but challenging without clear targets. Messaging focusing on whole foods, SSBs or dietary patterns might be more practical and impactful.
Recommendation strength	**Weak**: Given current evidence limitations, specific recommendations targeting added sugars alone should be interpreted with caution. Emphasis on food groups, particularly SSBs (see evidence-to-decision table below) may offer clearer guidance. A **weak** recommendation is suggested to limit added sugar intake, ideally within the context of broader food-based dietary guidelines.

Table 3. Evidence to Decision Table: SSBs.

Criterion	Evidence Summary
(1) Problem & importance	SSBs are the major source of added sugars in the diet. They have been linked to multiple adverse health outcomes, but updated evidence is needed as industry shifts toward reformulated products and changing consumption patterns.
(2) Certainty of evidence (per outcome)	**High GRADE Certainty**: Dental caries (57% higher risk) **Moderate GRADE Certainty**: T2D (39% higher risk), adult obesity (17% higher risk), depression (25% higher risk) **Low GRADE Certainty**: All-cause mortality (10% higher risk), CVD (20% higher risk), child obesity (20% higher risk) **Very Low GRADE Certainty**: Cancer (not significant), cognitive disorders (17% higher risk), NAFLD (39% higher risk) Significant linear dose-response was evident for all-cause mortality, CVD and T2D but less clear or not examined for other outcomes.
(3) Benefits vs harms	Strong and consistent evidence of harm across outcomes with no evidence of protective effects.
(4) Implementation considerations/ feasibility	Feasible through taxation, labeling, education and policies that make healthy/clean drinking water readily available in public places (including schools) and offering water as a default beverage choice. May face resistance due to habits and marketing.
Recommendation strength	**Strong:** Recommendation based on robust evidence across outcomes and populations. Clear public health messaging possible.

Table 4. Evidence to Decision Table: 100% fruit juice.

Criterion	Evidence Summary
(1) Problem & importance	Perceived as a healthy option to SSBs. Nuanced understanding is needed.
(2) Certainty of evidence (per outcome)	**High GRADE Certainty**: Child obesity (risk of higher BMI) **Low GRADE Certainty**: CVD (not significant), T2D (7% risk), Cancer (9% higher risk) **Very Low GRADE Certainty**: All-cause mortality, adult obesity Dose-response relationships are unclear.
(3) Benefits vs harms	The evidence on 100% fruit juice and health outcomes is mixed and varies by outcome and age group. **High-certainty evidence indicates that higher 100% juice intake is associated with increased risk of obesity in children**, while for adults' **associations with cardiovascular disease, type 2 diabetes, and cancer were of low certainty**, and **evidence for all-cause mortality and obesity was very low**. **Dose–response relationships remained unclear**, suggesting the need for moderation and further study.
(4) Implementation considerations/ feasibility	**100% fruit juice should not be considered metabolically equivalent to whole fruit**, given the absence of fiber and the rapid delivery of greater amounts of free sugars. Public health messaging should continue to emphasize **whole fruit as the preferred source of fruit intake**, while **limiting 100% juice to small, age-appropriate portions**, particularly in children. For adults, occasional moderate consumption may be acceptable, but habitual or high intake should be discouraged until stronger evidence supports the evidence.
Recommendation strength	**Weak**: As evidence for most health outcomes remains low in quality, with high-quality evidence observed only for childhood obesity, a precautionary approach is advisable. Recommendations should distinguish 100% fruit juice from whole fruit and encourage moderate consumption until more consistent long-term data emerge.

Table 5. Evidence to Decision Table: NSSBs.

Criterion	Evidence Summary
(1) Problem & importance	Perceived as a potential healthier option to SSBs. Nuanced understanding needed.
(2) Certainty of evidence (per outcome)	**Moderate GRADE Certainty**: All-cause mortality (13% risk), change in BMI (children), Alzheimer disease (42% risk) **Low GRADE Certainty**: CVD (17% risk), Adult obesity (39% risk), T2D (8% risk) **Very low GRADE Certainty**: Overall cancer (not significant) Dose response relationships remain unclear.
(3) Benefits vs harms	The evidence identified risks associated with high consumption of NSSBs but the dose-response nature of relationships was unclear. Some studies suggest possible neutral effects while no studies indicated beneficial effects. While NSSBs may reduce short-term energy or sugar intake when substituted for SSBs under controlled conditions, **there is no consistent evidence of long-term metabolic or cardioprotective benefit**. Moreover, emerging evidence raises concerns about possible adverse health effects suggesting that presumed benefits may not outweigh potential risks.
(4) Implementation considerations/ feasibility	Public messaging could emphasize the distinction between NSSBs and SSBs, without presenting NSSBs as a direct substitute, and underline that “sugar-free” does not automatically imply “risk-free”.
Recommendation strength	**Weak**: Given the limited and low-quality evidence, a cautious approach is advisable. Routine consumption of NSSBs may not be recommended until further research clarifies their health effects, dose-response relationships, and potential life-course impacts. Public health strategies may focus on promoting water and other unsweetened beverages as the preferred alternatives to SSBs.

Statement of Findings

Across the body of evidence, the strongest and most consistent effects were observed for **SSBs, which were associated with** increased risk of obesity, **T2D**, NAFLD, CVD, and all-cause mortality, with moderate-to-high certainty of evidence. **Dose–response analyses** demonstrated significant linear relationships for major outcomes: each additional daily serving of SSBs (≈350 mL, containing ~39 g added sugars) was associated with a **10% higher risk of all-cause mortality, 14% higher risk of CVD,** and **23% higher risk of T2D.**

Evidence for added sugars was surprisingly limited and inconsistent. Associations with all-cause mortality, CVD, and T2D were not statistically significant and were graded as Low or Very Low certainty. However, significant evidence was identified for NAFLD, with approximately 31% higher risk among individuals with higher added sugar intake (Low GRADE). Dose–response relationships remain unclear.

Evidence for 100% fruit juice was mixed and uncertain. Moderate consumption appeared largely neutral for most outcomes, but higher intakes were associated with increased risk of weight gain and T2D in several analyses, suggesting that fruit juice may not be considered metabolically equivalent to whole fruit. **Findings for NSSBs** were also inconsistent: no clear evidence of benefit was observed, but some studies reported possible, but weak, associations with higher risk of cardiometabolic disease and mortality.

The meta-evidence for children was limited. However, there was a clear evidence base for obesity risk. In children, higher consumption of SSBs (Low GRADE), 100% fruit juice (High GRADE), and NSSBs (Moderate GRADE) were all associated with increased risk of obesity. Public health guidance should emphasize water as a preferred beverage to SSBs, and recommendations that 100% fruit juice, if consumed, be **limited to small portions or diluted with water** to reduce sweetness and total sugar exposure.

Taken together, the evidence supports **public health strategies aimed at reducing SSB consumption, which typically represents the major source of added sugars in the diet.** Further high-quality research is needed to clarify dose–response relationships, strengthen the meta-evidence, and better understand the long-term safety and potential life-course effects of added sugars, 100% fruit juice, and NSSBs.

Discussion

This umbrella review synthesized the existing and most up-to-date meta-evidence on the associations between added sugars, SSBs, 100% fruit juice, and NSSBs with a variety of chronic disease-related health outcomes.

For SSBs, there was a consistent pattern of association with almost all outcomes examined, including evidence of dose-response for some. We identified High-quality evidence for dental caries (57% increased risk); Moderate-quality evidence for adult obesity (17% increased risk), depression (25%), and T2D (39%). Associations with other outcomes were observed, but the quality of evidence was lower (10% increased risk for

mortality, 20% for CVD, 39% for NAFLD, 17% for cognitive disorders, and 20% for obesity in children).

The evidence revealed a more nuanced picture for added sugars, 100% fruit juice, and NSSBs, highlighting critical gaps in the literature. For added sugars, our analysis revealed inconclusive and limited evidence regarding their association with all-cause mortality, CVD, and T2D (Very Low or Low-quality evidence), except for evidence showing a 31% risk of NAFLD (Low-quality evidence). Furthermore, mixed evidence was revealed for 100% fruit juice, often perceived as a healthier alternative to SSBs. While it may contribute to an increase in BMI in children and T2D, its effects on other outcomes remain uncertain. High NSSB consumption was linked to risks across outcomes such as all-cause mortality, CVD, T2D, and Alzheimer's disease. However, the nature of the associations remained unclear, with both linear and non-linear patterns observed for some outcomes, along with instances of neutral effects. Importantly, no evidence was identified for any beneficial associations between added sugars, SSBs, 100% fruit juice, or NSSBs consumption and any health outcomes.

Comparison with other studies

Overall, our findings are largely consistent with the conclusions presented in the most recent umbrella review on dietary sugar consumption and health published in 2023 [7]. Taken together, both umbrella reviews highlight the lack of meta-analyses and strong associations between added sugars and major chronic diseases, with the quality of evidence generally being Low to Moderate. Similarly, the evidence available for SSBs aligns, showing some associations with health outcomes such as obesity, T2D, and cardiovascular risks, but also with Moderate to Low evidence quality. That said, our study contributes valuable insights by incorporating a larger number of recent studies, which strengthens the overall evidence base.

Our findings also align with those of a previous umbrella review on 100% fruit juice, but with some important differences, resulting from the broader scope of their analysis, which included studies on broader biomarkers and clinical endpoints [9]. While that review found potential benefits, we did not observe similar results. In their analysis, approximately 20% of studies reported benefits (blood pressure, vascular function, inflammation, stroke mortality), 74.5% found no effect, and 5.9% identified adverse risks (with one meta-analysis each for CVD mortality, prostate cancer, and T2D). The pattern of adverse risks, especially concerning T2D and cancer, mirrors our updated findings. Indeed, in both studies, positive associations were found with cancer (although the authors focused on site-specific cancers), while the same meta-analysis also identified a positive association with T2D.

Although the evidence is overall uncertain, our findings for NSSBs are consistent with existing research suggesting that they may not serve as an appropriate substitute for SSBs. Similar to our updated findings, a 2023 umbrella review reported negative associations between NSSBs and all-cause mortality, T2D, and CVD [10]. Previous research has shown that consumption of diet beverages with alternative sweeteners can

lead to overall greater intake of sugars and calories. For example, children, who habitually consume diet beverages end up consuming more sugar and calories during the course of the day [35]. Also, several pregnancy cohort studies have shown that diet soda consumption during pregnancy increases the risk for obesity in the offspring [36–39]. Thus, although they may appear as potential alternatives, beverages with non-nutritive sweeteners, including artificial (eg sucralose) and natural (eg stevia) sweeteners could carry their own health risks and are not necessarily a simple or risk-free substitute for SSBs.

While the primary aim of our analysis was to evaluate the effects of NSSBs focusing on their *own effects*, substitution studies (for SSBs) were also considered during the extraction process. A meta-analysis of 14 cohort studies found that replacing SSBs with NSSBs was associated with a slight reduction in all-cause mortality (RR = 0.96, 95% CI: 0.94–0.98)[40]. However, the certainty of the evidence was Low GRADE. In terms of stroke, no significant benefit was observed from switching to NSSBs (RR = 1.03, 95% CI: 0.93–1.14), with very low-quality evidence, and for CHD, a modest protective effect was found (RR = 0.89, 95% CI: 0.81–0.98), but again, the certainty of the evidence was Low GRADE. For adults**,** the same meta-analysis found a small but significant reduction in overweight risk when replacing SSBs with NSSBs (RR = 0.88, 95% CI: 0.88–0.89). The evidence was of Low certainty based on GRADE. Similarly, another meta-analysis of RCTs on the effect of replacing SSBs with NSSBs in children showed a small reduction in BMI (MD = −0.114 kg/m², 95% CI: −0.207 to −0.021). However, the quality of evidence was Very Low GRADE [32]. As described above, while substituting NSSBs for SSBs may offer some slight health benefits, the evidence remains low to very low in quality. Additionally, there are risks associated with high consumption of NSSBs alone compared to either no consumption or reduced intake, with indications of harm across various outcomes, suggesting that the potential negative effects may outweigh the minimal benefits in certain cases.

Limitations

The primary limitation of this review lies in the number and quality of the underlying meta-analyses that have been previously reported on this topic. Many studies were observational, which inherently limits the ability to establish causal relationships. The GRADE assessments often resulted in Low or Very Low-quality evidence, particularly for outcomes like cancer and cardiovascular disease. This suggests that while associations between these exposures and health outcomes exist, further high-quality RCTs and robust longitudinal cohort studies are necessary to strengthen the evidence base.

Another related limitation is a lack of meta-analyses addressing various outcomes, particularly concerning added sugars and, to some extent, 100% fruit juice. There is a significant gap in meta-analyses specifically focused on children. While a substantial body of individual studies exists, particularly regarding the effects of added sugars on children, this literature has not been adequately synthesized in meta-analyses. This gap underscores the need for future meta-analytic work that integrates existing data on

children's health, which could significantly contribute to the evidence base and inform public health recommendations.

Additionally, assessing non-linear relationships between exposure and outcomes was not consistently feasible across studies, but it is crucial for making meaningful recommendations. While some dose-response relationships appeared linear, notably for SSBs, others showed signs of non-linearity, indicating that the effects of various sugar exposures may vary depending on the level of intake. **This underscores the need for more refined dose–response analyses in future research, as understanding these nuances is crucial for clarifying the true risk profile of consumption and informing actionable recommendations for added sugars and** 100% fruit juice**.**

Another limitation is the inconsistency in how exposures, particularly 100% fruit juice, are defined and measured across studies. Some of the studies classified any fruit drinks as 100% fruit juice (we excluded those from our analysis) while others grouped them with other sugary beverages. Future research should aim for standardized methods in defining and thus assessing and reporting these exposures.

Given the observational nature of most lead meta-analyses, many of the associations explored may be subject to residual confounding, and other unmeasured interactions or moderators could also play a significant role. These considerations are crucial when interpreting the findings. For instance, few studies have examined whether the link between NSSB intake and metabolic outcomes is influenced by broader dietary patterns. Some evidence from the Health Professionals Follow-up Study [41] and the CARDIA cohort [42] indicate that adverse associations were more pronounced among individuals with less healthy or 'Western' dietary habits, while little to no effect was observed in those following healthier diets. This potential influence of overall diet quality may have important implications for various associations, including those involving added sugars, as well as for the underlying dynamics and mechanisms. However, it’s worth noting that a non-negligeable number of lead meta-analyses (eight) were still rated as MODERATE or HIGH in terms of evidence strength (GRADE), a conclusion drawn after considering factors that commonly lead to the downgrading of observational studies.

A final important limitation of this review is the use of the GRADE framework to assess the quality of evidence, which inherently involves a degree of subjectivity. While GRADE is a widely used tool for evaluating evidence quality, it relies heavily on expert judgment regarding the validity of study results and the consistency of findings, which can introduce variability in assessments. Furthermore, some evaluation criteria, such as risk of bias or the precision of estimates, may be interpreted differently by different researchers, potentially leading to discrepancies in the classification of evidence quality. This subjectivity can influence the robustness of the conclusions drawn and limit the comparability between different studies or meta-analyses. Beyond subjectivity to the classification of evidence quality, not all meta-analyses employed a GRADE framework, making immediate cross-comparison of evidence quality difficult.

Public Health Implications

Despite these limitations, the findings of this umbrella review have important implications for public health policy and practice. **While current evidence is very limited for added sugars, it remains prudent to limit intake in accordance with the precautionary principle until more conclusive data becomes available.** Given the consistent evidence linking SSBs intake to a variety of adverse health outcomes, including childhood obesity, the overall recommendation for limiting SSB consumption is particularly strong and noteworthy. Public health interventions targeting SSB consumption, such as taxation, default offerings of water with meals, better public access to healthy drinking water including at schools, marketing, and clearer front-of-pack labeling, have shown promise in reducing consumption and improving population health outcomes and should be continuously implemented [43]. The food and beverage industry has typically used re-formulation by replacing added sugars in beverages with sweeteners, but as discussed below, sweeteners likely introduce other concerns.

The evidence suggests that reducing SSB consumption could not only decrease the burden of these chronic conditions but also serve as an effective entry point for addressing other related dietary behaviors. It is indeed worth noting that SSBs are often consumed alongside other unhealthy dietary patterns, including high intake of ultra-processed foods and low fiber intake [44], and are often a result of the food environment. Reducing SSB consumption and the overall availability of suboptimal dietary options could help curb other aspects of poor diet, potentially leading to broader improvements in diet-related behaviors and a reduction in chronic disease risk. While structural and institutional solutions are foundational, nutrition education remains key in helping both adults and children navigate the suboptimal food environment they are living in[45]. Finally, tackling SSB consumption may also serve as a gateway to addressing other related issues, such as screen time, poor sleep hygiene, or physical inactivity, especially if combined with complementary strategies targeting these health-risk behaviors[46,47].

On the other hand, 100% fruit juice, while often marketed as a healthier alternative to SSBs, should not be viewed as a substitute for whole fruit or a strategy for health improvement. Although the evidence is overall mixed, the findings of this review indicate that higher consumption of 100% fruit juice is linked to increased BMI and weight gain in children, as well as a slightly higher risk of developing T2D. Importantly, fruit and 100% fruit juice are not equivalent in their sugar delivery. A single serving of juice can contain the free sugars extracted from two to three pieces of fruit, concentrated in liquid form and largely composed of fructose, while often removing the fiber and other micronutrients. When consumed rapidly and in large amounts, this fructose load can overwhelm hepatic metabolism, promoting de novo lipogenesis and fat accumulation in the liver [48–50]. Given these metabolic concerns, along with the precautionary positions of other countries (e.g., Spain, the Netherlands, the UK, and France, which advise limiting or excluding fruit juice of all types) and the World Health Organization's recommendation to restrict free sugar intake[9], it would be prudent for the United States to adopt a more cautious stance than its current one. In the absence of high-quality evidence confirming

any health benefits, endorsing 100% fruit juice in U.S. dietary guidelines would not be justified. Public health messaging should instead emphasize moderation and the clear nutritional advantages of consuming whole fruits instead of drinking fruit juice.

Similar to 100% fruit juice, NSSB containing alternative sweeteners like sucralose, Ace-K, stevia, and monk fruit sweetener have also received significant attention as a potential alternative to SSBs. Although low- or zero-calorie NSSBs might in theory help individuals manage calorie intake, current evidence (while limited and uncertain) raises concerns about potential negative effects. Public health initiatives should therefore approach the marketing of these products with caution. Their widespread availability could inadvertently lead to overconsumption or foster a false sense of 'healthiness', which might undermine broader efforts to improve dietary habits. Additionally, there is a need for ongoing education to distinguish between non-nutritional sweeteners and natural, nutrient-rich beverages, ensuring that NSSBs are not perceived as a quick fix but rather integrated into a more balanced, holistic approach to health and nutrition.

Research Implications

The findings also point to several important areas for future research. First, there is a clear need for higher-quality evidence, particularly from RCTs, that can more definitively assess the causal relationship between added sugars, SSBs, 100% fruit juice, NSSBs, and health outcomes. The findings from this review, particularly the modest or non-significant associations, may reflect limitations in study design (observational studies are subject to residual confounding and moderation effects) rather than a true absence of effect.

Additionally, as noted above, non-linear relationships should be explored in greater depth. Understanding how varying levels of intake influence health risks at different thresholds could lead to more targeted and effective interventions. For instance, moderate consumption of 100% fruit juice may not be harmful, but high consumption could pose risks. Research should focus on identifying these thresholds and their potential impact on health outcomes.

Finally, although there is a wealth of individual studies demonstrating the health effects of added sugars, including significant metabolic and cognitive impacts in both adults and children, meta-analytic evidence remains limited. Our umbrella review, along with the most recent review on dietary sugars[7], highlights this gap in the literature. This emphasizes the urgent need for additional high-quality meta-analyses that draw on existing data to thoroughly assess the broader effects of added sugars, ultimately providing clearer, evidence-based conclusions that can guide future recommendations and public health policies. Finally, in addition to broader research on added sugars, further studies should investigate the long-term health outcomes in children across all exposures (SSBs, NSSBs, 100% fruit juice), as current research is largely limited to obesity and changes in BMI.

Summary and Conclusions

For added sugars, the overall evidence base for most outcomes was either lacking, inconsistent or graded as low quality, with the exception of evidence of a significant association with a 31% higher risk of non-alcoholic fatty liver disease (NAFLD; Low quality evidence). In contrast, the evidence for SSBs was strong and consistent across multiple outcomes, with significant associations observed for dental caries (57% higher risk; High), adult obesity (20%; Moderate), T2D (39%; Moderate), all-cause mortality (10%; Low), cardiovascular disease (20%; Low), childhood obesity (20%; Low), depression (25%; Moderate), and cognitive disorders (17%; Very Low). Dose–response analysis results for SSBs were mixed but did highlight linear associations, indicating that for every one can of soda per day (≈ 350 mL; ≈ 39g added sugar), risk increased by approximately 10% for all-cause mortality, 14% for cardiovascular disease, and 23% for T2D. High consumption of NSSBs was associated with harms across outcomes like all-cause mortality, CVD, T2D, and Alzheimer's disease. However, the nature of some associations was unclear, with both linear and non-linear patterns observed for some outcomes, as well as instances of neutral effects. For 100% fruit juice, the evidence was also mixed and limited but suggested a significant increase in childhood obesity (High) and a modest (7%) increase in T2D risk (Low), while some studies reported neutral associations. From a public-health perspective, the collective evidence indicates that the most promising opportunity to address added sugars is through strategies that promote reduction in consumption of SSB in favor of water and minimally processed beverages, and that encourage whole fruit over juice. By taking a careful and cautionary food-based approach, public-health policies can more effectively tackle the rising burden of obesity, type 2 diabetes, and other chronic diseases, while promoting overall population health.

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Appendix 1. Research strategy (added sugars, SSBs 100% fruit juice).

("added sugar*"[Title/Abstract] OR "sugar-sweetened beverage*"[Title/Abstract] OR SSB[Title/Abstract] OR SSBs[Title/Abstract] OR "sugary drink*"[Title/Abstract] OR "sugar sweetened beverage*"[Title/Abstract] OR "sweetened beverage*"[Title/Abstract] OR "free sugar*"[Title/Abstract] OR juice*[Title/Abstract])
AND
(obesity[Title/Abstract] OR overweight[Title/Abstract] OR "obesity"[MeSH Terms] OR "type 2 diabetes"[Title/Abstract] OR "diabetes mellitus, type 2"[MeSH Terms] OR "cardiovascular disease"[Title/Abstract] OR "cardiovascular diseases"[MeSH Terms] OR "myocardial infarction"[Title/Abstract] OR stroke[Title/Abstract] OR "heart attack"[Title/Abstract] OR "cardiovascular event*"[Title/Abstract] OR "non-alcoholic fatty liver disease"[Title/Abstract] OR NAFLD[Title/Abstract] OR "Fatty Liver"[MeSH Terms] OR Alzheimer*[Title/Abstract] OR "Alzheimer Disease"[MeSH Terms] OR dementia[Title/Abstract] OR "cognitive function"[Title/Abstract] OR cognition[Title/Abstract] OR memory[Title/Abstract] OR learning[Title/Abstract] OR "cognition disorders"[MeSH Terms] OR depression[Title/Abstract] OR anxiety[Title/Abstract] OR "major depressive disorder"[Title/Abstract] OR "anxiety disorders"[Title/Abstract] OR "dental caries"[Title/Abstract] OR "dental caries"[MeSH Terms] OR "tooth decay"[Title/Abstract] OR HbA1c[Title/Abstract] OR "glycated hemoglobin"[Title/Abstract] OR "hemoglobin A1c"[Title/Abstract] OR BMI[Title/Abstract] OR "body mass index"[Title/Abstract] OR "body weight"[Title/Abstract] OR "blood pressure"[Title/Abstract] OR hypertension[Title/Abstract] OR cancer[Title/Abstract] OR "neoplasms"[MeSH Terms] OR mortality[Title/Abstract] OR "mortality"[MeSH Terms])
AND
("meta-analysis"[pt] OR "meta-analysis"[Title])
AND
Humans[MeSH] AND ("2000/01/01"[Date - Publication] : "3000"[Date - Publication])

Appendix 2. Research strategy (NSSBs).

("artificially sweetened beverage*"[Title/Abstract] OR "artificial sweetened beverage*"[Title/Abstract] OR "non-nutritive sweetened beverage*"[Title/Abstract] OR "diet soda"[Title/Abstract] OR "diet soft drink*"[Title/Abstract] OR "low-calorie beverage*"[Title/Abstract] OR "ASB"[Title/Abstract] OR "NNS beverage*"[Title/Abstract] OR "low- and no-calorie sweetened beverage*"[Title/Abstract] OR "LNCSB"[Title/Abstract])
AND (obesity[Title/Abstract] OR overweight[Title/Abstract] OR "obesity"[MeSH Terms] OR "type 2 diabetes"[Title/Abstract] OR "diabetes mellitus, type 2"[MeSH Terms] OR "cardiovascular disease"[Title/Abstract] OR "cardiovascular diseases"[MeSH Terms] OR "myocardial infarction"[Title/Abstract] OR stroke[Title/Abstract] OR "heart attack"[Title/Abstract] OR "cardiovascular event*"[Title/Abstract] OR "non-alcoholic fatty liver disease"[Title/Abstract] OR NAFLD[Title/Abstract] OR "Fatty Liver"[MeSH Terms] OR Alzheimer*[Title/Abstract] OR "Alzheimer Disease"[MeSH Terms] OR dementia[Title/Abstract] OR "cognitive function"[Title/Abstract] OR cognition[Title/Abstract] OR memory[Title/Abstract] OR learning[Title/Abstract] OR "cognition disorders"[MeSH Terms] OR depression[Title/Abstract] OR anxiety[Title/Abstract] OR "major depressive disorder"[Title/Abstract] OR "anxiety disorders"[Title/Abstract] OR HbA1c[Title/Abstract] OR "glycated hemoglobin"[Title/Abstract] OR "hemoglobin A1c"[Title/Abstract] OR BMI[Title/Abstract] OR "body mass index"[Title/Abstract] OR "body weight"[Title/Abstract] OR "blood pressure"[Title/Abstract] OR hypertension[Title/Abstract] OR cancer[Title/Abstract] OR "neoplasms"[MeSH Terms] OR mortality[Title/Abstract] OR "mortality"[MeSH Terms])
AND ("meta-analysis"[pt] OR "meta-analysis"[Title])
AND Humans[MeSH]
AND ("2000/01/01"[Date - Publication] : "3000"[Date - Publication])

Appendix 3. Added Sugars, SSBs, 100% Fruit Juice: Evidence Table from Included Meta-Analyses (Articles highlighted in blue focused on a single exposure/outcome pair, while the first six columns provide general information about the article as a whole). *Please refer to the footnotes for the meanings of the acronyms.*

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Abbasalizad et al (2022)	August 2021	PubMed, Embase, Scopus, Cochrane, Web of Science	22 studies (BMI)	Observational studies with categories of SSB intake in children aged 2-18y	121,282 children	Change in BMI/weight	SSB	0.75 (0.35–1.15) - BMI unit	99.9%	No evidence for a nonlinear relation	None	JBI	No conflicts
	August 2021	PubMed, Embase, Scopus, Cochrane, Web of Science	15 (WC)	Observational studies with categories of SSB intake in children aged 2-18y	121,282 children	Waist circumference	SSB	2.35 cm; CI 1.34, 3.37	99.9%	No evidence for a nonlinear relation	None	JBI	No conflicts
	August 2021	PubMed, Embase, Scopus, Cochrane, Web of Science	5 (body fat)	Observational studies with categories of SSB intake in children aged 2-18y	121,282 children	Body fat	SSB	2.81; CI 2.21; 3.41 (% body fat)	96.9%	No evidence for a nonlinear relation	None	JBI	No conflicts
Ardeshirlarijani et al (2021)	January 31, 2019	PubMed/Medline, Web of Knowledge, Scopus, and EMBASE	7 cohorts	Healthy (free of any cardiovascular diseases, cancer, etc) adults populations with prospective cohort design with SSB exposure and WC outcome	24,007 adults	Waist circumference	SSB	1.14 (95% CI: 0.86, 1.51)	90,80%	NA	None	NOS	None reported
Asgari-Taee et al (2019)	December 2016	MEDLINE, Embase, Cochrane collaboration, ISI, and Google Scholar	4 cross-sectional studies	Studies with NAFLD as an outcome	6,326 adults	NAFLD	SSB	OR=1.40; 95% CI 1.07, 1.82	31.0%, P = 0.226	NA	None	None	No COI declared. Funded by Iran University of Medical Sciences (Grant no. 96-02-27-29952).
Auerbach et al (2017)	December 31, 2015	PubMed, Embase, CINAHL, and Cochrane databases	8 prospective cohort studies	Studies that examined 100% fruit juice consumption in children aged 1–18, assessed changes in BMI or BMI z-score, had a follow-up of at least 6 months, used experimental or cohort designs, and were published in English peer-reviewed journals	34, 470 children	Change in BMI/weight	100% fruit juice	0.003 (95% CI: 0.001 to 0.004) (energy adjusted, all ages)	11%; P = .34	NA	None	NOS	No COI declared. Funded by Ruth L. Kirschstein National Research Service of the National Institutes of Health through the University of Washington (grant T32HP10002).

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Bechthold et al (2019)	March 2017	PubMed and Embase	123 (total), 11 (SSB only)	Prospective study designs with information about the association for at least one of the following 12 food groups: whole grains/cereals, refined grains/cereals, vegetables, fruits, nuts, legumes, eggs, dairy products, fish, red meat, processed meat, SSB, included adults aged ≥18 years; and considered CHD, including myocardial infarction and other coronary artery diseases (like angina); stroke (haemorrhagic, ischemic); and HF as outcomes	8,740 adults	CHD	SSB	RR: 1.10; 95% CI 1.01 to 1.20	50%, p = 0.09	No evidence for a nonlinear relation	Moderate (GRADE)	Assessed but tool unknown	None reported
	March 2017	PubMed and Embase	n=123 (total), 11 (SSB only)	Prospective study designs with information about the association for at least one of the following 12 food groups: whole grains/cereals, refined grains/cereals, vegetables, fruits, nuts, legumes, eggs, dairy products, fish, red meat, processed meat, SSB, included adults aged ≥18 years; and considered CHD, including myocardial infarction and other coronary artery diseases (like angina); stroke (haemorrhagic, ischemic); and HF as outcomes	11,187 adults	Stroke	SSB	RR: 1.09; 95% CI 1.01 to 1.18	0%, p = 0.43	No evidence for a nonlinear relation	Moderate (GRADE)	Assessed but tool unknown	None reported
	March 2017	PubMed and Embase	n=123 (total), 11 (SSB only)	Prospective study designs with information about the association for at least one of the following 12 food groups: whole grains/cereals, refined grains/cereals, vegetables, fruits, nuts, legumes, eggs, dairy products, fish, red meat, processed meat, SSB, included adults aged ≥18 years; and considered CHD, including myocardial infarction and other coronary artery diseases (like angina); stroke (haemorrhagic, ischemic); and HF as outcomes	8,603 adults	Heart failure	SSB	RR: 1.11; 95% CI 0.88 to 1.39	81%, p = 0.02	NA	Low (GRADE)	Assessed but tool unknown	None reported
Bhagavathula et al (2022)	July 2021	PubMed, Medline, Web of Science and EmBase	8 cohort studies	Prospective cohorts, healthy adults >18y	1,252,547 adults	CVD mortality	SSB	RR: 1.14, 95% CI: 1.06–1.22	53.8%	No non-linear dose analysis conducted	None	NOS	No conflicts
Chen et al (2019)	January 2019	PubMed, Web of Science, Medline, Cochrane and EmBase	12 studies	Observational studies that examined SSB consumption in relation to nonalcoholic fatty liver disease	35,705 participants	NAFLD	SSB	1.39 (95% CI, 1.29–1.50)	42% (ns)	Non-linear	None	NOS	None reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Della Corte et all (2025)	July 2024	Medline, EmBase, CINAHL, Web of Science and Cochrane	29 cohorts (18 for SSB, 14 for fruit juice, 2 for added sugar)	Prospective cohort for >2 years and ascertained incident T2D and included healthy adults age 18+ from any racial/ethnic background and free of diabetes at baseline	541,288 (adults, SSB)	T2D	SSB	RR: 1.39; 95% CI: 1.26, 1.55	44.7%	Linear	Moderate (GRADE)	ROBINS-E tool	No conflicts
	July 2024	Medline, EmBase, CINAHL, Web of Science and Cochrane	29 cohorts (18 for SSB, 14 for fruit juice, 2 for added sugar)	Prospective cohort for >2 years and ascertained incident T2D and included healthy adults age 18+ from any racial/ethnic background and free of diabetes at baseline	SSB sample size = 541,288 with 43,532 cases; Fruit juice = 490,413 and 43,065 cases	T2D	Added sugars	RR: 0.92; 95% CI: 0.79, 1.07	0.0%	No evidence of a nonlinear dose-response association	Low (GRADE)	ROBINS-E tool	No conflicts
Deng et al (2014)	May 5, 2014	PubMed, EMBASE and Cochrane Library	total =18, SSB = 1 meta-analysis of 4 studies	Prospective studies investigating the relationship between a specific food or food groups and stroke outcome (including risk of stroke or stroke mortality)	259,176 participants	Stroke	SSB	RR=1·10; 95 % CI 1·00, 1·20	NA	NA	None	AMSTAR	None reported
Farhangi et al (2020)	April 20, 2020	PubMed, Scopus, Embase and Cochrane electronic databases	14 observational studies	Observational, original research publications, reported intake of SSBs (including sodas, carbonated drinks, non-100% fruit juices, syrup-based beverages, flavored sugary waters, sports and energy drinks, chocolate milk, yogurt drinks, lemonades, Coca-Cola, Sprite, orange juice, Nutrition Express, Red Bull, and sweetened teas) as the exposure and HTN, SBP, or DBP as outcomes, included children and adolescents under 19 years of age, and reported mean ± standard deviation (SD) of SBP or DBP or odds ratios (ORs) for HTN comparing highest versus lowest SSB consumption	93,873 participants (children, adolescents)	Hypertension	SSB	OR: 1.365; CI 1.145-1.626	0.0; P = 0.976	NA	None	AHRQ	No conflicts. Funded by Tabriz University of Medical Sciences
	April 20, 2020	PubMed, Scopus, Embase and Cochrane electronic databases	14 observational studies	Observational, original research publications, reported intake of SSBs (including sodas, carbonated drinks, non-100% fruit juices, syrup-based beverages, flavored sugary waters, sports and energy drinks, chocolate milk, yogurt drinks, lemonades, Coca-Cola, Sprite, orange juice, Nutrition Express, Red Bull, and sweetened teas) as the exposure and HTN, SBP, or DBP as outcomes, included children and adolescents under 19 years of age, and reported mean ± standard deviation (SD) of SBP or DBP or odds ratios (ORs) for HTN	93,873 participants (children, adolescents)	SBP	SSB	WMD: 1.67; CI 1.021-2.321	99.8; P < 0.001	No departure from linearity	None	AHRQ	No conflicts. Funded by Tabriz University of Medical Sciences

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
				comparing highest versus lowest SSB consumption									
	April 20, 2020	PubMed, Scopus, Embase and Cochrane electronic databases	14 observational studies	Observational, original research publications, reported intake of SSBs (including sodas, carbonated drinks, non-100% fruit juices, syrup-based beverages, flavored sugary waters, sports and energy drinks, chocolate milk, yogurt drinks, lemonades, Coca-Cola, Sprite, orange juice, Nutrition Express, Red Bull, and sweetened teas) as the exposure and HTN, SBP, or DBP as outcomes, included children and adolescents under 19 years of age, and reported mean ± standard deviation (SD) of SBP or DBP or odds ratios (ORs) for HTN comparing highest versus lowest SSB consumption	93,873 participants (children, adolescents)	DBP	SSB	WMD: 0.313; CI -0.131-0.757	99.4; P < 0.001	No departure from linearity	None	AHRQ	No conflicts. Funded by Tabriz University of Medical Sciences
Hu et al (2019)	June 2018	PubMed, Web of Science, Medline, Cochrane and EmBase	10 observational studies incl 4 cohort studies	Cohort or cross-sectional studies; diagnosis of depression and SSB data	365,289 participants	Depression	SSB	1.31 (95% CI 1.24-1.39)	29.2% (ns)	Non-linear	None	NOS	None reported
Huang et al (2014)	February 2013	PubMed, EMbase, and Cochrane Library Database	Four prospective studies	Studies were considered eligible if they met the following criteria: (1) a prospective study design; (2) the exposure of study was SSBs consumption; (3) the outcome was incident CHD; (4) reported relative risks (RRs) or hazards ratios (HRs) with 95% confidence intervals (CIs) or standard errors (SEs) for different categories of SSBs consumption; (5) described adjustment for potential confounding factors.	173,753 participants	CHD	SSB	1.17 (1.07:1.28)	0.0% (NS)	Linear dose-response but did not test for non-linearity	None	NOS	None reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Huang et al (2023)	May 10, 2022	PubMed, Embase, and Web of Science	19 cohorts (in 15 articles)	1) they were prospective studies conducted in human adults 18 y old; 2) the exposure investigated included at least one of the following categories of sugars: total sugars, added sugars, fructose, and sucrose; 3) outcomes were all-cause, CVD, or cancer mortality; and 4) reported effect sizes included hazard ratios (HRs) or relative risks (RRs). For dose-response meta-analysis, a quantitative measure of the intake for at least three levels of sugar intake or a risk estimate of the corresponding specific outcome (all-cause, CVD, or cancer mortality) for sugar intake on a continuous scale had to be available	852 to 195, 658 adults	All cause mortality	Added sugars	RR: 1.05 (95% CI, 0.97–1.14)	76.6%	No evidence for a nonlinear relation	None	NOS	None reported
	May 10, 2022	PubMed, Embase, and Web of Science	19 cohorts (in 15 articles)	1) they were prospective studies conducted in human adults 18 y old; 2) the exposure investigated included at least one of the following categories of sugars: total sugars, added sugars, fructose, and sucrose; 3) outcomes were all-cause, CVD, or cancer mortality; and 4) reported effect sizes included hazard ratios (HRs) or relative risks (RRs). For dose-response meta-analysis, a quantitative measure of the intake for at least three levels of sugar intake or a risk estimate of the corresponding specific outcome (all-cause, CVD, or cancer mortality) for sugar intake on a continuous scale had to be available	sample sizes in these studies ranged from 852 to 195 658, with an age range between 20 and >94 y	CVD mortality	Added sugars	RR: 1.08; 95% CI, 0.93–1.26	6.6%	No evidence for a nonlinear relation	None	NOS	None reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	May 10, 2022	PubMed, Embase, and Web of Science	19 cohorts (in 15 articles)	1) they were prospective studies conducted in human adults 18 y old; 2) the exposure investigated included at least one of the following categories of sugars: total sugars, added sugars, fructose, and sucrose; 3) outcomes were all-cause, CVD, or cancer mortality; and 4) reported effect sizes included hazard ratios (HRs) or relative risks (RRs). For dose-response meta-analysis, a quantitative measure of the intake for at least three levels of sugar intake or a risk estimate of the corresponding specific outcome (all-cause, CVD, or cancer mortality) for sugar intake on a continuous scale had to be available	sample sizes in these studies ranged from 852 to 195 658, with an age range between 20 and >94 y	Cancer mortality	Added sugars	RR = 0.99; 95% CI, 0.94–1.03	0.0%	Evidence for a nonlinear relation	None	NOS	None reported
Imamura et al (2015)	February 2014	PubMed, Ovid, Web of Knowledge	17 cohorts	Prospective design, examined SSB and incident T2D, adults over 18y and free of diabetes at baseline with at least 2y follow up	10,126,754 person years and 38,253 cases; adults mostly in USA and UK	T2D	SSB	RR: 1.13 (1.06 to 1.21)	79.8%	Linear	Moderate (GRADE)	CRB (non trial)	None reported
	February 2014	PubMed, Ovid, Web of Knowledge	17 cohorts	Prospective design, examined SSB and incident T2D, adults over 18y and free of diabetes at baseline with at least 2y follow up	10,126,754 person years and 38,253 cases; adults mostly in USA and UK	T2D	100% fruit juice	RR: 1.07 (1.01 to 1.14)	50.8%	Linear	Low (GRADE)	CRB (non trial)	None reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Jakobsen et al (2023)	August 2022	PubMed, EMBASE, SCOPUS, and Web of Science	60 observational studies	Cross-sectional studies and longitudinal studies were included if they were written in English and published in peer-reviewed journals from 1 January 1990 until 31 August 2022. Records were included if investigating otherwise healthy children or adolescents (mean age between 5 and 18 years of age) with overweight and/or obesity or a mixed population of children or adolescents with normal weight and children or adolescents with overweight/obesity. Records were excluded if examining only non-overweight children or adolescents (ISO-Body Mass Index (BMI) < 25 kg/m2), athletes or adolescents who underwent bariatric surgery. Also records investigating children or adolescents with diagnosed non-alcoholic-fatty-liver disease, diabetes, or other comorbidities were excluded.	242,061 participants	Obesity	SSB	OR:1.20 (1.09, 1.33)	79.34%	NA	None	NOS	Conflicts reported. Funded by Novo Nordisk Foundation, Sygeforsiking "danmark" and Arla Foods Amba (unrestricted grant)
Jayalath et al (2015)	November 11, 2014	MEDLINE, Embase, Cumulative Index to Nursing and Allied Health Literature, and the Cochrane	6 prospective cohort studies (n=240,508)	prospective cohorts that reported data on the association of SSBs and incident hypertension	n= 240,508 with 79,251 cases of hypertension observed over ≥3,197,528 person-years of follow-up	Hypertension	SSB	RR: 1.12; 95% CI: 1.06, 1.17	I^2 = 62%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by Canadian Institutes of Health Research (funding no. 129920)
Kazemi et al (2023)	November 2020	PubMed, Scopus, Web of Science	9 studies For all-cause mortality, 3 for CVD mortality and 4 for total cancer	Not reported	For all-cause: 748,934 participants and 97,787 events; For CVD mortality: 202,349 and 11,669 events; for cancer mortality: 402,256 and 29,396 events	All cause mortality	SSB	HR:1.10 (95% CI: 1.05–1.16)	63%	Linear	Low (GRADE)	NOS	Conflicts reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	November 2020	PubMed, Scopus, Web of Science	9 studies For all-cause mortality, 3 for CVD mortality and 4 for total cancer	Not reported	For all-cause: 748,934 participants and 97,787 events; For CVD mortality: 202,349 and 11,669 events; for cancer mortality: 402,256 and 29,396 events	CVD mortality	SSB	HR = 1.11 (95% CI: 1.06–1.16)	60%	Linear	NA	NOS	Conflicts reported
	November 2020	PubMed, Scopus, Web of Science	9 studies For all-cause mortality, 3 for CVD mortality and 4 for total cancer	Not reported	For all-cause: 748,934 participants and 97,787 events; For CVD mortality: 202,349 and 11,669 events; for cancer mortality: 402,256 and 29,396 events	Cancer mortality	SSB	HR = 1.02 (95% CI: 0.93–1.12)	59%	No dose response	Very Low (NutriGRADE)	NOS	Conflicts reported
Khan et al (2019)	January 2018	Medline, EmBase & Cochrane	24 cohort studies	Prospective cohort studies in people healthy at baseline	624,128 individuals; 11,856 incident cases of CVD and 12,224 CVD mortality cases; from Europe, USA and Australia; aged 21-79	CVD mortality	Added sugars	RR 1.03, [95% CI, 0.85, 1.26]	75%	Non-linear	Low (GRADE)	NOS	Conflicts reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Kim and Je (2016)	May 2015	PubMed, Embase and Web of Science	Six prospective studies (for SSBs)	Studies were included in the meta-analysis if they met the following criteria: a prospective cohort design; the exposure of interest was the consumption of SSBs or NSSBs; the outcome of interest was defined as incident hypertension or high blood pressure; relative risks (RRs) with 95% confidence intervals (CIs) were reported. Studies focused on patients with specific diseases were excluded.	246,822 subjects and 80,628 incident cases of hypertension (for SSBs)	Hypertension	SSB	RR=1.12 (95% CI: 1.07, 1.17)	59.5%, 95% CI: 0%, 84%; P = 0.03	Linear but no non-linear dose dependent analysis conducted	None	NOS	No conflicts reported. Funded by National Research Foundation of Korea (NRF) and the Ministry of Science, ICT & Future Planning (NRF-2014R1A1A1002736, NRF-2015R1A1A1A05001362)
Li et al (2022)	January 1, 2020	PubMed, Embase, Cochrane Library and Web of Science	15 cohorts	prospective cohort studies of adults researching the mortality risk and SSBs or NSSBs consumption	1,211,470 participants	All cause mortality	SSB	1.12; 95% CI, 1.06-1.19	74.3%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by United Fund of National Natural Science Foundation of China (grant no. U2004110)
	January 1, 2020	PubMed, Embase, Cochrane Library and Web of Science	15 cohorts	prospective cohort studies of adults researching the mortality risk and SSBs or NSSBs consumption	1,211,470 participants	CVD mortality	SSB	1.20 (95% CI, 1.05–1.38)	76.1%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by United Fund of National Natural Science Foundation of China (grant no. U2004110)
	January 1, 2020	PubMed, Embase, Cochrane Library and Web of Science	15 cohorts	prospective cohort studies of adults researching the mortality risk and SSBs or NSSBs consumption	1,211,470 participants	Cancer mortality	SSB	0.96 (95% CI, 0.84–1.10)	86.4%	NA	None	NOS	No conflicts reported. Funded by United Fund of National Natural Science Foundation of China (grant no. U2004110)
	January 1, 2020	PubMed, Embase, Cochrane Library and Web of Science	15 cohorts	prospective cohort studies of adults researching the mortality risk and SSBs or NSSBs consumption	1,211,470 participants	Other cause mortality	SSB	1.22 (95% CI, 1.01–1.47)	87.0%	NA	None	NOS	No conflicts reported. Funded by United Fund of National Natural Science Foundation of China (grant no. U2004110)
Liu et al (2019)	December 2, 2018	MEDLINE, EMBASE, and Cochrane	26 reports, including 15 prospective cohorts. 13 cohort comparisons (427 630 participants [n]; 120 553 cases) for SSBs; 2 cohort comparisons for 100% fruit juice (n=83 178; 46 811 cases)	All prospective cohort studies of ≥1 year duration that assessed the association of important food sources of fructose-containing sugars, including nonalcoholic beverages (eg, SSBs), grain and grain-based products, fruit and fruit-based products, dairy and dairy-based products, and sweets and desserts with incident hypertension in participants free of hypertension at the start of the study. If several studies provided results on the same outcome and used overlapping groups of individuals, we included the study with the longest follow-up.	930, 677 participants	Hypertension	SSB	RR=1.17 [95% CI, 1.11, 1.23]	66% (S)	Non-linear	Low (GRADE)	NOS	Conflicts reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
				Abstracts and unpublished studies were not included									
	December 2, 2018	MEDLINE, EMBASE, and Cochrane	26 reports, including 15 prospective cohorts. 13 cohort comparisons (427 630 participants [n]; 120 553 cases) for SSBs; 2 cohort comparisons for 100% fruit juice (n=83 178; 46 811 cases)	All prospective cohort studies of ≥1 year duration that assessed the association of important food sources of fructose-containing sugars, including nonalcoholic beverages (eg, SSBs), grain and grain-based products, fruit and fruit-based products, dairy and dairy-based products, and sweets and desserts with incident hypertension in participants free of hypertension at the start of the study. If several studies provided results on the same outcome and used overlapping groups of individuals, we included the study with the longest follow-up. Abstracts and unpublished studies were not included	930, 677 participants	Hypertension	100% fruit juice	RR=0.95 [95% CI, 0.85, 1.07]	85% (NS)	U shape	Low (GRADE)	NOS	Conflicts reported
Liu et al (2022)	May 20, 2022	PubMed and Web of Science databases	13 observational studies	Articles were included in the meta-analysis if they met the following criteria: 1) investigated the association between sugar-sweetened beverages (including soft drinks and fruit and vegetable juices) and cognitive decline, cognitive impairment, all-cause dementia, Alzheimer's disease (AD), or mild cognitive impairment (MCI); 2) reported hazard ratios (HRs), relative risks (RRs), or odds ratios (ORs) with 95% confidence intervals (CIs) for the association between SSB consumption and cognitive disorders; and 3) were published in English. Studies were excluded if they did not provide quantitative results for individual studies, focused on populations with serious health	242,014 participants all ages	Cognitive disorders	SSB	OR = 1.17, 95 % CI = 1.05-1.29	90.1%	NA	None	NOS (case control/cohort) and AHRQ (cross-sectional)	No conflicts reported. Funded by the National Natural Science Foundation of China [grant number 81903302], Young Elite Scientists Sponsorship Program by China Association for Science and Technology [grant number YESS20200151], and 345 Talent Project of Shengjing Hospital of China Medical University [grant number M0294]

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
				conditions (e.g., cardiovascular disease, type 2 diabetes, or cancer), or were animal studies, mechanistic research, or reviews.									
Liu et al (2023)	March 31, 2023	PubMed and Web of Science databases	27 studies were included (four observational focused on SSBs)	Studies were included if they were observational, reported associations between liver cancer and at least one of six food groups (grains, legumes, nuts, poultry, eggs, or sugar-sweetened beverages), provided or allowed calculation of risk estimates with 95% confidence intervals, and, in cases of overlapping populations, the study with the larger sample size was selected. Studies were excluded if they focused on food groups already covered in recent meta-analyses (e.g., fruits, vegetables, dairy, meats, fish), were non-original research (e.g., reviews, animal studies), lacked full-text access, or were duplicates.	Not provided (calculated: 1,100,932 for SSBs)	Liver cancer	SSB	OR=1.07 (0.93~1.24) (cf supp material)	22.1% (P=0.278)	NA	None	NOS	No conflicts reported. Funded by the National Natural Science Foundation of China (82103936), Natural Science Foundation of Zhejiang Province (LQ20H260008, LQ21H260001), Medical Health Science and Technology Project of Zhejiang Provincial Health Commission (2020KY195), Zhejiang Chinese Medical University Foundation (2020ZG16)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Liu et al (2023)	July 2022	PubMed and Web of Science	15 obs	Studies that investigated the associations between the intake of ≥1 food sources with added fructose (biscuits and cookies, cake, sugar-sweetened beverages [SSBs], sweets, candies, chocolate, or ice cream) and NAFLD in a general adult population	65,149	NAFLD	Added fructose	OR = 1.31, 95% CI = 1.17–1.48	87.7%	NA	None	NOS, AHRQ	None reported. Funded by National Natural Science, Foundation of China (grant number 81903302), the Young Elite Scientists Sponsorship Program by the China Association for Science and Technology (grant number YESS20200151), the 345 Talent Project of Shengjing Hospital of China Medical University (grant number M0294), and the Scientific Research Project of Liaoning Province Education Department (grant number LJKMZ20221149)
Liu et al (2024)	October 2023	PubMed, Web of Science, Embase and Scopus	154 studies (51 on SSBs)	Studies were included if they were observational (cohort, case-control, cross-sectional) involving toddlers and children aged 2–18, reported on children's food consumption (fruits, vegetables, sugar-sweetened beverages), parental education, or nutrition policies, and assessed childhood overweight or obesity as outcomes (excluding studies using only BMI or BMI z-scores). Included studies compared groups based on food intake frequency or preference, parental education levels, or exposure to nutrition policies versus non-exposure. Studies were excluded if they were case reports, reviews, involved children under 2 years or with diseases affecting eating or growth, or involved hospitalized patients.	3,343,808 children	Child Overweight/Obesity	SSB	High-income countries/regions (OR = 1.24; 95% CI = 1.13–1.36) - Figure 3 does not match values inserted in the main text	78.2% (cf Figure 4 instead of 3)	NA	None	NOS, JBI, NIH	No conflicts reported. Funded by Study of Diet and Nutrition Assessment and Intervention Technology (No.2020YFC2006300)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Laha et al (2021)	June 2020	PubMed, Web of Science and SCOPUS databases	27 (cohort + case-studies) included in the meta analysis but 64 in systematic review	Eligible cohort and case-control studies included adults without prior cancer (except nonmelanoma skin cancer) and assessed the association between sweet beverage intake and overall or site-specific cancer incidence, reporting HRs, RRs, or ORs with 95% CIs. Studies were excluded if they involved participants with a cancer history, focused on cancer survival or mortality, or were duplicates.	4,458,056 participants	Breast cancer mortality	SSB	1.14 (1.01-1.30)	0.0%	NA	None	ROBINS-E (cohort) and NOS (case control)	No conflicts reported. Funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund.
	June 2020	PubMed, Web of Science and SCOPUS databases	27 (cohort + case-studies) included in the meta analysis but 64 in systematic review	Eligible cohort and case-control studies included adults without prior cancer (except nonmelanoma skin cancer) and assessed the association between sweet beverage intake and overall or site-specific cancer incidence, reporting HRs, RRs, or ORs with 95% CIs. Studies were excluded if they involved participants with a cancer history, focused on cancer survival or mortality, or were duplicates.	4,458,056 participants	Breast PM cancer mortality	SSB	1.37 (0.99−1.88)	55.7%	NA	None	ROBINS-E (cohort) and NOS (case control)	No conflicts reported. Funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund.
	June 2020	PubMed, Web of Science and SCOPUS databases	27 (cohort + case-studies) included in the meta analysis but 64 in systematic review	Eligible cohort and case-control studies included adults without prior cancer (except nonmelanoma skin cancer) and assessed the association between sweet beverage intake and overall or site-specific cancer incidence, reporting HRs, RRs, or ORs with 95% CIs. Studies were excluded if they involved participants with a cancer history, focused on cancer survival or mortality, or were duplicates.	4,458,056 participants	Breast PostM cancer mortality	SSB	1.18 (0.79−1.75)	54.8%	NA	None	ROBINS-E (cohort) and NOS (case control)	No conflicts reported. Funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund.
	June 2020	PubMed, Web of Science and SCOPUS databases	27 (cohort + case-studies) included in the meta analysis but 64 in systematic review	Eligible cohort and case-control studies included adults without prior cancer (except nonmelanoma skin cancer) and assessed the association between sweet beverage intake and overall or site-specific cancer incidence, reporting HRs, RRs, or ORs with 95% CIs. Studies were excluded if they involved participants with a cancer history, focused on cancer survival or mortality, or were duplicates.	4,458,056 participants	Colorectal cancer mortality	SSB	1.18 (0.99−1.41)	0.0%	NA	None	ROBINS-E (cohort) and NOS (case control)	No conflicts reported. Funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund.

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	June 2020	PubMed, Web of Science and SCOPUS databases	27 (cohort + case-studies) included in the meta analysis but 64 in systematic review	Eligible cohort and case-control studies included adults without prior cancer (except nonmelanoma skin cancer) and assessed the association between sweet beverage intake and overall or site-specific cancer incidence, reporting HRs, RRs, or ORs with 95% CIs. Studies were excluded if they involved participants with a cancer history, focused on cancer survival or mortality, or were duplicates.	4,458,056 participants	Prostate cancer mortality	SSB	1.18 (1.10−1.27)	0.0%	NA	None	ROBINS-E (cohort) and NOS (case control)	No conflicts reported. Funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund.
	June 2020	PubMed, Web of Science and SCOPUS databases	27 (cohort + case-studies) included in the meta analysis but 64 in systematic review	Eligible cohort and case-control studies included adults without prior cancer (except nonmelanoma skin cancer) and assessed the association between sweet beverage intake and overall or site-specific cancer incidence, reporting HRs, RRs, or ORs with 95% CIs. Studies were excluded if they involved participants with a cancer history, focused on cancer survival or mortality, or were duplicates.	4,458,056 participants	Pancreatic cancer mortality	SSB	1.01 (0.92−1.11)	0.0%	NA	None	ROBINS-E (cohort) and NOS (case control)	No conflicts reported. Funded by the Institute of Health Carlos III through the grant CP15/00100 and PI18/00191 (cofounded by European Regional Development Fund.
Malik et al (2010)	May 2010	MEDLINE	11 obs	prospective cohort studies of SSB intake and risk of metabolic syndrome and type 2 diabetes	310,819 participants	T2D	SSB	RR: 1.26 (95% CI 1.12–1.41)	66%	NA	None	None	None reported
	May 2010	MEDLINE	11 obs	prospective cohort studies of SSB intake and risk of metabolic syndrome and type 2 diabetes	310,819 participants	MetS	SSB	RR: 1.20 (1.02–1.42)	76%	NA	None	None	None reported
Malik et al (2013)	March 2013	PubMed, EMBASE, and the Cochrane library	20 studies in children: 15 prospectives, 5 trials: 12 studies in adults: 7 prospectives, 5 trials	Original, English-language prospective cohort studies or clinical trials (≥2 weeks) in children or adults, assessing the effect of SSBs (not combined with other exposures) on body weight using multivariable-adjusted outcomes or group differences.	Children (15 cohort studies, n = 25,745; 5 trials, n = 2772) and adults (7 cohort studies, n = 174,252; 5 trials, n = 292)	Change in BMI	SSB	0.06 (95% CI: 0.02, 0.10)-unit increase in BMI (1-y change in BMI)	63.8%; P = 0.002	NA	None	NOS	No conflicts reported. Funded by NIH
	March 2013	PubMed, EMBASE, and the Cochrane library	20 studies in children: 15 prospectives, 5 trials: 12 studies in adults: 7 prospectives, 5 trials	Original, English-language prospective cohort studies or clinical trials (≥2 weeks) in children or adults, assessing the effect of SSBs (not combined with other exposures) on body weight using multivariable-adjusted outcomes or group differences.	Children (15 cohort studies, n = 25,745; 5 trials, n = 2772) and adults (7 cohort studies, n = 174,252; 5	Change in weight	SSB	0.22 kg (95% CI: 0.09, 0.34 kg)	70.2%; P < 0.001	NA	None	NOS	No conflicts reported. Funded by NIH

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
					trials, n = 292)								
	March 2013	PubMed, EMBASE, and the Cochrane library	20 studies in children: 15 prospectives, 5 trials: 12 studies in adults: 7 prospectives, 5 trials	Original, English-language prospective cohort studies or clinical trials (≥2 weeks) in children or adults, assessing the effect of SSBs (not combined with other exposures) on body weight using multivariable-adjusted outcomes or group differences.	Children (15 cohort studies, n = 25,745; 5 trials, n = 2772) and adults (7 cohort studies, n = 174,252; 5 trials, n = 292)	Change in BMI	SSB	[-0.17 (95% CI: -0.39, 0.05)]-unit in BMI	74.6%; P = 0.003	NA	None	CRB	No conflicts reported. Funded by NIH
	March 2013	PubMed, EMBASE, and the Cochrane library	20 studies in children: 15 prospectives, 5 trials: 12 studies in adults: 7 prospectives, 5 trials	Original, English-language prospective cohort studies or clinical trials (≥2 weeks) in children or adults, assessing the effect of SSBs (not combined with other exposures) on body weight using multivariable-adjusted outcomes or group differences.	Children (15 cohort studies, n = 25,745; 5 trials, n = 2772) and adults (7 cohort studies, n = 174,252; 5 trials, n = 292)	Change in weight	SSB	0.85 kg; 95% CI: 0.50; 1.20 kg	0.0%; P = 0.78	NA	None	CRB	No conflicts reported. Funded by NIH
Mattes et al (2011)	January 2009	PubMed, PsycINFO, Cochrane and prior reviews	10 RCTs	Human studies; more than 3 weeks; RCTs; includes an indicator of obesity outcome	Studies included were in children, teens and adults	Change in BMI/weight	SSB	0.58 (0.29; 0.88) standardized	not provided	Not tested	None	Cochrane Handbook for Systematic Reviews of Interventions	Conflicts reported
	January 2009	PubMed, PsycINFO, Cochrane and prior reviews	10 RCTs	Human studies; more than 3 weeks; RCTs; includes an indicator of obesity outcome	Studies included were in children, teens and adults	Change in BMI/weight	SSB	[−0.03 (−0.120;0.046)] Cf. Table 5	0%	Not tested	None	Cochrane Handbook for Systematic Reviews of Interventions	Conflicts reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
McKeown et al (2018)	N/A	Not applicable (internal meta analysis)	11 cohorts	Being part of the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium + Participants within each cohort were excluded from the present analysis when they had type 2 diabetes (prevalent or self-reported), were taking medication for type 2 diabetes, had fasting glucose ≥7 mmol/l (≥126 mg/dl) or were not fasting at blood draw. Participants were also excluded if they had implausible dietary data based on cohort-specific cut-points or missing genotype data.	34,748 adults	Fasting glucose	SSB	β ± SE 0.014 ± 0.004 [mmol/l]	0%	NA	None	None	Conflicts reported. Funded in part by the US Department of Agriculture, under agreement No. 58-1950-0-014. MAH is supported by R01 DK100425. CES is supported by K08 HL112845. JBM is supported by K24DK080140 and U01DK078616. KLY is supported by KL2TR001109.
	N/A	Not applicable (internal meta analysis)	11 cohorts	Being part of the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium + Participants within each cohort were excluded from the present analysis when they had type 2 diabetes (prevalent or self-reported), were taking medication for type 2 diabetes, had fasting glucose ≥7 mmol/l (≥126 mg/dl) or were not fasting at blood draw. Participants were also excluded if they had implausible dietary data based on cohort-specific cut-points or missing genotype data.	34,748 adults	Fasting insulin	SSB	0.030 ± 0.005 [log e pmol/l]	48%	NA	None	None	Conflicts reported. Funded in part by the US Department of Agriculture, under agreement No. 58-1950-0-014. MAH is supported by R01 DK100425. CES is supported by K08 HL112845. JBM is supported by K24DK080140 and U01DK078616. KLY is supported by KL2TR001109.
Meng et al (2021)	June 20, 2020	PubMed, Embase, and Ovid databases	17 prospective cohort studies	1) prospective design (cohort, case-cohort, or nested case-control); (2) SSBs or NSSBs reported as exposure in ≥2 categories, with T2D, CVDs, or all-cause mortality as outcomes; (3) healthy baseline population; (4) reported RRs, HRs, ORs with 95% CIs or sufficient data to calculate them; (5) published in English. Excluded= duplicates, letters, comments, reviews, meta-analyses, studies with incomplete or unreliable data, and those without full texts.	645,658 participants	T2D	SSB	1.29; 95% CI: 1.23–1.34	29.9%, P= 0.102	Linear	None	NOS	No conflicts reported. Funded by the China key research and development program (Grant No. 2018YFE0206300-02), the National Natural Science Foundation of China (Grant No. 81803234), and Xinghua Industrial Research Centre for Food Science and Human Health, China Agricultural University.

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	June 20, 2020	PubMed, Embase, and Ovid databases	17 prospective cohort studies	1) prospective design (cohort, case-cohort, or nested case-control); (2) SSBs or NSSBs reported as exposure in ≥2 categories, with T2D, CVDs, or all-cause mortality as outcomes; (3) healthy baseline population; (4) reported RRs, HRs, ORs with 95% CIs or sufficient data to calculate them; (5) published in English. Excluded= duplicates, letters, comments, reviews, meta-analyses, studies with incomplete or unreliable data, and those without full texts.	645,658 participants	CVD	SSB	RR: 1.17; 95% CI: 1.12–1.23	14.7%, P = 0.293	Linear	None	NOS	No conflicts reported. Funded by the China key research and development program (Grant No. 2018YFE0206300-02), the National Natural Science Foundation of China (Grant No. 81803234), and Xinghua Industrial Research Centre for Food Science and Human Health, China Agricultural University.
	June 20, 2020	PubMed, Embase, and Ovid databases	17 prospective cohort studies	1) prospective design (cohort, case-cohort, or nested case-control); (2) SSBs or NSSBs reported as exposure in ≥2 categories, with T2D, CVDs, or all-cause mortality as outcomes; (3) healthy baseline population; (4) reported RRs, HRs, ORs with 95% CIs or sufficient data to calculate them; (5) published in English. Excluded= duplicates, letters, comments, reviews, meta-analyses, studies with incomplete or unreliable data, and those without full texts.	645,658 participants	All cause mortality	SSB	RR: 1.14; 95% CI: 1.04–1.24	83.0%, P < 0.001	Non-linear	None	NOS	No conflicts reported. Funded by the China key research and development program (Grant No. 2018YFE0206300-02), the National Natural Science Foundation of China (Grant No. 81803234), and Xinghua Industrial Research Centre for Food Science and Human Health, China Agricultural University.
Milajerdi et al (2019)	December 2017	PubMed, MEDLINE, SCOPUS, EMBASE, and Google Scholar	Five cohort and four case–control publications	Included: Cohort and case-control studies in adults reporting HR, RR, or OR for the association between SSBs, sodas, and carbonated drinks with pancreatic cancer risk; only the most complete data from duplicate datasets used	2,041,689 participants	Pancreatic cancer	SSB	RR: 1.06; 95%CI: 0.87 to 1.29	65.4%, P=0.02	No evidence for a nonlinear dose-response relationship. No linear dose reponse.	None	NOS	No conflicts reported. Funded by Iran National Science Foundation (INSF).
	December 2017	PubMed, MEDLINE, SCOPUS, EMBASE, and Google Scholar	Five cohort and four case–control publications	Included: Cohort and case-control studies in adults reporting HR, RR, or OR for the association between SSBs, sodas, and carbonated drinks with pancreatic cancer risk; only the most complete data from duplicate datasets used	2,041,689 participants	Pancreatic cancer	SSB	RR: 1.11; 95% CI: 0.92 to 1.35	22.4%, P=0.27	NA	None	NOS	No conflicts reported. Funded by Iran National Science Foundation (INSF).

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Muñoz et al (2022)	June 2022	Pubmed and SCOPUS	14 (9 cross-sectional and 5 cohort)	Studies assessing the SSBs-MetS, soft drinks-MetS, or bottled fruit juices-MetS, or energy drinks-MetS, or milkshakes-MetS relationships in population-based epidemiological studies (cross-sectional or longitudinal studies) and conducted in human adults.	62,693 adults (cross-sectional), 28,932 adults (cohort)	MetS	SSB	OR 1.35, 95%CI 1.15,1.58	57%	NA	None	JBI	No conflicts reported. CIBERCV and grants PI17/01709, PI19/00020, and PI19/00948 from the Instituto de Salud Carlos III.
	June 2022	Pubmed and SCOPUS	14 (9 cross-sectional and 5 cohort)	Studies assessing the SSBs-MetS, soft drinks-MetS, or bottled fruit juices-MetS, or energy drinks-MetS, or milkshakes-MetS relationships in population-based epidemiological studies (cross-sectional or longitudinal studies) and conducted in human adults.	62,693 adults (cross-sectional), 28,932 adults (cohort)	MetS	SSB	OR 1.18, 95%CI 1.06,1.32	70%	NA	None	JBI	No conflicts reported. CIBERCV and grants PI17/01709, PI19/00020, and PI19/00948 from the Instituto de Salud Carlos III.
Narain et al (2016)	July 2015	Medline and EMBASE	7 cohorts	studies that considered soft drink intake and risk of mortality, myocardial infarction or stroke	308,420 adults (age range 34–75 years)	Stroke	SSB	RR 1.13, 95% CI 1.02–1.24	0%	NA	None	Reported but unknown tool	No conflicts reported. Funded by North Staffs Heart Committee
	July 2015	Medline and EMBASE	8 cohorts	studies that considered soft drink intake and risk of mortality, myocardial infarction or stroke	308,420 adults (age range 34–75 years)	Myocardial infarction	SSB	RR 1.22, 95% CI 1.14–1.30	8%	NA	None	Reported but unknown tool	No conflicts reported. Funded by North Staffs Heart Committee
	July 2015	Medline and EMBASE	9 cohorts	studies that considered soft drink intake and risk of mortality, myocardial infarction or stroke	308,420 adults (age range 34–75 years)	Vascular events	SSB	RR: 1.09 (0.82, 1.45)	NA	NA	None	Reported but unknown tool	No conflicts reported. Funded by North Staffs Heart Committee
	July 2015	Medline and EMBASE	10 cohorts	studies that considered soft drink intake and risk of mortality, myocardial infarction or stroke	308,420 adults (age range 34–75 years)	All cause mortality	SSB	RR: 1.03 (95% CI 0.91–1.18)	75%	NA	None	Reported but unknown tool	No conflicts reported. Funded by North Staffs Heart Committee
Nguyen et al (2023)	September 8, 2022	MEDLINE, Embase, and Cochrane databases	85 articles including 48 in children (40 cohorts; 8 RCTs, n = 2783) and 37 in adults (21 cohorts,; 16 RCTs, n = 1343)	Study Types Included: prospective cohort studies (≥6 months), RCTs (≥2 weeks) assessing addition/subtraction of SSBs, population: Children (<18 y) and adults (≥18 y), SSBs defined as beverages with added sugar (e.g., soft drinks, fruit drinks), comparators in RCTs: noncaloric beverages	in children (40 cohorts, n = 91,713; 8 RCTs, n = 2783) and in adults (21 cohorts, n = 448,661; 16 RCTs, n = 1343)	Change in BMI	SSB	.07-kg/m2 (95% CI: 0.04 kg/m2, 0.10 kg/m2	82%, P < 0.01	Linear, no departure from linearity	Low (GRADE)	NOS	Conflicts reported
	September 8, 2022	MEDLINE, Embase, and Cochrane databases	85 articles including 48 in children (40 cohorts; 8 RCTs, n = 2783) and 37 in adults (21 cohorts,; 16	Study Types Included: prospective cohort studies (≥6 months), RCTs (≥2 weeks) assessing addition/subtraction of SSBs, population: Children (<18 y) and adults (≥18 y), SSBs defined as beverages with added sugar (e.g., soft drinks, fruit drinks), comparators in RCTs: noncaloric beverages	in children (40 cohorts, n = 91,713; 8 RCTs, n = 2783) and in adults (21 cohorts, n = 448,661; 16	Change in weight	SSB	0.42-kg (95% CI: 0.26 kg, 0.58 kg)	90%, P < 0.01	Linear, no departure from linearity	Low (GRADE)	NOS	Conflicts reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
			RCTs, n = 1343)		RCTs, n = 1343)								
	September 8, 2022	MEDLINE, Embase, and Cochrane databases	85 articles including 48 in children (40 cohorts; 8 RCTs, n = 2783) and 37 in adults (21 cohorts,; 16 RCTs, n = 1343)	Study Types Included: prospective cohort studies (≥6 months), RCTs (≥2 weeks) assessing addition/subtraction of SSBs, population: Children (<18 y) and adults (≥18 y), SSBs defined as beverages with added sugar (e.g., soft drinks, fruit drinks), comparators in RCTs: noncaloric beverages	in children (40 cohorts, n = 91,713; 8 RCTs, n = 2783) and in adults (21 cohorts, n = 448,661; 16 RCTs, n = 1343)	Change in BMI	SSB	−0.21 kg/m2; 95% CI: −0.40 kg/m2, −0.01 kg/m2	99%, P < 0.01	NA	Low (GRADE)	CRB	Conflicts reported
	September 8, 2022	MEDLINE, Embase, and Cochrane databases	85 articles including 48 in children (40 cohorts; 8 RCTs, n = 2783) and 37 in adults (21 cohorts,; 16 RCTs, n = 1343)	Study Types Included: prospective cohort studies (≥6 months), RCTs (≥2 weeks) assessing addition/subtraction of SSBs, population: Children (<18 y) and adults (≥18 y), SSBs defined as beverages with added sugar (e.g., soft drinks, fruit drinks), comparators in RCTs: noncaloric beverages	in children (40 cohorts, n = 91,713; 8 RCTs, n = 2783) and in adults (21 cohorts, n = 448,661; 16 RCTs, n = 1343)	Change in weight	SSB	0.83 kg; 95% CI: 0.47 kg, 1.19 kg	87%, P < 0.01	Linear, no departure from linearity	Moderate (GRADE)	CRB	Conflicts reported
Nguyen et al (2024)	May 18, 2023	MEDLINE, Embase, and Cochrane	42 articles (17 obs. studies in children; 6 obs. studies and 19 RCTs in adults)	Prospective cohort (≥6 months) and RCTs (≥2 weeks). Studies with an isocaloric control, a multimodal intervention, or combined 100% fruit juice with other foods, supplements, or lifestyle factors were excluded.	45,851 children; 268 095 adults	Change in BMI/weight	100% fruit juice	0.03 BMI unit (95% CI, 0.01-0.05)	85%; P <.001	NA	Moderate (NutriGRADE)	Newcastle-Ottawa Scale (NOS)	Conflicts reported. Funded by the Ontario Graduate Scholarship, Peterborough KM Hunter Charitable Foundation Graduate Award, Dalton Whitebread Scholarship Fund, and SMART Healthy Cities Trainee Award
	May 18, 2024	MEDLINE, Embase, and Cochrane	42 articles (17 obs. studies in children; 6 obs. studies and 19 RCTs in adults)	Prospective cohort (≥6 months) and RCTs (≥2 weeks). Studies with an isocaloric control, a multimodal intervention, or combined 100% fruit juice with other foods, supplements, or lifestyle factors were excluded.	45,851 children; 268 095 adults	Change in BMI/weight	100% fruit juice	0.07 kg; (95% CI, −0.06-0.20 kg)	97%, P <.001	NS	Low (NutriGRADE)	NOS	Conflicts reported. Funded by the Ontario Graduate Scholarship, Peterborough KM Hunter Charitable Foundation Graduate Award, Dalton Whitebread Scholarship Fund, and SMART Healthy Cities Trainee Award

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	May 18, 2025	MEDLINE, Embase, and Cochrane	42 articles (17 obs. studies in children; 6 obs. studies and 19 RCTs in adults)	Prospective cohort (≥6 months) and RCTs (≥2 weeks). Studies with an isocaloric control, a multimodal intervention, or combined 100% fruit juice with other foods, supplements, or lifestyle factors were excluded.	45,851 children; 268 095 adults	Change in BMI/weight	100% fruit juice	−0.53 kg; (95% CI, −1.55 to 0.48 kg)	95%, P <.001	NA	Moderate (NutriGRADE)	Cochrane Risk-Bias (CRB)	Conflicts reported. Funded by the Ontario Graduate Scholarship, Peterborough KM Hunter Charitable Foundation Graduate Award, Dalton Whitebread Scholarship Fund, and SMART Healthy Cities Trainee Award
Pan et al (2022)	September 20, 2019	PubMed, Embase, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and PsycINFO	Thirteen prospective studies	Prospective cohort studies that used multivariable analyses (Cox proportional hazards or logistic regression models) to examine the association between sugar-sweetened beverages (SSBs), artificially sweetened beverages (NSSBs), or 100% fruit juice and the risk of all-cause, cancer, or cardiovascular mortality. Abstracts reporting multivariable results were also considered. Studies were excluded if more than 20% of participants had major chronic illnesses at baseline. For studies based on the same cohort and outcome, only the most recent or longest follow-up publication was included; if follow-up duration was identical, the study with the larger sample size was selected	1,539,127 participants (mean age of 58.33years)	All cause mortality	SSB	HR=1.11(95% CI: 1.05 to1.19) - Table S7	83%	Non-linear	Low (GRADE)	NOS	None reported. Funded by Ministry of Science and technology of China (2019YFC1709805) and Gansu Provincial Hospital
	September 20, 2019	PubMed, Embase, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and PsycINFO	Thirteen prospective studies	Prospective cohort studies that used multivariable analyses (Cox proportional hazards or logistic regression models) to examine the association between sugar-sweetened beverages (SSBs), artificially sweetened beverages (NSSBs), or 100% fruit juice and the risk of all-cause, cancer, or cardiovascular mortality. Abstracts reporting multivariable results were also considered. Studies were excluded if more than 20% of participants had major chronic illnesses at baseline. For studies based on the same cohort and outcome, only the most recent or longest follow-up publication was included; if follow-up duration was	1,539,127 participants (mean age of 58.33years)	All cause mortality	100% fruit juice	HR=1.0(95%CI: 0.78 to1.29) - Table S7	83%	NA	Very low (GRADE)	NOS	None reported. Funded by Ministry of Science and technology of China (2019YFC1709805) and Gansu Provincial Hospital

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
				identical, the study with the larger sample size was selected									
	September 20, 2019	PubMed, Embase, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and PsycINFO	Thirteen prospective studies	Prospective cohort studies that used multivariable analyses (Cox proportional hazards or logistic regression models) to examine the association between sugar-sweetened beverages (SSBs), artificially sweetened beverages (NSSBs), or 100% fruit juice and the risk of all-cause, cancer, or cardiovascular mortality. Abstracts reporting multivariable results were also considered. Studies were excluded if more than 20% of participants had major chronic illnesses at baseline. For studies based on the same cohort and outcome, only the most recent or longest follow-up publication was included; if follow-up duration was identical, the study with the larger sample size was selected	1,539,127 participants (mean age of 58.33years)	CVD mortality	SSB	HR=1.14 (95%CI: 1.02 to1.27) - Table S7	59%	Non-linear	Low (GRADE)	NOS	None reported. Funded by Ministry of Science and technology of China (2019YFC1709805) and Gansu Provincial Hospital

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	September 20, 2019	PubMed, Embase, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and PsycINFO	Thirteen prospective studies	Prospective cohort studies that used multivariable analyses (Cox proportional hazards or logistic regression models) to examine the association between sugar-sweetened beverages (SSBs), artificially sweetened beverages (NSSBs), or 100% fruit juice and the risk of all-cause, cancer, or cardiovascular mortality. Abstracts reporting multivariable results were also considered. Studies were excluded if more than 20% of participants had major chronic illnesses at baseline. For studies based on the same cohort and outcome, only the most recent or longest follow-up publication was included; if follow-up duration was identical, the study with the larger sample size was selected	1,539,127 participants (mean age of 58.33years)	CVD mortality	100% fruit juice	HR =1.20; 95%CI 1.01 to 1.42 - Table S7	0%	NA	Very low (GRADE)	NOS	None reported. Funded by Ministry of Science and technology of China (2019YFC1709805) and Gansu Provincial Hospital
	September 20, 2019	PubMed, Embase, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and PsycINFO	Thirteen prospective studies	Prospective cohort studies that used multivariable analyses (Cox proportional hazards or logistic regression models) to examine the association between sugar-sweetened beverages (SSBs), artificially sweetened beverages (NSSBs), or 100% fruit juice and the risk of all-cause, cancer, or cardiovascular mortality. Abstracts reporting multivariable results were also considered. Studies were excluded if more than 20% of participants had major chronic illnesses at baseline. For studies based on the same cohort and outcome, only the most recent or longest follow-up publication was included; if follow-up duration was identical, the study with the larger sample size was selected	1,539,127 participants (mean age of 58.33years)	Cancer mortality	SSB	HR=1.04 (95% CI: 0.97 to 1.11) - Table S7	44%	Linear	Very low (GRADE)	NOS	None reported. Funded by Ministry of Science and technology of China (2019YFC1709805) and Gansu Provincial Hospital
Pan et al (2023)	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-	4,518,547 adults	Cancer	SSB	RR: 1.07 (0.95 to 1.22)	Not provided	Non-linear	Very low (GRADE)	NOS	No reported conflicts

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
				control studies, studies with >20% chronically ill participants at baseline were excluded.									
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Breast cancer	SSB	RR: 1.17 (1.00 to 1.37)	66%	Linear, no departure from linearity	Moderate (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Colorectal cancer	SSB	RR: 1.10 (1.04 to 1.15)	0%	Non-linear	Moderate (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Endometrial cancer	SSB	RR: 1.01 (0.99 to 1.03)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Gastric cancer	SSB	RR: 1.00 (0.85 to 1.17)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Kidney cancer	SSB	RR: 1.06 (0.98 to 1.15)	Not provided	Linear, no departure from linearity	Low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Leukimia	SSB	RR: 1.06 (0.73 to 1.54)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Multiple myeloma	SSB	RR: 1.18 (0.90 to 1.55)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts

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	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Non Hodgkin lymphoma	SSB	RR: 1.07 (0.92 to 1.23)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Pancreatic cancer	SSB	RR: 1.08 (0.97 to 1.21)	Not provided	Non-linear	Low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Prostate cancer	SSB	RR: 1.10 (1.00 to 1.22)	0%	Non-linear	Low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Cancer	100% fruit juice	RR: 1.09 (0.98 to 1.21)	41%	NA	Low (GRADE)	NOS	No reported conflicts

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Breast cancer	100% fruit juice	RR: 1.07 (0.96 to 1.18)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Colorectal cancer	100% fruit juice	RR: 1.21 (1.00 to 1.47)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Endometrial cancer	100% fruit juice	RR: 1.05 (1.00 to 1.10)	Not provided	NA	Very low (GRADE)	NOS	No reported conflicts
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Pancreatic cancer	100% fruit juice	RR: 0.91 (0.61 to 1.35)	Not provided	Linear, no departure from linearity	Very low (GRADE)	NOS	No reported conflicts

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	June 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohorts	Included: prospective cohort studies (including abstracts with multivariate results), adults aged ≥18 years, reported adjusted RR, HR, or OR for SSBs, NSSBs, or 100% fruit juice, outcomes: overall or site-specific cancer risk, only one version per cohort (most recent or informative). Studies with cancer patients at baseline, cross-sectional and case-control studies, studies with >20% chronically ill participants at baseline were excluded.	4,518,547 adults	Prostate cancer	100% fruit juice	RR: 1.13 (0.93 to 1.39)	Not provided	Non-linear	Very low (GRADE)	NOS	No reported conflicts
Poorolajal et al (2020)	November 2018	Web of Science, PubMed, and Scopus	199 (observational)	Observational studies addressing the associations between overweight/obesity in children/adolescents aged between 5 to 19 years and associated risk factors were analyzed.	1,636,049	Obesity	SSB	OR: 1.24 (1.07, 1.43)	I^2 = 78% (SSB)	NA	None	NOS	The Vice-Chancellor of Research and Technology, Hamadan University of Medical Sciences funded this study (No. 9610266919). No COI reported
Qin et al (2020)	September 2019	PubMed,EMBASE, Web of Science, and Open Grey	39 obs total	prospective cohort studies investigating the associations of SSB and NSSB and obesity, T2D, HTN, and all-cause mortality in adults	56,579 participants	Obesity	SSB	1.20 (95% CI 1.10–1.31)	1.4%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by National Science Foundation of China
	September 2019	PubMed,EMBASE, Web of Science, and Open Grey	39 obs total	prospective cohort studies investigating the associations of SSB and NSSB and obesity, T2D, HTN, and all-cause mortality in adults	1,010,392 participants	T2D	SSB	1.27 (95% CI 1.18–1.36)	80.1%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by National Science Foundation of China
	September 2019	PubMed,EMBASE, Web of Science, and Open Grey	39 obs total	prospective cohort studies investigating the associations of SSB and NSSB and obesity, T2D, HTN, and all-cause mortality in adults	312,156 participants	Hypertension	SSB	1.13 (95% CI 1.10–1.16)	39.8%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by National Science Foundation of China
	September 2019	PubMed,EMBASE, Web of Science, and Open Grey	39 obs total	prospective cohort studies investigating the associations of SSB and NSSB and obesity, T2D, HTN, and all-cause mortality in adults	1,125,834 participants	All cause mortality	SSB	1.10 (95% CI 1.02–1.17)	80.2%	No evidence for a nonlinear relation	None	NOS	No conflicts reported. Funded by National Science Foundation of China

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Rousham et al (2022)	December 23, 2020	PubMed (MEDLINE), Cochrane CENTRAL, and Embase	60 studies from 71 articles were included. Most studies were observational (59/60 and one RCT), and no included studies were from low-income countries	Quantitative human studies of children where age at intervention or exposure was between birth and ≤10.9 y, published from January 1971 with no restriction on publication language, studies reporting (greater) consumption of unhealthy foods and beverages compared with no or low consumption, studies reporting on growth and body composition	72 to 16,058 (children)	BMI	SSB	β = 0.01; 95% CI: −0.00, 0.02	73.66%	NA	Low (GRADE)	ROBINS-I and RoB2 tools	No conflicts reported. Funding support was received from the Food and Nutrition Action in Health Systems unit, Department of Nutrition and Food Safety
	December 23, 2020	PubMed (MEDLINE), Cochrane CENTRAL, and Embase	60 studies from 71 articles were included. Most studies were observational (59/60 and one RCT), and no included studies were from low-income countries	Quantitative human studies of children where age at intervention or exposure was between birth and ≤10.9 y, published from January 1971 with no restriction on publication language, studies reporting (greater) consumption of unhealthy foods and beverages compared with no or low consumption, studies reporting on growth and body composition	72 to 16,058 (children)	BMI zscore	SSB	β = 0.10; 95% CI: −0.11, 0.31	0.0%	NA	Low (GRADE)	ROBINS-I and RoB2 tools	No conflicts reported. Funding support was received from the Food and Nutrition Action in Health Systems unit, Department of Nutrition and Food Safety
	December 23, 2020	PubMed (MEDLINE), Cochrane CENTRAL, and Embase	60 studies from 71 articles were included. Most studies were observational (59/60 and one RCT), and no included studies were from low-income countries	Quantitative human studies of children where age at intervention or exposure was between birth and ≤10.9 y, published from January 1971 with no restriction on publication language, studies reporting (greater) consumption of unhealthy foods and beverages compared with no or low consumption, studies reporting on growth and body composition	72 to 16,058 (children)	Body fat	SSB	β = 1.86; 95% CI: 0.38, 3.34	22.8%	NA	Low (GRADE)	ROBINS-I and RoB2 tools	No conflicts reported. Funding support was received from the Food and Nutrition Action in Health Systems unit, Department of Nutrition and Food Safety

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	December 23, 2020	PubMed (MEDLINE), Cochrane CENTRAL, and Embase	60 studies from 71 articles were included. Most studies were observational (59/60 and one RCT), and no included studies were from low-income countries	Quantitative human studies of children where age at intervention or exposure was between birth and ≤10.9 y, published from January 1971 with no restriction on publication language, studies reporting (greater) consumption of unhealthy foods and beverages compared with no or low consumption, studies reporting on growth and body composition	72 to 16,058 (children)	BMI z score	100% fruit juice	β=0.01; 95% CI: 0.00, 0.01	0.0%	NA	Low (GRADE)	ROBINS-I and RoB2 tools	No conflicts reported. Funding support was received from the Food and Nutrition Action in Health Systems unit, Department of Nutrition and Food Safety
Ruanpeng et al (2017)	May 2015	MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, and Cochrane Central Register of Controlled Trials	11 obs	The inclusion criteria were (i) randomized controlled trials (RCTs) or observational studies (case-control, cohort studies or cross-sectional) published as original studies to appraise the risk of obesity in patients consuming either sugar or artificially sweetened soda, (ii) odds ratios, relative risks, hazard ratios or standardized incidence ratio with 95% CIs were presented and (iii) a reference group composed of participants who did not consume soda. No limits were implemented to language.	11 studies for the association between obesity and sugar-sweetened soda (Europe, USA, Iran, Australia)	Obesity	SSB	RR: 1.18 (95% CI, 1.10–1.27)	40%	NA	None	NOS	No conflicts reported
Santos et al (2022)	December 2021	Pubmed, Lilacs, Web of Science, Cochrane, Embase, and Scopus databases	27 longitudinal studies	The review included longitudinal (cohort) studies involving adults aged 20 and over from the general population (excluding those with pre-existing conditions or institutionalized), examining the link between SSB intake and at least one of the following outcomes: type 2 diabetes, obesity, coronary heart disease (CHD), or stroke, regardless of follow-up length. Studies were excluded if they were reviews, cross-sectional, intervention-based, animal or in vitro studies, or if they analyzed overall dietary patterns without isolating results for SSBs. No restrictions were placed on language or publication date.	1,500,000 participants	T2D	SSB	RR = 1.20; 95% C.I. 1.13-1.28	70%; p-value < 0.01	NA	None	NOS (Cf Table 1)	No conflicts reported. This study was supported by Brazilian National IResearch Council (grant number 442801/2019-0)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	December 2021	Pubmed, Lilacs, Web of Science, Cochrane, Embase, and Scopus databases	27 longitudinal studies	The review included longitudinal (cohort) studies involving adults aged 20 and over from the general population (excluding those with pre-existing conditions or institutionalized), examining the link between SSB intake and at least one of the following outcomes: type 2 diabetes, obesity, coronary heart disease (CHD), or stroke, regardless of follow-up length. Studies were excluded if they were reviews, cross-sectional, intervention-based, animal or in vitro studies, or if they analyzed overall dietary patterns without isolating results for SSBs. No restrictions were placed on language or publication date.	1,500,000 participants	Obesity	SSB	RR = 1.17; 95% C.I. 1.10-1.25	36%; p-value = 0.20	NA	None	NOS (Cf Table 1)	No conflicts reported. This study was supported by Brazilian National IResearch Council (grant number 442801/2019-0)
	December 2021	Pubmed, Lilacs, Web of Science, Cochrane, Embase, and Scopus databases	27 longitudinal studies	The review included longitudinal (cohort) studies involving adults aged 20 and over from the general population (excluding those with pre-existing conditions or institutionalized), examining the link between SSB intake and at least one of the following outcomes: type 2 diabetes, obesity, coronary heart disease (CHD), or stroke, regardless of follow-up length. Studies were excluded if they were reviews, cross-sectional, intervention-based, animal or in vitro studies, or if they analyzed overall dietary patterns without isolating results for SSBs. No restrictions were placed on language or publication date.	1,500,000 participants	CHD	SSB	RR = 1.15; 9% C.I. 1.06-1.25	66%; p-value = 0.06	NA	None	NOS (Cf Table 1)	No conflicts reported. This study was supported by Brazilian National IResearch Council (grant number 442801/2019-0)
	December 2021	Pubmed, Lilacs, Web of Science, Cochrane, Embase, and Scopus databases	27 longitudinal studies	The review included longitudinal (cohort) studies involving adults aged 20 and over from the general population (excluding those with pre-existing conditions or institutionalized), examining the link between SSB intake and at least one of the following outcomes: type 2 diabetes, obesity, coronary heart disease (CHD), or stroke, regardless of follow-up length. Studies were excluded if they were reviews, cross-sectional, intervention-based, animal or in vitro studies, or if they analyzed overall dietary patterns without isolating results for SSBs. No	1,500,000 participants	Stroke	SSB	RR = 1.10; 9% C.I. 1.01-1.19	43%; p-value = 0.14	NA	None	NOS (Cf Table 1)	No conflicts reported. This study was supported by Brazilian National IResearch Council (grant number 442801/2019-0)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
				restrictions were placed on language or publication date.									
Schlesinger et al (2019)	August 2018	PubMed and Web of Science	9 prospective studies used for SSB analysis	prospective study with information on SSB and outcome based on weight gain or waist circumference	Adults (18+)	Obesity	SSB	RR: 1.20 (95% CI: 1.01, 1.43)	23%	Linear	Very low (NutriGRADE)	NutriGrade	No conflicts reported
	August 2018	PubMed and Web of Science	9 prospective studies used for SSB analysis	prospective study with information on SSB and outcome based on weight gain or waist circumference	Adults (18+)	Abdominal obesity	SSB	RR: 1.34 (95% CI: 1.13, 1.59)	90%	Non-linear	Very low (NutriGRADE)	NutriGrade	No conflicts reported
	August 2018	PubMed and Web of Science	9 prospective studies used for SSB analysis	prospective study with information on SSB and outcome based on weight gain or waist circumference	Adults (18+)	Weight gain	SSB	RR: 1.23 (95% CI: 1.11, 1.37)	0%	No dose response	Very low (NutriGRADE)	NutriGrade	No conflicts reported
Schwingshackl et al (2017)	February 2017	PubMed, Embase, Medline (Ovid), Cochrane Central, and Google Scholar	88 including 10 on SSBs (mainly based cohorts)	Studies were included in the meta-analysis if they met all of the following criteria: (1) prospective design studies (cohort studies, nested case–control studies, case-cohort studies, follow-up of RCTs) that were peer-reviewed and available in full-text; (2) information about the association for ≥1 of the following twelve food groups: whole grains/cereals, refined grains/cereals, vegetables, fruits, nuts, legumes, eggs, dairy products, fish, red meat, processed meat, and SSB on risk of T2D; (3) Participants ≥18 years; and (4) considering T2D as outcome (study population had to be free of T2D at the onset of the study).	25,600 participants	T2D	SSB	RR: 1.30; 95% CI 1.20–1.40	34%	Non-linear	High (NutriGRADE)	NOS	No conflicts reported. Funded by NHS BRC grant (Interventional Public Health)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Schwingshackl et al (2017)	June 2017	PubMed, Scopus, and Web of Science	28 reports were included in the meta-analysis	This review included cohort, case-cohort, nested case-control studies, and RCT follow-ups that assessed the association between intake of at least one of 12 food groups; including sugar-sweetened beverages (SSBs); and the risk of developing hypertension in adults (≥18 years), defined by new onset of elevated blood pressure or initiation of antihypertensive medication during follow-up.	Five studies including 81,495 incident hypertension cases were included in the meta-analysis comparing extreme intake categories (range of intake: 0−457 mL/d)	Hypertension	SSB	1.12; 95% CI: 1.06, 1.18	59%; P = 0.04	No evidence for a nonlinear relation	Low (NutriGRADE)	NutriGrade	No conflicts reported
Schwingshackl et al (2018)	December 2016	PubMed, Embase, and Google Scholar	103 prospective studies including 5 on SSBs	Included peer-reviewed cohort-type studies with full text, involving adults (≥18 y), reporting on at least one of 12 key food groups. Focused on all-cause mortality; excluded studies on chronically ill populations or those reporting only cause-specific mortality.	81,407 mortality cases (in adults)	All cause mortality	SSB	RR: 1.02; 95% CI: 0.97, 1.06	78%, p< 0.01	No evidence for non-linear	Very low (NutriGRADE)	NutriGrade	No conflicts reported. Funded by National Health Service Biomedical Research Centre
Schwingshackl et al (2018)	April 2017	PubMed and Embase	83 obs (n=3 for SSB)	prospective studies investigating the association between these 12 food groups and risk of CRC	2,464 CRC	Colorectal cancer	SSB	RR: 1.09; 95% CI 0.97, 1.22	46%	NA	Low (GRADE)	NutriGrade	None reported
Sun et al (2023)	February 10, 2022	PubMed, Embase, and the Cochrane Library	64 (16 cohorts for SSBs)	prospective cohort studies analyzing the association between at least 1 dietary source of fructose and CVD, CHD, and stroke	118,586 participants	CVD	SSB	RR: 1.20 (95% CI: 1.07, 1.34)	0%, P = 0.73	Linear, no departure from linearity	Low (GRADE)	NOS	No conflicts reported. Funded by National Natural Science Foundation of China (82170360 and 82200912) and the Natural Science Foundation of Sichuan Province (2022NSFSC0673)
	February 10, 2022	PubMed, Embase, and the Cochrane Library	64 (16 cohorts for SSBs)	prospective cohort studies analyzing the association between at least 1 dietary source of fructose and CVD, CHD, and stroke	118,586 participants	CHD	SSB	RR: 1.21 (1.05, 1.39)	50.1%, P = 0.11	Linear, no departure from linearity	Low (GRADE)	NOS	No conflicts reported. Funded by National Natural Science Foundation of China (82170360 and 82200912) and the Natural Science Foundation of Sichuan Province (2022NSFSC0673)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	February 10, 2022	PubMed, Embase, and the Cochrane Library	64 (16 cohorts for SSBs)	prospective cohort studies analyzing the association between at least 1 dietary source of fructose and CVD, CHD, and stroke	118,586 participants	Stroke	SSB	RR: 1.14 (1.04, 1.24)	27.2%, P=0.194	Linear, no departure from linearity	Moderate (GRADE)	NOS	No conflicts reported. Funded by National Natural Science Foundation of China (82170360 and 82200912) and the Natural Science Foundation of Sichuan Province (2022NSFSC0673)
	February 10, 2022	PubMed, Embase, and the Cochrane Library	64 (16 cohorts for SSBs)	prospective cohort studies analyzing the association between at least 1 dietary source of fructose and CVD, CHD, and stroke	118,586 participants	CVD mortality	SSB	RR: 1.16 (1.06, 1.27)	43.4%, P=0.089	Linear, no departure from linearity	Moderate (GRADE)	NOS	No conflicts reported. Funded by National Natural Science Foundation of China (82170360 and 82200912) and the Natural Science Foundation of Sichuan Province (2022NSFSC0673)
Sun et al (2023)	January 2021	PubMed, EMBase, Cochrane, Science Direct, Web of Science	10 studies (7 cohort and 3 cross-sectional)	Literature inclusion criteria: (1) Study population: adults aged 30 years or older. (2) Study type: all study types. (3) Interference measures or exposure factors of the study: SSBs. (4) Outcome of the study: Cognitive functional status, mild cognitive impairment, prevalence of dementia and Alzheimer's disease. Literature exclusion criteria: (1) The language of the literature is not English or Chinese. (2) Non-clinical studies. (3) Literature for which data extraction was not possible. (4) Study subjects with serious health problems and severe psychiatric system disorders. (5) The study design was problematic and incomprehensive.	333 - 16,948 sample size	Cognitive function	SSB	1.59, 95% CI: 0.93–2.74	68%	NA	None	JBI and NOS	No conflicts reported

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	January 2021	PubMed, EMBase, Cochrane, Science Direct, Web of Science	10 studies (7 cohort and 3 cross-sectional)	Literature inclusion criteria: (1) Study population: adults aged 30 years or older. (2) Study type: all study types. (3) Interference measures or exposure factors of the study: SSBs. (4) Outcome of the study: Cognitive functional status, mild cognitive impairment, prevalence of dementia and Alzheimer's disease. Literature exclusion criteria: (1) The language of the literature is not English or Chinese. (2) Non-clinical studies. (3) Literature for which data extraction was not possible. (4) Study subjects with serious health problems and severe psychiatric system disorders. (5) The study design was problematic and incomprehensive.	333 - 16,948 sample size	Dementia	SSB	HR=2.77, 95% CI: 2.24–3.43	0%	NA	None	JBI and NOS	No conflicts reported
	January 2021	PubMed, EMBase, Cochrane, Science Direct, Web of Science	10 studies (7 cohort and 3 cross-sectional)	Literature inclusion criteria: (1) Study population: adults aged 30 years or older. (2) Study type: all study types. (3) Interference measures or exposure factors of the study: SSBs. (4) Outcome of the study: Cognitive functional status, mild cognitive impairment, prevalence of dementia and Alzheimer's disease. Literature exclusion criteria: (1) The language of the literature is not English or Chinese. (2) Non-clinical studies. (3) Literature for which data extraction was not possible. (4) Study subjects with serious health problems and severe psychiatric system disorders. (5) The study design was problematic and incomprehensive.	333 - 16,948 sample size	Alzheimer	SSB	HR=2.63, 95% CI: 1.70–4.05	0%	NA	None	JBI and NOS	No conflicts reported
Taneri et al (2022)	January 29, 2021	MEDLINE (via Ovid), Embase, Web of Science Core Collection, Cochrane Library, and Google Scholar	40 cohort studies(n=12 SSBs)	adult, cohort studies, evaluated risk of all-cause mortality	1,351,875 participants for SSB specific analysis (n=12 studies); adults	All cause mortality	SSB	RR = 1.11, 95% CI, 1.04, 1.18	82%	NA	None	NOS	None reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
Valenzuela et al (2021)	January 24, 2019	Medline, Embase, Cochrane Library, SciELO, LILACS, OpenGrey and HMIC	38 cross-sectional studies	participants from general populations; consumption of any type of SSB; high SSB consumers compared with a lower consumption group including non-consumers; dental caries [measured by the decayed, missing and filled teeth or surfaces indices for primary or adult teeth (DMFT/dmft or DMFS/dmfs), or by the early childhood caries index], and/or dental erosion (no restriction in measurement as no general consensus on a standard index has been reached by dental academics) measured at two or more SSB consumption levels	13,920 participants	Dental caries	SSB	OR: 1.57 (1.28–1.92)	73.7%	Non-linear	High (GRADE)	None (publication bias analysis not considered a RoB)	None reported. Funded by CONICYT Becas-Chile Doctoral Scholarship [Folio Number 72180286]
	January 24, 2019	Medline, Embase, Cochrane Library, SciELO, LILACS, OpenGrey and HMIC	38 cross-sectional studies	participants from general populations; consumption of any type of SSB; high SSB consumers compared with a lower consumption group including non-consumers; dental caries [measured by the decayed, missing and filled teeth or surfaces indices for primary or adult teeth (DMFT/dmft or DMFS/dmfs), or by the early childhood caries index], and/or dental erosion (no restriction in measurement as no general consensus on a standard index has been reached by dental academics) measured at two or more SSB consumption levels	9,111 participants	Erosion (teeth)	SSB	OR: 1.43 (1.01–2.03)	87.9%	NA	Moderate (GRADE)	None (publication bias analysis not considered a RoB)	None reported. Funded by CONICYT Becas-Chile Doctoral Scholarship [Folio Number 72180286]
Wang et al (2022)	November 10, 2021	PubMed, Cochrane library, Embase, Web of Science	32 obs	(1) prospective cohort studies; (2) the exposure was SSB intake, and the outcomes were incidents of stroke, depression, cancer, or mortality; (3) the participants were healthy adults at enrollment and aged ≥ 18 years; (4) for dose-response analysis, the levels of SSB consumption should be ranked at least three categories	3,505,329 (13,485 stroke events)	Stroke	SSB	RR 1.12, 95% CI 1.03-1.23	29.9%	Linear	None	NOS	No conflicts reported. Funded by Natural Science Foundation of Jiangsu Province (BK20201435)
	November 10, 2021	PubMed, Cochrane library, Embase, Web of Science	33 obs	(1) prospective cohort studies; (2) the exposure was SSB intake, and the outcomes were incidents of stroke, depression, cancer, or mortality; (3) the participants were healthy adults at enrollment and aged ≥ 18 years; (4) for dose-response analysis, the levels of SSB consumption should be ranked at least three categories	3,505,329 participants (3,694 depression	Depression	SSB	RR: 1.25, 1.11-1.41	0%	Non-linear	None	NOS	No conflicts reported. Funded by Natural Science Foundation of Jiangsu Province (BK20201435)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	November 10, 2021	PubMed, Cochrane library, Embase, Web of Science	32 obs	(1) prospective cohort studies; (2) the exposure was SSB intake, and the outcomes were incidents of stroke, depression, cancer, or mortality; (3) the participants were healthy adults at enrollment and aged ≥ 18 years; (4) for dose-response analysis, the levels of SSB consumption should be ranked at least three categories	3,505,329 participants (14,166 cancer)	Cancer	SSB	RR: 1.10, 1.03-1.17	0%	Non-linear	None	NOS	No conflicts reported. Funded by Natural Science Foundation of Jiangsu Province (BK20201435)
	November 10, 2021	PubMed, Cochrane library, Embase, Web of Science	35 obs	(1) prospective cohort studies; (2) the exposure was SSB intake, and the outcomes were incidents of stroke, depression, cancer, or mortality; (3) the participants were healthy adults at enrollment and aged ≥ 18 years; (4) for dose-response analysis, the levels of SSB consumption should be ranked at least three categories	3,505,329 participants (99,126 death)	All cause mortality	SSB	RR: 1.08, 1.05-1.11	68.2%	Linear	None	NOS	No conflicts reported. Funded by Natural Science Foundation of Jiangsu Province (BK20201435)
Xi et al (2015)	May 2014	PubMed and EmBase	6 cohort studies for HT; 4 for CHD; 4 for stroke	Cohort studies on SSB with incident cases of HT, CVD or stroke	240,726	Hypertension	SSB	RR 1·10, 95 % CI 1·06, 1·15	46.7%	Linear	None	None	No conflicts reported
	May 2014	PubMed and EmBase	6 cohort studies for HT; 4 for CHD; 4 for stroke	Cohort studies on SSB with incident cases of HT, CVD or stroke	194,664	CHD	SSB	RR 1·16, 95 % CI 1·06, 1·27	0%	Linear	None	None	No conflicts reported
	May 2014	PubMed and EmBase	6 cohort studies for HT; 4 for CHD; 4 for stroke	Cohort studies on SSB with incident cases of HT, CVD or stroke	259,176	Stroke	SSB	RR 1·10, 95 % CI 1·00, 1·20	43%	No dose response	None	None	No conflicts reported
Yang et al (2022)	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	CVD	SSB	1.14 (1.00–1.31)	71.3%	NA	None	NOS	No conflicts reported
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	CHD	SSB	1.17 (1.07–1.28)	38.2%	NA	None	NOS	No conflicts reported
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	Stroke	SSB	1.17 (1.07–1.28)	43.6%	NA	None	NOS	No conflicts reported
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	CVD mortality	SSB	1.21 (1.07–1.36)	62.4%	NA	None	NOS	No conflicts reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	CVD	Added sugars	RR: 1.08 (0.86–1.36)	71.3%	NA	None	NOS	No conflicts reported
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	CHD	Added sugars	RR: 1.22 (1.04–1.42)	38.2%	NA	None	NOS	No conflicts reported
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	Stroke	Added sugars	RR: 1.10 (0.92, 1.33)	43.6%	NA	None	NOS	No conflicts reported
	April 2022	EmBase, Ovid, Medline	21 cohort studies	Human cohort studies with measures of added sugars/SSB and cardiovascular outcomes; excluded interventions and non-English	Not specified	CVD mortality	Added sugars	RR: 1.12 (0.96–1.32)	62.4%	NA	None	NOS	No conflicts reported
Yin et al (2021)	December 1, 2019	PubMed and Embase	11 (SSB)	1) the authors reported data from an original, peer-reviewed study (not reviews, conferences, and letters); 2) the study had a prospective design; 3) the authors reported RRs, HRs, or ORs with 95% CIs for ≥3 quantitative categories of SSB or LCSB consumption; 4) the investigators reported ≥1 of the outcomes of CVD risk, including incidence of total CVD, coronary heart disease, stroke, or CVD mortality	SBs comprised 16,937,316 person-years of follow-up, 16,915 incident CVD cases (7396 coronary heart disease cases, 6598 stroke cases), and 18,042 CVD deaths. Most were conducted in the US (followed by Europe and Asia)	CVD	SSB	RR: 1.09 (1.01 to 1.18)	28.8%	Linear, no departure from linearity	None	NOS	Conflicts reported. Funded by National Key Research and Development Program of China (2017YFC1600500), and the Major International (Regional) Joint Research Project (NSFC 81820108027)

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
	December 1, 2019	PubMed and Embase	11 (SSB)	1) the authors reported data from an original, peer-reviewed study (not reviews, conferences, and letters); 2) the study had a prospective design; 3) the authors reported RRs, HRs, or ORs with 95% CIs for ≥3 quantitative categories of SSB or LCSB consumption; 4) the investigators reported ≥1 of the outcomes of CVD risk, including incidence of total CVD, coronary heart disease, stroke, or CVD mortality	SBs comprised 16,937,316 person-years of follow-up, 16,915 incident CVD cases (7396 coronary heart disease cases, 6598 stroke cases), and 18,042 CVD deaths. Most were conducted in the US (followed by Europe and Asia)	CVD mortality	SSB	RR: 1.20 (1.10 to 1.31)	11.7%	Linear, no departure from linearity	None	NOS	Conflicts reported. Funded by National Key Research and Development Program of China (2017YFC1600500), and the Major International (Regional) Joint Research Project (NSFC 81820108027)
Zhang et al (2021)	March 2020	PubMed, EmBase, Web of Science, Cochrane, ProQuest, ClinicalTrials.Gov	10 studies for all-cause mortality; 10 for CVD mortality and 4 for cancer mortality; follow-up from 5.9 to 31 years	Prospective study, peer-reviewed	965,851 (114,935 deaths)	All cause mortality	SSB	HR: 1.08; 95% CI: 1.04, 1.12	70.5%	Linear	High (NutriGRADE)	NOS	None reported
	March 2020	PubMed, EmBase, Web of Science, Cochrane, ProQuest, ClinicalTrials.Gov	10 studies for all-cause mortality; 10 for CVD mortality and 4 for cancer mortality; follow-up from 5.9 to 31 years	Prospective study, peer-reviewed	898,005 with 24,365 deaths	CVD mortality	SSB	HR: 1.08; 95% CI: 1.04, 1.12	16.4%	Linear	High (NutriGRADE)	NOS	None reported
	March 2020	PubMed, EmBase, Web of Science, Cochrane, ProQuest, ClinicalTrials.Gov	10 studies for all-cause mortality; 10 for CVD mortality and 4 for cancer mortality; follow-up	Prospective study, peer-reviewed	For all-cause mortality, sample was 965,851 with 114,935 deaths; for CVD	Cancer mortality	SSB	HR: 1.02; 95% CI: 0.96, 1.09)	69.9%	No dose response	Low (GRADE)	NOS	None reported

Authors	Date of Last Search	Databases	No. RCTs (and/or Total studies)	Eligibility criteria	Population	Outcome type	Exposure	Pooled effect(s) & model	Heterog. (I^2)	NON-linear dose-dependent analysis	GRADE	RoB (Risk of Bias) method	Funding/COI
			from 5.9 to 31 years		mortality was 898,005 with 24,365 deaths; Studies from USA, Europe and Asia; male and female adults; middle-age or elderly; mostly all healthy at baseline								
Zhao et al (2024)	February 2021	PubMed, EmBase and Web of Science	23 studies for HT and 12 for BP	Population based cohorts, definition of HT based on BP, taking meds or doctor diagnosed	619,745 for HT	Hypertension	SSB	RR 1.27 [95% CI, 1.17–1.38]	89.10%	Non-linear	Very Low (AMSTAR 2 tool)	NOS	No conflicts reported

AMSTAR: A Measurement Tool to Assess Systematic Reviews; BMI: body mass index; BP: blood pressure; CI: confidence interval; CHD: Coronary Heart Disease ; CRB: Cochrane Risk Bias; CRC: colorectal cancer; CVD: cardiovascular diseases; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; HR: hazard ratio; HT: hypertension; MD: mean difference; NAFLD: Non-Alcoholic Fatty Liver Disease; NOS: Newcastle Ottawa Scale; OR: odds ratio; PM: pre menopause; PostM: post menopause; RCTs: randomized controlled trials; RR: relative risks; SSBs: sugar-sweetened beverages; T2D: type 2 diabetes.

Appendix 4. NSSBs: Evidence Table of Included Meta-Analyses (articles highlighted in blue are those that examined multiple exposure/outcome pairs). *Please refer to the footnotes for the meanings of the acronyms*.

Citation	*Last search date*	*Databases search*	*No. RCTs (and/or Total studies)*	*Eligibility criteria*	*Intervention/ Exposure*	*Outcomes*	*RR and CI*	*Heterog. (I^2)*	*Dose response*	*Certainty (GRADE?)*	*RoB (Risk of Bias) method*
Bhagavathula et al (2022)	July 31, 2021	PubMed/MEDLINE, Web of Science, and Embase	8 cohort studies	(1) population-based prospective cohorts; (2) conducted among adult (≥18 years) population; (3) investigated the SBs consumption, such as sugar-sweetened beverages (SSB) and artificial-sweetened beverages (ASB), by Food Frequency Questionnaire (FFQs) or The Diet History Questionnaire (DHQs); (4) indicated a definite outcome for cardiovascular mortality; (5) evaluated the association between SB consumption and risk of cardiovascular mortality by the effect sizes of odds ratios (ORs), relative risks or risk ratios (RRs), or hazard ratios (HRs) with 95% confidence intervals (CI); (6) published in English. Daily consumption of at least one glass or serving (250 ml) of SB was considered the lowest-threshold and two or more glasses (serving) as the highest-threshold.	ASB	CVD mortality	RR: 1.02 (0.96, 01.08)	I^2 = 0.0%	Not Tested	Not Stated	Newcastle-Ottawa Scale
Chen et al (2024)	August 2023	Medline, Embase, Web of Science, and Cochrane CENTRAL databases	11 prospective cohort studies	A study was included for the analysis if it (1) was a prospective cohort study; 2) had assessment of the association between ASB consumption and mortality among generally healthy adults; and 3) provided risk estimates for three or more levels of ASB consumption	ASB	All cause mortality	RR: 1.13 (01.06, 1.21)	I^2 = 66.3%	Non linear	Moderate	Not Specified

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				with mortality or a dose-response estimate.							
		Medline, Embase, Web of Science, and Cochrane CENTRAL databases	11 prospective cohort studies	A study was included for the analysis if it (1) was a prospective cohort study; 2) had assessment of the association between ASB consumption and mortality among generally healthy adults; and 3) provided risk estimates for three or more levels of ASB consumption with mortality or a dose-response estimate.	ASB	CVD mortality	RR: 1.26 (1.10, 1.44)	I^2 = 52.0%	Linear (no evidence of non linearity)	Moderate	Not Specified
		Medline, Embase, Web of Science, and Cochrane CENTRAL databases	11 prospective cohort studies	A study was included for the analysis if it (1) was a prospective cohort study; 2) had assessment of the association between ASB consumption and mortality among generally healthy adults; and 3) provided risk estimates for three or more levels of ASB consumption with mortality or a dose-response estimate.	ASB	Cancer mortality	RR: 0.99 (0.96, 01.03)	I^2 = 21.7%	No evidence of non linearity, no evidence of linearity	Low	Not Specified
Espinosa et al (2024)	April 2024	PubMed, EMBASE, the Cochrane library, LILACS, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform databases	4 RCTs and 8 prospective cohort studies	A search was conducted for RCTs (≥4 weeks duration) comparing non-nutritive sweeteners (NNS) with control groups (water/placebo or caloric comparators like sugar, fructose, milk, and tea). Prospective cohort studies examining the association between NNS intake and BMI changes were also included. Studies focused on children (2–9 years), adolescents (10–19 years), and young adults (20–24 years), with an expanded age range (10–24 years) to capture broader developmental stages. Sensitivity analyses will	NNS beverages	BMI	MD = −0.114 kg/m2, CI (−0.207, −0.021)	I^2 = 87.02%	Not Stated	Very Low	ROBINS

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				exclude 20–24 years to evaluate results for the 10–19 years group. Cross-sectional, ecologic studies, and non-research articles (reviews, abstracts, commentaries) were excluded due to confounding and reverse causation risks.							
		PubMed, EMBASE, the Cochrane library, LILACS, ClinicalTrials.gov, and the WHO International Clinical Trials Registry Platform databases	4 RCTs and 8 prospective cohort studies	RCTs (≥4 weeks duration) comparing non-nutritive sweeteners (NNS) with control groups (water/placebo or caloric comparators like sugar, fructose, milk, and tea). Prospective cohort studies examining the association between NNS intake and BMI changes were also included. Studies focused on children (2–9 years), adolescents (10–19 years), and young adults (20–24 years), with an expanded age range (10–24 years) to capture broader developmental stages. Sensitivity analyses will exclude 20–24 years to evaluate results for the 10–19 years group. Cross-sectional, ecologic studies, and non-research articles (reviews, abstracts, commentaries) were excluded due to confounding and reverse causation risks.	NNS beverages	BMI	MD= 0.05 kg/m2, CI (−0.03, 0.13)	I^2 = 75.06%	Not Stated	Moderate	Newcastle-Ottawa Scale
Imamura et al (2016)	February 2014	PubMed, Embase, Ovid, and Web of Knowledge	10 studies	Prospective design, assessed the consumption of beverages and incident type 2 diabetes, and recruited adults free of diabetes and aged 18 years or older	ASB	Type 2 diabetes	higher consumption of artificially sweetened beverages by one serving per day was associated with a 25% greater	I^2 = 70%	No evidence of non linearity	Low	CRB

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
							incidence of type 2 diabetes (95% confidence interval 18% to 33%; I2=70%) before adjustment for adiposity. After adjustment, the estimate of 25% greater incidence was attenuated to 8% (2.1% to 15%).				
Jouni et al (2025)	September 2024	PubMed, Scopus, and Web of Science	7 studies	1) observational research using nested case-control, or prospective cohort; 2) carried out on healthy adults (≥18 years); 3) declared the use of sugar and artificially sweetened beverages; 4) reported the estimated risk of AD as an outcome variable as a measure of the result; and 5) recorded ORs, RRs, or HRs in addition to 95% CIs.	ASB	AD	RR: 1.42 (1.14, 1.78)	I^2 = 0.0%	Linear (no evidence of non linearity)	Moderate	ROBINS
Kim and Je (2016)	May 2015	PubMed, Embase and Web of Science	4 cohort studies	Adults (18+), human studies published in English, prospective cohort design; the exposure of interest was the consumption of SSBs or ASBs; the outcome of interest was defined as incident hypertension or high blood pressure	ASB	Hypertension	RR: 1.09 (95% CI: 1.06, 1.11)	Not Stated	Unclear, results for non-linearity dose-response analysis not given	Not Stated	Newcastle-Ottawa Scale
Li et al (2022)	January 1, 2020	PubMed, Embase, Cochrane Library and Web of Science	5 cohort studies	Adults, prospective cohort studies, SSB or ASB consumption, more than one reference group, and all-cause mortality or CVD mortality as outcomes	ASB	All-cause mortality	HR: 1.12 (1.04–1.21)	I^2 = 79.3%	Linear	Not Stated	Newcastle-Ottawa Scale
		PubMed, Embase, Cochrane Library and Web of Science	3 cohort studies	Adults, prospective cohort studies, SSB or ASB consumption, more than one reference group, and all-cause mortality or CVD mortality as outcomes	ASB	Cancer (mortality)	HR: 1.04, (0.97–1.12)	Not Stated	No evidence of non linearity	Not Stated	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
		PubMed, Embase, Cochrane Library and Web of Science	3 cohort studies	Adults, prospective cohort studies, SSB or ASB consumption, more than one reference group, and all-cause mortality or CVD mortality as outcomes	ASB	CVD (mortality)	HR: 1.23 (1.00–1.50)	I^2 = 82.5%	Linear	Not Stated	Newcastle-Ottawa Scale
Meng et al (2021)	June 20, 2020	PubMed, Embase, and Ovid	8 prospective studies on all-cause mortality	Adults (18+), prospective design, measured SSB or ASB as exposures, a healthy study population at baseline, published in English	ASB	All-cause mortality	RR: 1.15, (1.06–1.24)	I^2 = 78.9%	Non-linear	Not Stated	Newcastle-Ottawa Scale
		PubMed, Embase, and Ovid	10 prospective studies on CVDs	Adults (18+), prospective design, measured SSB or ASB as exposures, a healthy study population at baseline, published in English	ASB	CVD	RR: 1.17, (1.06–1.29)	I^2 = 57.4%	Non-linear	Not Stated	Newcastle-Ottawa Scale
		PubMed, Embase, and Ovid	17 prospective studies on T2D	Adults (18+), prospective design, measured SSB or ASB as exposures, a healthy study population at baseline, published in English	ASB	Type 2 diabetes	RR: 1.18, (1.08–1.29)	I^2 = 53.5%	Non-linear	Not Stated	Newcastle-Ottawa Scale
Narain et al (2016)	July 2015	Medline and EMBASE	7 cohort studies	Prospective cohorts, no language restricton, ages 34-75	ASB	All-cause mortality	**Incremental increase**: The pooled results suggest a one-serving per day increase in ASB consumption was associated with a greater risk of stroke (RR 1.08, 95% CI 1.03–1.14), but not of MI or vascular events **High vs low**: The pooled results of two studies suggest ASB consumption is associated with a greater risk of stroke (RR 1.14, 95% CI 1.04–1.26) and the results from	I^2 = 73%	Non linearity not properly tested	Not Stated	Not Specified

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
							one study suggest a greater risk of vascular events (RR 1.44, 95% CI 1.02–2.03). No significant difference was observed for MI or mortality				
		Medline and EMBASE	7 cohort studies	Prospective cohorts, no language restricton, ages 34-76	ASB	Myocardial infarction	**Incremental increase**: The pooled results suggest a one-serving per day increase in ASB consumption was associated with a greater risk of stroke (RR 1.08, 95% CI 1.03–1.14), but not of MI or vascular events **High vs low**: The pooled results of two studies suggest ASB consumption is associated with a greater risk of stroke (RR 1.14, 95% CI 1.04–1.26) and the results from one study suggest a greater risk of vascular events (RR 1.44, 95% CI 1.02–2.03). No significant difference was observed for MI or mortality	I^2 = 59%	Non linearity not properly tested	Not Stated	Not Specified
		Medline and EMBASE	7 cohort studies	Prospective cohorts, no language restricton, ages 34-77	ASB	Stroke	**Incremental increase**: The pooled results suggest a one-serving per day increase in ASB consumption was associated with a	I^2 = 0%	Non linearity not properly tested	Not Stated	Not Specified

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
							greater risk of stroke (RR 1.08, 95% CI 1.03–1.14), but not of MI or vascular events **High vs low**: The pooled results of two studies suggest ASB consumption is associated with a greater risk of stroke (RR 1.14, 95% CI 1.04–1.26) and the results from one study suggest a greater risk of vascular events (RR 1.44, 95% CI 1.02–2.03). No significant difference was observed for MI or mortality				
Pan et al (2022)	September 2019	Embase, Web of Science, Cochrane Central Register of Controlled Trials, and PsycINFO	13 prospective studies	Prospective cohort studies that assessed the association of SSBs, ASBs, or 100% fruit juice with mortality risk from all-cause, cancer, or cardiovascular diseases using multivariable analysis (Cox proportional hazards models or logistic regression models). Abstracts that reported the results of multivariable analysis were also included in our review. Studies were excluded if more than 20% of the samples in cohorts had major chronic illness at baseline. When studies were from the same cohort with the same outcomes	ASB	All-cause mortality	HR: 1.12, (01.05, 1.20)	I^2 = 78%	Linear (no evidence of non linear)	Low	NOS

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				of interest, we only included the latest or the longest follow up publication; if the duration of follow-up was the same, we included publications with the most participants.							
		Embase, Web of Science, Cochrane Central Register of Controlled Trials, and PsycINFO	13 prospective studies	Prospective cohort studies that assessed the association of SSBs, ASBs, or 100% fruit juice with mortality risk from all-cause, cancer, or cardiovascular diseases using multivariable analysis (Cox proportional hazards models or logistic regression models). Abstracts that reported the results of multivariable analysis were also included in our review. Studies were excluded if more than 20% of the samples in cohorts had major chronic illness at baseline. When studies were from the same cohort with the same outcomes of interest, we only included the latest or the longest follow up publication; if the duration of follow-up was the same, we included publications with the most participants.	ASB	Cancer (mortality)	HR: 1.02 (0.92, 1.13)	I^2 = 50%	Linear	Very low	Newcastle-Ottawa Scale
		Embase, Web of Science, Cochrane Central Register of Controlled Trials, and PsycINFO	13 prospective studies	Prospective cohort studies that assessed the association of SSBs, ASBs, or 100% fruit juice with mortality risk from all-cause, cancer, or cardiovascular diseases	ASB	CVD (mortality)	HR: 1.13 (01.03, 1.24)	I^2 = 0%	Non-linear	Low	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				using multivariable analysis (Cox proportional hazards models or logistic regression models). Abstracts that reported the results of multivariable analysis were also included in our review. Studies were excluded if more than 20% of the samples in cohorts had major chronic illness at baseline. When studies were from the same cohort with the same outcomes of interest, we only included the latest or the longest follow up publication; if the duration of follow-up was the same, we included publications with the most participants.							
Pan et al (2023)	Jun 20th, 2022	Embase, PubMed, Web of Science, and the Cochrane Library	37 cohort studies	Prospective cohort studies with participants aged 18 or older were included if they reported adequately adjusted effect estimates (relative risk (RR), hazard ratio (HR), or odds ratio (OR)) with 95% confidence intervals (CIs). Studies examining the association between SSBs (beverages with added sugar), ASBs (low-calorie, non-carbonated, caffeinated or caffeine-free drinks), or 100% fruit juice intake and the risk of overall or specific cancers were included. Abstracts reporting multivariate analysis results were also considered. Studies were excluded if	ASB	Overall cancer	RR: 1.00 (0.87, 1.15)	I^2 = 10.8%	Not Stated	Very low	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				they involved cancer patients at baseline, were cross-sectional or case-control, or had more than 20% of participants with chronic illness at baseline. Only the latest or most informative data from the same cohort with relevant exposure and outcome data were included. No age limit or publication status restrictions were applied.							
Qin et al (2020)	June 20, 2020	PubMed, Embase, and Ovid	39 studies	Prospective cohort, measured ASB, reported T2D, CVDs, and all-cause mortality as outcomes, healthy population at baseline, published in English	ASB	All-cause mortality	1.15, (1.07–1.23)	I^2 = 77.8%	Non-linear	Not Stated	Newcastle–Ottawa Scale
		PubMed, Embase, and Ovid	39 studies	Prospective cohort, measured ASB, reported T2D, CVDs, and all-cause mortality as outcomes, healthy population at baseline, published in English	ASB	Hypertension	1.13, (1.10–1.15)	I^2 = 47.9%	Non-linear	Not Stated	Newcastle–Ottawa Scale
		PubMed, Embase, and Ovid	39 studies	Prospective cohort, measured ASB, reported T2D, CVDs, and all-cause mortality as outcomes, healthy population at baseline, published in English	ASB	Obesity	1.39, (0.96–2.01)	I^2 = 89.2%	No evidence of non linearity	Not Stated	Newcastle–Ottawa Scale
		PubMed, Embase, and Ovid	39 studies	Prospective cohort, measured ASB, reported T2D, CVDs, and all-cause mortality as outcomes, healthy population at baseline, published in English	ASB	Type 2 diabetes	1.20, (1.05–1.38)	I^2 = 91.5%	No evidence of non linearity	Not Stated	Newcastle–Ottawa Scale
Querioz et al (2025)	June 2024	Medline, Embase, and Cochrane databases	6 observational studies	(1) prospective cohorts; (2) studies providing comparable data for the outcomes; (3) studies providing data of patients that drink ASBs in	ASB	All cause mortality	HR 1.14, (01.03, 1.26)	I^2 = 79%	Not Stated	Not Stated	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				comparison to those who don't or have minimum consumption; (4) studies available for review in English and full text. We excluded from this analysis studies: (1) no comparison group; (2) mixing the use of ASBs with other beverages in the same group; (3) data available in a non-comparable measure; (4) not having the outcomes being evaluated.							
		Medline, Embase, and Cochrane databases	5 observational studies	(1) prospective cohorts; (2) studies providing comparable data for the outcomes; (3) studies providing data of patients that drink ASBs in comparison to those who don't or have minimum consumption; (4) studies available for review in English and full text. We excluded from this analysis studies: (1) no comparison group; (2) mixing the use of ASBs with other beverages in the same group; (3) data available in a non-comparable measure; (4) not having the outcomes being evaluated.	ASB	CVD mortality	HR 1.29, (1.1, 1.53)	I^2 = 63%	Not Stated	Not Stated	Newcastle-Ottawa Scale
		Medline, Embase, and Cochrane databases	4 observational studies	(1) prospective cohorts; (2) studies providing comparable data for the outcomes; (3) studies providing data of patients that drink ASBs in comparison to those who don't or have minimum consumption; (4) studies available for review in English and full text. We excluded from this analysis studies: (1) no comparison	ASB	Stroke	HR 1.15, (01.01, 1.32)	I^2 = 25%	Not Stated	Not Stated	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				group; (2) mixing the use of ASBs with other beverages in the same group; (3) data available in a non-comparable measure; (4) not having the outcomes being evaluated.							
		Medline, Embase, and Cochrane databases	2 observational studies	(1) prospective cohorts; (2) studies providing comparable data for the outcomes; (3) studies providing data of patients that drink ASBs in comparison to those who don't or have minimum consumption; (4) studies available for review in English and full text. We excluded from this analysis studies: (1) no comparison group; (2) mixing the use of ASBs with other beverages in the same group; (3) data available in a non-comparable measure; (4) not having the outcomes being evaluated.	ASB	CHD	HR 1.13 (0.89, 1.43)	I^2 = 87%	Not Stated	Not Stated	Newcastle-Ottawa Scale
Ruanpeng et al (2017)	May 2015	MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, and Cochrane Central Register of Controlled Trials	3 cohort studies	(i) randomized controlled trials (RCTs) or observational studies (case-control, cohort studies or cross-sectional) published as original studies to appraise the risk of obesity in patients consuming either sugar or artificially sweetened soda, (ii) odds ratios, relative risks, hazard ratios or standardized incidence ratio with 95% CIs were presented and (iii) a reference group composed of participants who did not consume soda. No limits were implemented to language.	ASB	Obesity	1.59, (1.22-2.08)	I^2 = 36%	Not Stated	Not Stated	Not Stated

Citation	*Last search date*	*Databases search*	*No. RCTs (and/or Total studies)*	*Eligibility criteria*	*Intervention/ Exposure*	*Outcomes*	*RR and CI*	*Heterog. (I^2)*	*Dose response*	*Certainty (GRADE?)*	*RoB (Risk of Bias) method*
Taneri et al (2022)	January 29, 2021	MEDLINE (via Ovid), Embase, Web of Science Core Collection, Cochrane Library, and Google Scholar	6 cohort studies	Adults (18+), prospective design, evaluated consumption of UPF, evaluated all-cause mortality, human subjects	ASB	All-cause mortality	RR = 1.14, (1.05, 1.22)	I^2 = 76.2%	Not Stated	Not Stated	Newcastle-Ottawa Scale
Yang et al (2022)	April 1, 2022	Embase, Medline, Emcare	2 cohort studies	Inclusion criteria: 1. All human participants; 2. Prospective cohort studies that examined the association between added sugar, SSB or ASB and cardiovascular outcomes; 3. The exposure interest was the dietary intake of added sugar, SSB or ASB; 4. Outcome: The outcomes were defined as coronary heart disease (defined as nonfatal myocardial infarction, angina, coronary revascularization (i.e., percutaneous transluminal coronary angioplasty or coronary artery bypass surgery, or coronary heart disease death), stroke (defined as fatal or nonfatal stroke), and composite cardiovascular disease (comprised of coronary heart disease and stroke). Outcomes were defined and diagnosed according to selfreported, medical record or clinical examination. Exclusion criteria: 1. Intervention studies, review papers, comment, letters, news, notes, protocols, papers or abstracts from conference proceedings.	ASB	CVD	RR: 1.21 (0.98, 1.50)	I^2 = 71.3%	Not Stated	Not Stated	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
				2. Articles without an abstract or full text in English.							
Yin et al (2022)	September 2022	PubMed and Web of Science	17 cohort studies	(1) studies with a prospective cohort design; (2) ASB intake as the exposure; (3) cancer incidence as the outcome; and (4) reported estimates of risk ratio (RR) or hazard ratio (HR) and corresponding 95% confidence intervals (CIs) in the highest versus lowest categories. Studies that treated the exposure as a continuous variable were excluded, as RRs or HRs for the highest versus lowest category could not be obtained. For dose–response analysis, studies should provide a quantitative measurement of intake, the number of cases, and follow-up person-years for each category (or sufficient data to calculate them) with at least three categories classified based on the dosage of exposure	ASB	Overall cancer	RR = 1.03 (0.96, 1.11)	I^2 = 53.0%	No linear, no non linear associations	Very low	ROBINS E
Zhang et al (2021)	March 2020	PubMed, EMBASE, Web of Science, Cochrane Library, ProQuest, ClinicalTrials.gov, and the International Clinical Trials Registry Platform	7 cohort studies	Prospective studies - observational or intervention, SSB consumption, mortality, no language restriction	ASB	All-cause mortality	HRs (95% CIs) across different doses (0, 1, 1.5, 2, and 2.5 servings/d) were 1.00, 1.01 (0.99, 1.03), 1.04 (1.02, 1.07), 1.08 (1.05, 1.11), and 1.13 (1.09, 1.18)	Not Stated	J-shaped	Low	Newcastle-Ottawa Scale
		PubMed, EMBASE, Web of Science, Cochrane Library, ProQuest, ClinicalTrials.gov, and the International	3 cohort studies	Prospective studies - observational or intervention, SSB consumption, mortality, no language restriction	ASB	Cancer mortality	Not Stated	Not Stated	No association	Low	Newcastle-Ottawa Scale

Citation	Last search date	Databases search	No. RCTs (and/or Total studies)	Eligibility criteria	Intervention/ Exposure	Outcomes	RR and CI	Heterog. (I^2)	Dose response	Certainty (GRADE?)	RoB (Risk of Bias) method
		Clinical Trials Registry Platform									
		PubMed, EMBASE, Web of Science, Cochrane Library, ProQuest, ClinicalTrials.gov, and the International Clinical Trials Registry Platform	4 cohort studies	Prospective studies - observational or intervention, SSB consumption, mortality, no language restriction	ASB	CVD mortality	HRs (95% CIs) across different doses 1,00, 1.01 (0.96, 1.07), 1.07 (1.01, 1.13), 1.15 (1.08, 1.23), and 1.25 (1.14, 1.37)	Not Stated	J-shaped	Moderate	Newcastle-Ottawa Scale
Zhao et al (2024)	February 2, 2021	PubMed, EMBASE and Web of Science	35 cohort studies	Studies were included if: (1) they were population-based cohort or cross-sectional studies; (2) the subject of interest was intake of sugar (including fructose, sucrose, glucose, SSBs, ASBs, added sugar, and total sugar); (3) the definition of hypertension included SBP ≥ 140 mmHg or DBP ≥90mmHg, self-report of taking antihypertensive medicine, self-report of doctor-diagnosed hypertension, or hypertension as categorized by the International Classification of Disease (10th revision for hypertension, ICD10: 110 or ICD9:401); and (4) the studies reported quantitative estimates and their 95% confidence intervals (CIs) or standard errors (or sufficient data to calculate these estimates). If multiple articles were published for the same study, we included data from the study with the most detailed report and/or the largest sample size. We excluded reviews, comments, letters, and editorials.	ASB	Hypertension	RR: 1.14 (01.09, 1.18)	I^2 = 72.90%	No evidence of a non-linear dose–response	Very low	AMSTAR 2

ASB: artificially sweetened beverages; AMSTAR: A Measurement Tool to Assess Systematic Reviews; BMI: body mass index; BP: blood pressure; CI: confidence interval; CHD: Coronary Heart Disease ; CRB: Cochrane Risk Bias; CRC: colorectal cancer; CVD: cardiovascular diseases; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; HR: hazard ratio; HT: hypertension; MD: mean difference; NAFLD: Non-Alcoholic Fatty Liver Disease; NOS: Newcastle Ottawa Scale; NSSBs: non-sugar sweetened beverages; OR: odds ratio; RCTs: randomized controlled trials; RR: relative risks; T2D: type 2 diabetes.

Appendix 5. Added Sugars, SSBs, 100% Fruit Juice: Quality Appraisal of Included Meta-Analyses (articles highlighted in blue are those that examined only one exposure/outcome pair). *Please refer to the footnotes for the meanings of the acronyms*.

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
Abbasalizad et al (2022)	Change in BMI/weight	SSB	Moderate	Good methodology, but no GRADE (meta quality evidence)
	Waist circumference	SSB	Moderate	Good methodology, but no GRADE (meta quality evidence)
	Body fat	SSB	Moderate	Good methodology, but no GRADE (meta quality evidence)
Ardeshirlarijani et al (2021)	Waist circumference	SSB	Moderate	Overall, good but lack GRADE rating
Asgari-Taee et al (2019)	NAFLD	SSB	Moderate	Good methodology but no GRADE rating and risk of bias analysis
Auerbach et al (2017)	Change in BMI/weight	100% fruit juice	Moderate	Good methodology, missing GRADE rating
Bechthold et al (2019)	CHD	SSB	High	
	Stroke	SSB	High	
	Heart failure	SSB	High	
Bhagavathula et al (2022)	CVD mortality	SSB	Moderate	Good methodology, but no GRADE (meta quality evidence)
Chen et al (2019)	NAFLD	SSB	Moderate	Strong study, lack reports on GRADE
Della Corte et al (2025)	T2D	SSB	High	Very thorough and recent study
	T2D	Added sugars	High	Very thorough and recent study
Deng et al (2014)	Stroke	SSB	Moderate	Lacking GRADE rating
Farhangi et al (2020)	Hypertension	SSB	Moderate	Good methodology but still based on observational studies
	SBP	SSB	Moderate	Good methodology but still based on observational studies
	DBP	SSB	Moderate	Good methodology but still based on observational studies
Hu et al (2019)	Depression	SSB	Moderate	Strong study, lack reports on GRADE
Huang et al (2014)	CHD	SSB	Moderate	Good methodology but no GRADE rating

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
Huang et al (2023)	All cause mortality	Added sugars	Moderate	Good methodology, no GRADE
	CVD mortality	Added sugars	Moderate	Good methodology, no GRADE
	Cancer mortality	Added sugars	Moderate	Good methodology, no GRADE
Imamura et al (2015)	T2D	SSB	High	Strong study
	T2D	100% fruit juice	High	Strong study
Jakobsen et al (2023)	Obesity	SSB	Moderate	Good methodology, but no GRADE (meta quality evidence)
Jayalath et al (2015)	Hypertension	SSB	Moderate	Good methodology, GRADE rating missing
Kazemi et al (2023)	All cause mortality	SSB	High	Good methodology
	CVD mortality	SSB	Moderate	Good methodology, but no GRADE for CVD mortality
	Cancer mortality	SSB	High	Good methodology
Khan et al (2019)	CVD mortality	Added sugars	High	Good study analyzing multiple cohorts with rigorous methods
Kim and Je (2016)	Hypertension	SSB	Moderate	Good methodology but no GRADE rating
Li et al (2022)	All cause mortality	SSB	Moderate	
	CVD mortality	SSB	Moderate	
	Cancer mortality	SSB	Moderate	
	Other cause mortality	SSB	Moderate	
Liu et al (2019)	Hypertension	SSB	High	Good methodology
	Hypertension	100% fruit juice	High	Good methodology
Liu et al (2022)	Cognitive disorders	SSB	Moderate	Good methodology but no GRADE rating
Liu et al (2023)	Liver cancer	SSB	Moderate	Good methodology but no GRADE rating
Liu et al (2023)	NAFLD	Added fructose	Moderate	Despite no grade, great MA on NAFLD
Liu et al (2024)	Child Overweight/Obesity	SSB	Moderate	Good methodology but missing GRADE rating + reporting issues here and there
Llaha et al (2021)	Breast cancer mortality	SSB	Moderate	Good methodology but no GRADE rating

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
	Breast PM cancer mortality	SSB	Moderate	Good methodology but no GRADE rating
	Breast PostM cancer mortality	SSB	Moderate	Good methodology but no GRADE rating
	Colorectal cancer mortality	SSB	Moderate	Good methodology but no GRADE rating
	Prostate cancer mortality	SSB	Moderate	Good methodology but no GRADE rating
	Pancreatic cancer mortality	SSB	Moderate	Good methodology but no GRADE rating
Malik et al (2010)	T2D	SSB	Low to Moderate	No GRADE and risk of bias used
	MetS	SSB	Low to Moderate	No GRADE and risk of bias used
Malik et al (2013)	Change in BMI	SSB	Moderate	Comprehensive methodology but no GRADE rating
	Change in weight	SSB	Moderate	Comprehensive methodology but no GRADE rating
	Change in BMI	SSB	Moderate	Comprehensive methodology but no GRADE rating
	Change in weight	SSB	Moderate	Comprehensive methodology but no GRADE rating
Mattes et al (2011)	Change in BMI/weight	SSB	Moderate	Overall methodology not so comprehensive, GRADE missing
	Change in BMI/weight	SSB	Moderate	Overall methodology not so comprehensive, GRADE missing
McKeown et al (2018)	Fasting glucose	SSB	Moderate	Good methodology but no risk of bias evaluation and GRADE rating
	Fasting insulin	SSB	Moderate	Good methodology but no risk of bias evaluation and GRADE rating
Meng et al (2021)	T2D	SSB	Moderate	Good methodology but still based on observational studies
	CVD	SSB	Moderate	Good methodology but still based on observational studies
	All cause mortality	SSB	Moderate	Good methodology but still based on observational studies
Milajerdi et al (2019)	Pancreatic cancer	SSB	Moderate	Good methodology but no GRADE

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
	Pancreatic cancer	SSB	Moderate	Good methodology but no GRADE
Muñoz et al (2022)	MetS	SSB	Moderate	Good methodology, GRADE rating missing
	MetS	SSB	Moderate	Good methodology, GRADE rating missing
Narain et al (2016)	Stroke	SSB	Moderate	No GRADE, no specific risk of bias tool
	Myocardial infarction	SSB	Moderate	No GRADE, no specific risk of bias tool
	Vascular events	SSB	Moderate	No GRADE, no specific risk of bias tool
	All cause mortality	SSB	Moderate	No GRADE, no specific risk of bias tool
Nguyen et al (2023)	Change in BMI	SSB	High	Comprehensive methodology + ratings (evidence quality)
	Change in weight	SSB	High	Comprehensive methodology + ratings (evidence quality)
	Change in BMI	SSB	High	Comprehensive methodology + ratings (evidence quality)
	Change in weight	SSB	High	Comprehensive methodology + ratings (evidence quality)
Nguyen et al (2024)	Change in BMI/weight	100% fruit juice	High	Well conducted (may be clearer on some minor reporting aspects)
	Change in BMI/weight	100% fruit juice	High	Well conducted (may be clearer on some minor reporting aspects)
	Change in BMI/weight	100% fruit juice	High	Well conducted (may be clearer on some minor reporting aspects)
Pan et al (2022)	All cause mortality	SSB	High	Good methodology
	All cause mortality	100% fruit juice	High	Good methodology
	CVD mortality	SSB	High	Good methodology
	CVD mortality	100% fruit juice	High	Good methodology
	Cancer mortality	SSB	High	Good methodology
Pan et al (2023)	Cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Breast cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
	Colorectal cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Endometrial cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Gastric cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Kidney cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Leukimia	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Multiple myeloma	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Non Hodgkin lymphoma	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Pancreatic cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Prostate cancer	SSB	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Cancer	100% fruit juice	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Breast cancer	100% fruit juice	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Colorectal cancer	100% fruit juice	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Endometrial cancer	100% fruit juice	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Pancreatic cancer	100% fruit juice	Moderate	Good methodology, reporting heterogeneity was inconsistent
	Prostate cancer	100% fruit juice	Moderate	Good methodology, reporting heterogeneity was inconsistent
Poorolajal et al (2020)	Obesity	SSB	Moderate	Good methodology but no GRADE rating
Qin et al (2020)	Obesity	SSB	Moderate	Good methodology but no GRADING
	T2D	SSB	Moderate	Good methodology but no GRADING
	Hypertension	SSB	Moderate	Good methodology but no GRADING

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
	All cause mortality	SSB	Moderate	Good methodology but no GRADING
Rousham et al (2022)	BMI	SSB	High	Good methodology
	BMI z-score	SSB	High	Good methodology
	Body fat	SSB	High	Good methodology
	BMI z-score	100% fruit juice	High	Good methodology
Ruanpeng et al (2017)	Obesity	SSB	Moderate	No GRADE rating
Santos et al (2022)	T2D	SSB	Moderate	Good methodology but no GRADE rating
	Obesity	SSB	Moderate	Good methodology but no GRADE rating
	CHD	SSB	Moderate	Good methodology but no GRADE rating
	Stroke	SSB	Moderate	Good methodology but no GRADE rating
Schlesinger et al (2019)	Obesity	SSB	Moderate	Good methodology, only two database screened
	Abdominal obesity	SSB	High	Good methodology
	Weight gain	SSB	High	Good methodology
Schwingshackl et al (2017)	T2D	SSB	High	Good methodology
Schwingshackl et al (2017)	Hypertension	SSB	Moderate	Good methodology but no GRADE rating
Schwingshackl et al (2018)	All cause mortality	SSB	High	Clear methodology, transparency in reporting
Schwingshackl et al (2018)	Colorectal cancer	SSB	High	Clear methodology
Sun et al (2023)	CVD	SSB	High	
	CHD	SSB	High	
	Stroke	SSB	High	
	CVD mortality	SSB	High	
Sun et al (2023)	Cognitive function	SSB	High	Good methodology, but no GRADE (meta quality evidence)

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
	Dementia	SSB	High	Good methodology, but no GRADE (meta quality evidence)
	Alzheimer	SSB	High	Good methodology, but no GRADE (meta quality evidence)
Taneri et al (2022)	All cause mortality	SSB	Moderate	Good methodology, but no GRADE (meta quality evidence)
Valenzuela et al (2021)	Dental caries	SSB	Moderate	Missing a proper RoB analysis
	Erosion (teeth)	SSB	Moderate	Missing a proper RoB analysis
Wang et al (2022)	Stroke	SSB	Moderate	Clear, concise, great analysis but no GRADE rating
	Depression	SSB	Moderate	Clear, concise, great analysis but no GRADE rating
	Cancer	SSB	Moderate	Clear, concise, great analysis but no GRADE rating
	All cause mortality	SSB	Moderate	Clear, concise, great analysis but no GRADE rating
Xi et al (2015)	Hypertension	SSB	High	Missing a proper RoB analysis and GRADE rating
	CHD	SSB	High	Missing a proper RoB analysis and GRADE rating
	Stroke	SSB	High	Missing a proper RoB analysis and GRADE rating
Yang et al (2022)	CVD	SSB	Moderate	Comprehensive study applying good methodology, GRADE rating missing
	CHD	SSB	Moderate	Comprehensive study applying good methodology, GRADE rating missing
	Stroke	SSB	Moderate	Comprehensive study applying good methodology, GRADE rating missing
	CVD mortality	SSB	Moderate	Comprehensive study applying good methodology, GRADE rating missing
	CVD	Added sugars	Moderate	Comprehensive study applying good methodology, GRADE rating missing
	CHD	Added sugars	Moderate	Comprehensive study applying good methodology, GRADE rating missing

Authors	Outcome type	Exposure	Quality	One line rationale for final decision
	Stroke	Added sugars	Moderate	Comprehensive study applying good methodology, GRADE rating missing
	CVD mortality	Added sugars	Moderate	Comprehensive study applying good methodology, GRADE rating missing
Yin et al (2021)	CVD	SSB	Moderate	No GRADE, and not as many database searches
	CVD mortality	SSB	Moderate	No GRADE, and not as many database searches
Zhang et al (2021)	All cause mortality	SSB	High	Good methodology
	CVD mortality	SSB	High	Good methodology
	Cancer mortality	SSB	High	Good methodology
Zhao et al (2024)	Hypertension	SSB	High	Comprehensive methodology

BMI: body mass index; CHD: Coronary Heart Disease; CVD: cardiovascular diseases; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; NAFLD: Non-Alcoholic Fatty Liver Disease; PM: pre menopause; PostM: post menopause; SSBs: sugar-sweetened beverages; T2D: type 2 diabetes.

Appendix 6. NSSBs: Quality Appraisal of Included Meta-Analyses (articles highlighted in blue are those that examined multiple exposure/outcome pairs). *Please refer to the footnotes for the meanings of the acronyms*.

Citation	*Intervention/Exposure*	*Outcomes*	*Quality Appraisal*	*One line rationale for final decision*
Bhagavathula et al (2022)	ASB	CVD mortality	Moderate	No GRADE given.
Chen et al (2024)	ASB	All cause mortality	High	Comprehensive methodology
	ASB	CVD mortality	High	Comprehensive methodology
	ASB	Cancer mortality	High	Comprehensive methodology
Espinosa et al (2024)	NNS beverages	BMI	High	Comprehensive methodology
Imamura et al (2016)	ASB	Type 2 diabetes	High	Comprehensive study
Jouni et al (2025)	ASB	AD	High	Comprehensive methodology
Kim and Je (2016)	ASB	Hypertension	Moderate	No GRADE given.
Li et al (2022)	ASB	All-cause mortality	Moderate	No GRADE given.
	ASB	Cancer (mortality)	Moderate	No GRADE given.
	ASB	CVD (mortality)	Moderate	No GRADE given.
Meng et al (2021)	ASB	All-cause mortality	Moderate	No GRADE given.
	ASB	CVD	Moderate	No GRADE given.
	ASB	Type 2 diabetes	Moderate	No GRADE given.
Narain et al (2016)	ASB	All-cause mortality	Low	No GRADE given, no standardized risk of bias, no non-pooled results, no heterogeneity
	ASB	Myocardial infarction	Low	No GRADE given, no standardized risk of bias, no non-pooled results, no heterogeneity
	ASB	Stroke	Low	No GRADE given, no standardized risk of bias, no non-pooled results, no heterogeneity
Pan et al (2022)	ASB	All-cause mortality	High	Clear and comprehensive
	ASB	Cancer (mortality)	High	Clear and comprehensive
	ASB	CVD (mortality)	High	Clear and comprehensive
Pan et al (2023)	ASB	Overall cancer	High	Comprehensive methodology
Qin et al (2020)	ASB	All-cause mortality	Moderate	No GRADE given.
	ASB	Hypertension	Moderate	No GRADE given.
	ASB	Obesity	Moderate	No GRADE given
	ASB	Type 2 diabetes	Moderate	No GRADE given

Citation	*Intervention/Exposure*	*Outcomes*	*Quality Appraisal*	*One line rationale for final decision*
Querioz et al (2025)	ASB	All cause mortality	Moderate	No GRADE given.
	ASB	CVD mortality	Moderate	No GRADE given.
	ASB	Stroke	Moderate	No GRADE given.
	ASB	CHD	Moderate	No GRADE given.
Ruanpeng et al (2017)	ASB	Obesity	Moderate	No GRADE, no risk of bias assessment
Taneri et al (2022)	ASB	All-cause mortality	Moderate	No GRADE given.
Yang et al (2022)	ASB	CVD	Moderate	No GRADE given.
Yin et al (2022)	ASB	Overall cancer	High	Comprehensive methodology
Zhang et al (2021)	ASB	All-cause mortality	High	Comprehensive methodology
	ASB	Cancer mortality	High	Comprehensive methodology
	ASB	CVD mortality	High	Comprehensive methodology
Zhao et al (2024)	ASB	Hypertension	High	Comprehensive methodology

ASB: artificially sweetened beverages; BMI: body mass index; CHD: Coronary Heart Disease; CVD: cardiovascular diseases; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; NSSBs: non-sugar sweetened beverages; T2D: type 2 diabetes.

Appendix 4.3. Refined Carbohydrates, Insulin Resistance & Chronic Disease

THE ROLE OF REFINED CARBOHYDRATES IN DRIVING INSULIN RESISTANCE AND CHRONIC DISEASE

A Narrative Review

Benjamin Bikman, PhD
Department of Cell Biology and Physiology
Brigham Young University

Introduction: Insulin Resistance and Its Public Health Significance

Insulin resistance (IR) is a metabolic disorder where peripheral tissues exhibit reduced responsiveness to insulin, impairing glucose uptake and driving compensatory hyperinsulinemia, a key contributor to type 2 diabetes and cardiovascular disease. Fasting insulin levels serve as a critical and accessible marker for detecting IR, validated as a reliable indicator in diverse populations, including obese individuals [1,2]. The Homeostatic Model Assessment (HOMA-IR), derived from fasting insulin and glucose, further supports IR assessment [3]. Other surrogate markers, like the triglyceride-to-HDL cholesterol ratio (TG:HDL), reflect IR-associated dyslipidemia [4], but fasting insulin remains a cornerstone for clinical evaluation and research.

IR is central to the metabolic syndrome and underlies multiple chronic diseases. It increases the risk of type 2 diabetes (T2D) fivefold, raises cardiovascular disease (CVD) risk two- to threefold, and contributes to non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome (PCOS), and certain cancers [5]. Mechanistically, IR is linked with chronic inflammation, oxidative stress, mitochondrial dysfunction, and ectopic fat deposition, which collectively exacerbate multi-organ damage.

Poor metabolic health represents the most critical and widespread public health crisis in the U.S., with profound implications for chronic disease burden and healthcare costs. Analysis of NHANES 2009–2016 data revealed that only 12.2% of U.S. adults met criteria for metabolic health, defined by optimal waist circumference, blood pressure, glucose/HbA1c, triglycerides, and HDL cholesterol [6]. Strikingly, even among normal-weight individuals, just 16.2% were metabolically healthy, highlighting that poor metabolic health pervades all body types and affects millions across diverse demographics, exacerbating risks for diabetes, cardiovascular disease, and other preventable conditions.

Dietary factors are central to this crisis. Refined carbohydrates—including white flour, sucrose, and high-fructose corn syrup—are stripped of fiber, bran, and micronutrients during industrial processing. They are characterized by high glycemic index (GI) and glycemic load (GL), producing rapid spikes in glucose and insulin compared with intact, fiber-rich carbohydrate sources, as well as non-carbohydrate nutrients, like fats and proteins. The 2021 international GI/GL tables, which catalog more than 4,000 foods, consistently place refined breads, cereals, and rice at the higher end of the glycemic spectrum [7].

Since the 1970s, U.S. dietary guidelines have prioritized reducing dietary fat to mitigate cardiovascular risk, advocating that 45–65% of daily caloric intake derive from carbohydrates, without distinguishing between refined and unrefined sources. This guidance, combined with shifting consumer preferences toward perceived 'heart-healthy' low-fat options, encouraged the food industry to market and proliferate low-fat, high-carbohydrate products rich in refined grains and added sugars. This interplay between policy, consumer demand, and industry marketing contributed to carbohydrates becoming the dominant energy source in many diets [8,9]. In the U.S.,

carbohydrates have become the dominant energy source, accounting for about 50-55% of daily intake post-guidelines, with similar trends observed globally [10]. This shift, influenced by guidelines and economic factors, exceeds contributions from fats and proteins, which minimally influence blood glucose and insulin dynamics [11]. This dietary shift paralleled significant public health challenges, with obesity prevalence in the U.S. escalating from approximately 15% in 1980 to over 40% by 2018 and diabetes prevalence rising from roughly 5% to 14% over the same period [12]. The widespread availability and marketing of affordable, carbohydrate-heavy processed foods have entrenched these dietary patterns, contributing to the growing burden of metabolic diseases.

This narrative review synthesizes evidence from PubMed and Google Scholar searches (2000-2025), prioritizing meta-analyses, RCTs, and cohorts on refined carbs and IR. Inclusion focused on studies with direct metabolic outcomes.

Evidence Synthesis

Hyperinsulinemia as a Key Driver of Insulin Resistance

While hyperinsulinemia has long been interpreted as a compensatory response to reduced insulin sensitivity, evidence now supports its role as a causal driver of IR. Prolonged exposure to elevated insulin levels compromises multiple aspects of insulin physiology. Hyperinsulinemia also elevates pro-inflammatory cytokines such as TNF-α and IL-6, which further impair insulin sensitivity.

Animal and human studies support this causal view. In an animal model, chronic insulin infusion reduced insulin-mediated glucose disposal by nearly 40%, an effect reversed when infusion ceased [13]. Similar effects are seen in shorter-term studies [14]. Similarly, deletion of leptin receptors specifically in β-cells led to hyperinsulinemia preceding the development of IR and obesity [15]. In humans, fasting hyperinsulinemia independently predicted T2D in Pima Indians, even after adjustment for insulin sensitivity measured by clamp techniques [16]. Patients with insulinomas or those receiving chronic insulin therapy also develop features of IR, confirming that insulin excess itself can impair insulin action. A 2021 review emphasized the plausibility of hyperinsulinemia as a primary driver of metabolic disease [17].

Refined Carbohydrates as Inducers of Hyperinsulinemia

Carbohydrates elicit the strongest and most sustained insulin response among macronutrients, with dietary protein provoking a moderate response and dietary fat eliciting no direct insulin secretion. In human studies, protein consumption stimulates a modest insulin release, particularly in individuals with type 2 diabetes, driven by amino acids and gut hormones, while fat intake produces negligible insulin responses in both nondiabetic and diabetic individuals [18,19]. Refined carbohydrates, with their high glycemic index (GI) and glycemic load (GL), produce disproportionately large and rapid insulin excursions. The GI/GL tables confirm that refined breads, cereals, and rice

consistently provoke higher postprandial insulin demands than intact grains and legumes [7].

Importantly, any degree of processing magnifies this effect, including even carbohydrate-rich foods that are considered “whole grain”. Indeed, a crossover trial showed that breads made with milled whole wheat flour elicited glucose and insulin responses nearly indistinguishable from white bread [20]. These findings demonstrate that the differences in processing between “whole grain” and “refined grain” carbohydrates manifest in negligible metabolic benefits.

Definitions of Carbohydrate Quality

The assessment of carbohydrate quality is pivotal in understanding its implications for insulin resistance and chronic disease, as not all carbohydrates exert equivalent metabolic effects; refined carbohydrates, characterized by rapid digestion and minimal fiber or nutrient density, promote hyperglycemia and hyperinsulinemia, whereas high-quality sources like fiber-rich vegetables mitigate these risks through slower glucose absorption and improved satiety [21]. Scientists have developed various metrics to quantify carbohydrate quality, drawing from nutritional databases, dietary recalls, and clinical trials measuring physiological responses such as postprandial glycemia. These tools enable researchers to differentiate refined from unrefined carbohydrates and link dietary patterns to outcomes like type 2 diabetes and cardiovascular disease, emphasizing factors beyond mere quantity, such as fiber content, glycemic response, and processing level [22].

Key metrics include the Glycemic Index (GI), which ranks foods based on their incremental blood glucose rise relative to a reference like glucose (GI=100), calculated via the area under the 2-hour glucose curve after consuming 50g of available carbohydrates; high-GI foods, often refined starches, are associated with elevated insulin resistance [23]. The Glycemic Load (GL) refines this by incorporating serving size (GL = GI × grams of available carbohydrate / 100), providing a more accurate predictor of overall dietary glycemic impact and its role in chronic disease progression [24]. Composite indices, such as the Carbohydrate Quality Index (CQI), integrate multiple dimensions—including fiber intake, whole-to-refined grain ratios, and solid-form carbohydrates—scored against population tertiles to evaluate diet quality holistically; higher CQI scores correlate with reduced metabolic syndrome prevalence [25].

Additional ratio-based metrics, like the carbohydrate-to-fiber ratio (e.g., <10:1 for high quality), penalize low-fiber refined products while incorporating free sugars to further distinguish beneficial sources; for instance, a 10:1:1 threshold (carbs:fiber:free sugars) reclassifies fiber-dense staples as superior, challenging oversimplified views of refined carbohydrates' harm [26]. The Carbohydrate Food Quality Score (CFQS) extends this by profiling grains and non-grains on fiber, sugars, sodium, potassium, and whole grain content, aligning with guidelines to inform public health strategies against insulin resistance [27]. These metrics collectively underscore the need to prioritize unrefined

carbohydrates in mitigating chronic disease risks, with ongoing research validating their predictive power.

Refined Carbohydrates, Satiety, and Reward

Refined carbohydrates not only drive exaggerated insulin responses but also promote behavioral patterns that amplify metabolic load. The satiety index demonstrates that refined carbohydrates products and sweets are among the least satiating foods when consumed in equal-calorie portions, while protein-rich and fiber-dense foods scored highest [28]. This low satiety response encourages overconsumption and frequent snacking, increasing exposure to hyperinsulinemic excursions.

Additionally, refined carbohydrates activate reward pathways in the brain. Experimental work in animals and humans has shown that sugar and refined starches can stimulate dopaminergic signaling in the mesolimbic system, producing addictive-like consumption patterns [29,30]. These hedonic effects, coupled with low satiety, create a feedback loop of frequent, high-volume refined carbohydrate intake, reinforcing chronic hyperinsulinemia and accelerating IR development.

Evidence Linking Refined Carbohydrates to Insulin Resistance and Related Chronic Disease

Epidemiological evidence consistently implicates refined carbohydrate consumption in the development of insulin resistance and its sequelae. Prospective cohort studies show clear associations with type 2 diabetes (T2D), the most common long-term outcome of insulin resistance. A meta-analysis of 21 cohorts found that each 5-unit increase in dietary glycemic index (GI) increased T2D risk by 8%, and individuals consuming the highest-GI diets had a 14% higher risk compared with those consuming the lowest [31]. Another meta-analysis of seven cohorts reported that higher white rice intake was associated with significantly increased T2D risk [32].

Given that cardiovascular disease (CVD) is both a result of insulin resistance and the most common cause of death in the United States, it is important to evaluate this connection. The PURE study, which included more than 149,000 individuals from 21 countries, reported that consuming seven or more servings of refined grains per day was associated with higher risks of all-cause mortality and major CVD events compared with consuming fewer than two servings daily [33]. While some meta-analyses suggest null associations when refined grains are narrowly defined (excluding indulgent foods) [34], the overall evidence indicates no cardiovascular protection and possible harm at higher intakes.

Clinical trial evidence further supports a causal role for refined carbohydrates in driving insulin resistance. A large 2025 meta-analysis of 174 randomized trials reported that carbohydrate-restricted diets produced consistent improvements across multiple cardiometabolic outcomes, including lower systolic and diastolic blood pressure, reductions in triglycerides and inflammatory markers, and improvements in HDL cholesterol [35]. These broad effects strengthen the argument that carbohydrate load,

rather than dietary fat, is the more critical dietary determinant of insulin sensitivity and downstream vascular risk.

Beyond general cardiometabolic markers, trials targeting carbohydrate quality show specific benefits for glycemic control. A 2021 BMJ meta-analysis of randomized trials in patients with type 2 diabetes found that low-glycemic index and low-glycemic load diets led to meaningful improvements in HbA1c, fasting glucose, and blood lipids compared with higher-GI diets [36]. Complementary evidence comes from resistant starch interventions, which reduce the postprandial glycemic impact of meals. Supplementation studies in patients with obesity and type 2 diabetes demonstrate improvements in fasting glucose and HOMA-IR [37,38]. Together, these findings highlight that lowering both the quantity and quality of refined carbohydrate exposure improves laboratory markers directly linked to insulin resistance.

Even more striking are results from ketogenic and very low-carbohydrate diets (<50 g/day). A systematic review and meta-analysis in patients with type 2 diabetes reported clear reductions in HbA1c, fasting glucose, body weight, waist circumference, and triglycerides, alongside increases in HDL cholesterol [39]. A second meta-analysis of randomized controlled trials in overweight and obese adults confirmed that ketogenic diets produced greater improvements in HbA1c and HOMA-IR than low-fat diets, with consistently more favorable effects on triglycerides and HDL cholesterol [40]. These improvements in both glycemic control and direct measures of insulin resistance underscore the physiological advantage of carbohydrate restriction over conventional low-fat strategies.

Finally, head-to-head randomized trials reinforce these conclusions at the individual study level. In the DIRECT trial, participants assigned to a low-carbohydrate diet achieved greater reductions in fasting insulin than those assigned to a calorie-restricted low-fat diet, despite comparable weight loss [41]. Similarly, the A TO Z trial demonstrated superior reductions in fasting insulin among overweight women following a very low-carbohydrate diet compared to a low-fat comparator [42]. These direct comparisons illustrate that the benefits of carbohydrate restriction extend beyond weight reduction alone, reflecting improvements in insulin dynamics that are not matched by low-fat dietary patterns.

Additional RCTs corroborate these findings. For instance, in the DIETFITS trial, a low-carb diet led to greater improvements in insulin sensitivity (measured by 30-min insulin response) compared to low-fat in overweight adults [43]. Similarly, a 2020 RCT in adults with metabolic syndrome showed that a low-carb diet reduced HOMA-IR more effectively than a low-fat diet over 12 weeks [44]. These trials collectively demonstrate benefits beyond weight loss.

Evidence supports not only limiting refined grains and sugars but also replacing them with minimally processed, fiber-rich foods such as intact vegetables and fruits. For example, an RCT replacing refined grains with vegetables and legumes improved HOMA-IR and fasting insulin in adults with T2D over 6 months [45]. Another trial

substituting refined carbs with fiber-rich fruits and vegetables reduced postprandial insulin excursions and enhanced satiety [46]. Mechanistically, minimally processed plant foods slow glucose absorption, lower postprandial insulin excursions, and promote satiety through their intact fiber and micronutrient content. Importantly, the benefits appear strongest when "whole grains" are consumed in intact forms (e.g., oats, barley, brown rice) rather than milled flours, as trials have shown that breads made from whole wheat flour elicit glucose and insulin responses nearly identical to refined white bread [20].

Taken together, the convergence of meta-analyses, targeted dietary interventions, and head-to-head randomized trials provides robust evidence that limiting refined carbohydrate intake and replacing it with lower-GI alternatives substantially improves insulin resistance. These effects are evident across a spectrum of outcomes, from clinical endpoints to direct laboratory markers such as HbA1c, fasting glucose, HOMA-IR, and fasting insulin.

Low-fat diets, insulin resistance, and the calorie/carbohydrate confound

Of course, the dominant view regarding diet and insulin resistance is that reducing calories through a reduction in dietary fat is ideal. Most "low-fat" trials are explicitly calorie-restricted weight-loss interventions, which introduces a substantial confounding variable—namely, reduced carbohydrate consumption. Efforts to reduce total calories also lowers absolute carbohydrate grams compared with participants' baseline diets, so the "low-fat" arm is typically lower-carb than habitual intake.

Reported improvements in insulin resistance with "low-fat" diets therefore conflate fat reduction with reduced energy and carbohydrate exposure. For example, the Women's Health Initiative Dietary Modification Trial measured fasting insulin and HOMA-IR serially. The low-fat intervention produced small early improvements that attenuated over time, consistent with weight and calorie reduction rather than a specific benefit of fat itself [47]. Furthermore, when calories are held constant, higher carbohydrate loads raise insulin responses. In isocaloric, tightly controlled feeding trials, replacing dietary carbohydrate with fats improves fasting insulin, suggesting that carbohydrate per se increases insulin demand independent of calories [48].

Optimal Replacements for Refined Carbohydrates

Non-starchy vegetables show robust evidence for improving IR, with RCTs demonstrating reductions in HOMA-IR when replacing refined carbs [49]. Fruits, when consumed whole, offer benefits via fiber but may not match vegetables due to fructose; meta-analyses indicate moderate IR improvements with whole fruit intake [50]. Prioritizing proteins and fats should focus on plant-based sources (e.g., nuts, seeds) to align with CVD risk reduction, as evidenced by trials showing better outcomes than animal-heavy diets [51]. This avoids historical high-saturated fat pitfalls.

Evidence in Children and Adolescents

The crisis of poor metabolic health is not limited to adults but profoundly impacts U.S. children and adolescents, a demographic where early interventions could yield lifelong

benefits. Data from the National Health and Nutrition Examination Survey (NHANES) 2007–2016 indicate that less than 25% of adolescents aged 12–19 years achieve optimal metabolic health [52]. This low prevalence highlights the pervasive nature of metabolic disturbances even in youth, driven in part by dietary patterns heavy in refined carbohydrates that promote insulin resistance from an early age. Randomized controlled trials (RCTs) in this vulnerable population provide compelling evidence that reducing refined carbohydrate intake can mitigate these risks. For instance, one RCT in obese children compared carbohydrate-modified diets (focusing on lower glycemic load options) to standard portion-controlled approaches and found significant improvements in insulin resistance markers, including reduced fasting insulin levels and enhanced glucose disposal, without compromising safety or growth [53]. Building on this, studies emphasizing strategic replacements of refined carbohydrates with fiber-rich vegetables and high-quality proteins demonstrate even more pronounced benefits, often surpassing traditional low-fat diets. A notable pilot study in adolescents with type 2 diabetes explored a very-low-energy diet rich in proteins and low-starch/sugar vegetables, revealing that adherent participants achieved rapid weight loss, substantial reductions in liver fat, and complete reversal of diabetes in many cases, accompanied by marked improvements in insulin sensitivity [54]. These pediatric trials underscore a consistent theme: prioritizing carbohydrate quality over mere caloric restriction not only addresses insulin resistance more effectively but also fosters sustainable metabolic improvements, potentially averting the escalation to chronic diseases later in life. Extending such interventions to younger age groups, including pre-pubertal children, warrants further exploration to maximize preventive impact.

Discussion and Implications

The weight of evidence indicates that refined carbohydrates, by provoking sustained hyperinsulinemia, are major contributors to insulin resistance and related chronic diseases. Mechanistically, they overwhelm β-cell secretory pathways, downregulate insulin signaling cascades, promote ectopic fat accumulation, and amplify inflammatory signaling. Behaviorally, they encourage overeating through low satiety and hedonic reinforcement, compounding metabolic stress.

The epidemiological evidence is strongest for T2D, with consistent dose-response relationships. Evidence for CVD and mortality is weaker but suggests that refined grains confer no protection and may be harmful at high intakes. Randomized trials confirm that reducing refined carbohydrate intake improves glycemic control, insulin sensitivity, and related markers, though the effects are generally modest unless carbohydrate restriction is substantial.

Policy implications are significant. Public health guidelines should move beyond generic macronutrient percentages to emphasize carbohydrate quality. Refined carbohydrates should be explicitly limited, replaced with low-GI, fiber-rich carbohydrates such as intact vegetables and fruits. At the same time, efforts should prioritize dietary protein and fat. Practical measures include reforming school lunch programs to prioritize reducing

sources of refined carbohydrates, implementing clear front-of-package labeling, and incentivizing urban agriculture to increase access to natural sources of proteins and fats. Industry practices that promote refined, hyperpalatable carbohydrates should be counterbalanced by education campaigns that emphasize satiety, metabolic health, and the risks of chronic hyperinsulinemia.

Future research should prioritize long-term randomized controlled trials lasting more than one year to assess hard endpoints such as diabetes incidence and cardiovascular outcomes. Trials should span the lifespan, including the first 1000 days and pre-pubertal children, where interventions could yield lifelong metabolic benefits [55]. Greater standardization of what constitutes “refined carbohydrates” is also needed, incorporating not only fiber content but also particle size and processing methods. Additionally, emerging evidence on the gut microbiome suggests that loss of fermentable fiber from refined carbohydrate diets reduces short-chain fatty acid production, which may further impair insulin sensitivity; this warrants more investigation.

Conclusion

Refined carbohydrates are uniquely capable of driving hyperinsulinemia and insulin resistance, not only through their rapid glycemic effects but also through behavioral mechanisms of low satiety and high reward that further exacerbate the hyperinsulinemia. Cohort studies demonstrate increased risk of T2D and suggest harm for cardiovascular outcomes at high intakes, while clinical trials show improvements in insulin sensitivity and glycemic control when refined carbohydrates are reduced or replaced with low-GI alternatives. Historical dietary guidelines that emphasized total carbohydrate intake without distinguishing between refined and unrefined sources contributed to their overconsumption, with lasting public health consequences. Restricting refined carbohydrates, while promoting intact, low-GI, fiber-rich alternatives, as well as protein and fat, represents an evidence-based, practical, and urgently needed strategy to mitigate insulin resistance and the chronic disease epidemic.

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Appendix 4.4. Whole Grains, Refined Carbohydrates, Fiber, Glycemic Index & Disease

ASSOCIATIONS BETWEEN WHOLE GRAINS, REFINED CARBOHYDRATES, DIETARY FIBERS, AND GLYCEMIC INDEX AND CARDIOMETABOLIC DISEASES, COLORECTAL CANCER, AND MORTALITY

An Umbrella Review

Michael Goran, PhD
Professor and Vice Chair for Research
Department of Pediatrics, Keck School of Medicine
Children's Hospital of Los Angeles
University of Southern California

Daisy Zamora, PhD
Department of Physical Medicine and Rehabilitation
The University of North Carolina at Chapel Hill

Benjamin T. Bikman, PhD
Department of Cell Biology and Physiology
Brigham Young University

Abstract

Objectives: Refined carbohydrates are prevalent in Western diets and have been shown to be adversely associated with cardiometabolic diseases and mortality, whereas other studies show protective effects of whole grain carbohydrates and total fiber intake. The main objective of this umbrella review was to conduct an update synthesis of prior meta-analyses to evaluate these associations with clinical outcomes.

Methods: We searched PubMed, Embase, Web of Science, Cochrane, Ovid MEDLINE, and Scopus from January 2018 to September 2025 for meta-analyses of prospective cohorts or randomized controlled trials examining whole grains, refined carbohydrates, total fibers, and GI against the pre-specified outcomes of all-cause mortality, cardiovascular disease (CVD), obesity, and type 2 diabetes (T2D). In many studies colorectal cancer was also examined and was also included in our analysis. Data were extracted and cross-checked, quality assessed via an adapted ROBIS tool, and certainty rated using GRADE. Lead meta-analyses were selected based on recency, quality, and comprehensiveness.

Results: From 19 meta-analyses, higher whole grain intake was associated with lower risk of all-cause mortality (RR: 0.83, 95% CI 0.78-0.89; High GRADE), CVD (RR: 0.85, 95% CI 0.79-0.96; High GRADE), CRC (RR: 0.87, 95% CI 0.76-0.96; Moderate GRADE), obesity (RR: 0.85, 95% CI 0.79-0.91; Low GRADE), and T2D (RR: 0.67, CI 0.58-0.78; Low GRADE). Higher dietary fiber was associated with lower risk of all-cause mortality (RR: 0.83, CI 0.78-0.88; Moderate GRADE), CRC (RR: 0.84, 95% CI 0.78-0.89; Moderate GRADE), T2D (RR: 0.92, CI 0.88-0.96; Moderate GRADE), and coronary heart disease (RR: 0.8, 95% CI 0.61-1.04; Low GRADE). Dose-response analysis identified significant risk reductions per 30g/day of whole grain consumption (ranging between 6% reduction for all-cause mortality and colorectal cancer to 24% for type 2 diabetes), and an optimal intake of 25-29g/day for total fiber. Refined grain intake showed no significant associations with mortality or cardiovascular disease (Low GRADE). High dietary glycemic index was associated with increased risk of all-cause mortality (RR: 1.08, 95% CI 1.05-1.12; Very Low GRADE), CVD (RR: 1.15, 95% CI1.11-1.19; Low Grade), diabetes-related cancers (RR: 1.05, 95% CI 1.02-1.08; Very Low GRADE), and T2D (RR: 1.27, 95% CI 1.21-1.34; Low GRADE).

Conclusion: The evidence indicates that whole grains and dietary fiber confer protective effects for all outcomes examined with moderate to high certainty in a dose-responsive fashion, while refined grains show limited evidence of harm, and high-GI diets appear detrimental although this was rated as low to very low certainty. These findings support dietary recommendations focused on carbohydrate quality that would promote consumption of whole-grain foods and fiber-rich foods to replace refined or processed carbohydrates.

Introduction

The high prevalence of cardiometabolic disorders, including obesity, type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and certain cancers, represents one of the most pressing public health challenges of the modern era. Worldwide, obesity affects over 1 billion adults, with prevalence projected to continue rising steeply through 2030 in many regions (1). In the United States alone, more than 40% of adults live with obesity, a condition inextricably linked to insulin resistance—a hallmark of metabolic dysfunction that predisposes individuals to a cascade of comorbidities, including hypertension, dyslipidemia, and all-cause mortality (2,3). Emerging evidence underscores that dietary factors, particularly the quality and quantity of carbohydrates consumed, play a pivotal role in the etiology and progression of these disorders (4).

Refined carbohydrates are carbohydrates which have gone through processing, and are the most-consumed macronutrient in the world, accounting for over 70% of all calories in some regions (5). Indeed, the multinational Prospective Urban and Rural Epidemiological (PURE) study of over 135,000 participants across 18 countries found that the highest quintile of carbohydrate intake (77.2% of calories) was associated with a 28% increased risk of all-cause mortality compared to moderate levels (5). A follow-up study from the same cohort showed that poor carbohydrate quality, as determined by a high glycemic index, was associated with increased risk of cardiovascular disease and overall mortality (6). Accordingly carbohydrates, and especially refined carbohydrates have garnered increasing scrutiny for their potential to aggravate cardiometabolic health. Unlike whole foods, which retain fiber and micronutrients that may mitigate glycemic excursions, refined carbohydrates, especially grains, undergo processing that strips away these protective components, resulting in rapid digestion and absorption (7). This leads to pronounced postprandial hyperglycemia and hyperinsulinemia, which over time may impair metabolic processes and accelerate the development of T2DM and CVD (8). For instance, diets high in refined carbohydrates have been associated with elevated triglycerides, reduced high-density lipoprotein (HDL) cholesterol, and increased visceral adiposity—key features of the metabolic syndrome (9). In contrast, dietary patterns emphasizing whole grains, fibers, and lower GI foods appear to confer protective effects, reducing inflammation and improving endothelial function through mechanisms involving enhanced mitochondrial bioenergetics and reduced oxidative stress (10).

Despite these insights, the literature on carbohydrate quality and health outcomes remains fragmented. Numerous systematic reviews and meta-analyses have explored associations between refined grains, whole grains, total dietary fibers, and GI with clinical endpoints such as all-cause mortality, CVD events, colorectal cancer, obesity, and T2DM (4,7,8,11). However, findings are inconsistent; while some report dose-dependent risks with higher refined grain intake, others suggest null or modest effects, potentially confounded by variations in study design, population characteristics, and adjustment for covariates like physical activity and total energy intake (12). This heterogeneity underscores the need for a comprehensive and updated synthesis of

existing evidence to discern patterns and evaluate the certainty of associations across the different relevant exposures related to carbohydrate quality. Umbrella reviews, which aggregate data from multiple meta-analyses, offer a robust framework to address these gaps, providing higher-level insights into the strength and direction of relationships while accounting for methodological quality and bias (13).

In light of these considerations, the objective of this umbrella review was to systematically evaluate and synthesize evidence from existing meta-analyses on the associations between carbohydrate quality and clinical outcomes. We examined refined grains as the primary exposure, as well as other carbohydrate quality indicators of whole grains, total dietary fibers, and glycemic index, relative to key clinical outcomes including all-cause mortality, CVD (encompassing coronary heart disease and stroke), colorectal cancer, obesity, and T2DM. By applying rigorous quality appraisal tools and certainty assessments, such as an adapted Risk of Bias in Systematic Reviews (ROBIS) framework and Grading of Recommendations Assessment, Development, and Evaluation (GRADE) criteria, we aimed to clarify the extent to which higher consumption of refined carbohydrates elevates disease risk and to identify priorities for future research.

Methods

This umbrella review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, with adaptations for overviews of reviews as outlined in established methodological framework (13). All stages of the review, including screening, extraction, and appraisal, were performed independently by at least two reviewers, with discrepancies resolved through consensus and discussion. We searched PubMed (n=636), Embase (n=610), and Cochrane (n=19) for systematic reviews and meta-analyses published between Jan 2018 and Sept 2025 on carbohydrate quality including the exposures of refined carbohydrates, whole grains, total fiber, glycemic index (GI) and glycemic load (GL) relative to the major health outcomes of all-cause mortality, CVD, type 2 diabetes, and obesity. Many of the studies that we identified also included cancer-related outcomes, mostly colorectal cancer and also diabetes-related cancers. Therefore, these cancers were also considered in our analysis. Detailed search strings are available in the **Appendix**.

Eligibility Criteria

Eligible studies included self-identified meta-analyses that used reproducible methods, including a documented search strategy, explicit eligibility criteria, and a clear data extraction process. Studies must have been published between September 1, 2018, and September 1, 2025, in the English language, and focus on adults aged 18 years and older who were healthy at baseline. The exposures of interest were indicators of carbohydrate quality, such as dietary fiber intake, whole grain consumption, and dietary glycemic index or glycemic load. Comparators may include lower intake of these indicators or the substitution of refined grains with less processed carbohydrate sources, such as whole grains, fruits, vegetables, or legumes. Comparisons in which

diets were matched for macronutrient composition and include lifestyle modifications like exercise were included but not strictly required. Studies must have reported at least one of the following outcomes: all-cause mortality, incident type 2 diabetes, cardiovascular disease (including coronary heart disease, stroke, or composite CVD), or obesity (defined as incident obesity or weight gain). Umbrella reviews were included only if they included a distinct systematic synthesis of carbohydrate quality indicators, such as cross-review comparisons, GRADE evidence assessments, or quantitative integration.

Studies were excluded if they were narrative reviews, scoping reviews, umbrella reviews lacking original synthesis, or single primary studies. Also excluded were reviews of cross-sectional or ecological studies, studies limited to children or pregnant/lactating populations (unless adult-only results can be separated), and reviews that report only on intermediate biomarkers without addressing the primary outcomes. Reviews focusing solely on total carbohydrate intake without differentiating quality, or on dietary patterns not specific to refined carbohydrate content, were not eligible. Additionally, reviews centered on weight-loss interventions or limited to participants undergoing treatment for chronic diseases—unless findings generalize to primary prevention—were excluded. Interventions using isolated fiber supplements in powder form rather than food-based dietary fiber were not eligible. Non–peer-reviewed sources such as preprints, theses, conference abstracts, letters, and editorials, as well as non-English publications, were excluded. Finally, all included reviews must provide a quantitative meta-analysis of at least one eligible outcome.

Study Selection

Citations were imported into Covidence software for deduplication and screening. Titles and abstracts were screened in duplicate, followed by full-text assessment against eligibility criteria. Reasons for exclusion were documented at each stage, and a PRISMA flow diagram was generated to summarize the selection process.

Data Extraction

Data from eligible meta-analyses were extracted using a standardized template piloted on five reviews. Extraction was performed and cross-checked by three reviewers with discrepancies resolved by discussion. Extracted items included: citation details, last search date, databases searched, eligibility criteria, population characteristics (e.g., sample size, age, sex, regions), number of included studies and RCTs, outcomes, interventions/exposures (e.g., refined vs. whole grains, dose-response type), pooled effect estimates (e.g., relative risk [RR], hazard ratio [HR], mean difference [MD] with 95% confidence intervals [CI]), heterogeneity (I^2), risk of bias methods (e.g., Cochrane RoB, Newcastle-Ottawa Scale [NOS]), certainty assessments (e.g., GRADE), and funding/conflicts of interest. An evidence table was compiled for each meta-analysis, cross-referenced with an Excel spreadsheet for organization. Although refined grains were conceptualized as the comparator in the protocol, in practice several meta-

analyses reported whole grain intake as an exposure, and we summarized these findings as a distinct category.

Quality Appraisal

Methodological quality was assessed concurrently with extraction using an adapted version of the Risk of Bias in Systematic Reviews (ROBIS) tool (14). Four domains were evaluated: (1) clarity and pre-specification of eligibility criteria; (2) adequacy of search strategy (e.g., multiple databases, transparent methods); (3) accuracy of data collection and risk of bias assessment in included studies; and (4) appropriateness of synthesis methods and reporting. Each review received a global rating: high quality (low concern in ≥3 domains, no serious flaws in the remaining); moderate quality (sound methods but one domain unclear or with concerns); or low quality (serious concerns in ≥2 domains or major flaws, e.g., no risk of bias assessment). Ratings were assigned independently by all reviewers with consensus achieved through discussion.

Synthesis of Results

Meta-analyses were organized by exposure (refined grains, whole grains, total fibers, GI) and outcome, prioritizing clinical endpoints (e.g., all-cause mortality over surrogates). For each exposure-outcome pair, a lead meta-analysis was selected based on: (1) recency (latest search date); (2) quality (high rating preferred); (3) comprehensiveness (most included studies/RCTs); and (4) relevance (e.g., all-cause outcomes for mortality, CVD, cancer). Ties were resolved by favoring RCTs over observational data. Summary effect estimates, heterogeneity, and dose-response details were tabulated.

Certainty of evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach (15). Where GRADE ratings were reported in original meta-analyses, they were adopted; otherwise three reviewers applied ratings (high, moderate, low, very low) based on GRADE criteria with justifications for upgrading or downgrading provided and agreed upon by consensus. A Summary of Findings (SoF) table was constructed, including effect interpretations and GRADE rationales.

Where included in the original neta-analysis, we also extracted information on subgroup analyses which explored modifiers such as sex, obesity status, and geographic region (e.g., US, Europe, Asia), as well as sensitivity analyses and any information on publication bias such as funnel plots or Egger's test.

Results

Study Selection (Figure 1)

A total of 1,265 unique citations were identified from database searches and gray literature. After deduplication, 904 records were screened by title and abstract, resulting in 38 full-text articles assessed for eligibility. Nineteen were excluded (see PRISMA

diagram for details). This left 19 meta-analyses for inclusion and the process is summarized on **Figure 1**.

Characteristics of Included Reviews

The included meta-analyses were published between 2019 and 2024, with most (85%) focusing on prospective cohorts from regions including the US, Europe, Asia, and multi-country cohorts. Populations were primarily adults without baseline chronic disease (mean age ~56 years, follow-up ~12.6 years). Dietary assessment was predominantly via food frequency questionnaires (FFQs). Exposures included highest vs. lowest intake comparisons, with adjustments for confounders like age, sex, adiposity, smoking, energy intake, and physical activity. Key studies reported dose-response analyses (linear or nonlinear) and subgroup explorations (e.g., by sex, obesity status). Funding sources were noted in 60% of reviews, with no conflicts in most; a few declared industry ties but reported no influence. Detailed characteristics are tabulated in the supplementary evidence table (cross-referenced from the Excel spreadsheet). For example, lead meta-analyses covered 10–40 primary studies each, with sample sizes ranging from 117,885 to 3.9 million person-years.

Quality of Included Meta-Analysis

Using the adapted ROBIS tool, 90% of meta-analyses were rated high quality (low concern across ≥3 domains, e.g., comprehensive searches in multiple databases like PubMed, Embase, and Cochrane; appropriate synthesis with random-effects models; risk of bias assessments via NOS or ROBIN-I). The remaining 10% were moderate quality, primarily due to limited search scope or outdated evidence (e.g., searches pre-2020). No low-quality reviews were included after screening. Common strengths included duplicate extraction and GRADE certainty ratings and potential publication bias in smaller meta-analyses.

Synthesis of Findings

Meta-analyses were grouped by exposure and outcome, with lead selections as follows. For whole grains, Hu et al. (4) for mortality and CVD, Reynolds et al. (7) for cancer and T2DM, and Schlesinger et al. (16) for obesity. For refined grains, Hu et al.(4) for all outcomes available and identified. For total fibers, Mirrafiei et al. (10) for mortality, Reynolds et al (7) for cancer, and Hardy et al. (17) for CVD and T2DM. For GI, Jenkins et al. (8) for all outcomes available and identified. All of these lead meta-analyses had a high-quality rating and had an author-assigned GRADE certainty of evidence ranging from Very Low to High as discussed below and summarized in **Table 1** and **Table 2**.

Findings for Whole Grains

The evidence for whole grains was robust, high in quality and consistent across outcomes (**Tables 1 and 2**). Higher whole grain consumption was associated with 17% lower risk of all-cause mortality (High GRADE), 15% lower risk of cardiovascular disease (High GRADE), 13% lower risk for colorectal cancer (Moderate GRADE), 15% lower risk of obesity (Low GRADE) and 33% lower risk of type 2 diabetes (Low

GRADE). Overall, the direction of association is strongly protective, particularly for cardiometabolic outcomes.

Findings for Total Fiber

The evidence for dietary fiber demonstrated significant protective effects for multiple outcomes. Higher fiber intake was associated with 17% lower risk of all-cause mortality (Moderate GRADE), 16% lower risk for colorectal cancer (Moderate GRADE), 8% lower risk for type 2 diabetes (Moderate GRADE and supported by trial evidence on intermediate biomarkers), and 20% lower risk for coronary heart disease (Low GRADE). Overall, the beneficial effects of fiber appear broad but with variable strength depending on the outcome.

Findings for Refined Grains

For refined grains, our analysis only identified associations with all-cause mortality and cardiovascular disease, but neither reached statistical significance, and both were graded as low GRADE certainty because of wide confidence intervals, high heterogeneity, and inconsistency across studies.

Findings for Glycemic Index (GI)

High GI was associated with increased risk across outcomes, though the certainty of evidence was rated as Low or Very Low. Elevated GI was linked to 8% higher risk of all-cause mortality (Very Low GRADE), 15% higher risk of cardiovascular disease (Low GRADE), 5% higher risk of diabetes-related cancers (Very Low GRADE), and 27% higher risk of type 2 diabetes (Low GRADE). While the direction of association was consistent, the evidence was downgraded for inconsistency, indirectness, and observational bias.

Subgroup analyses from lead meta-analysis showed stronger risks for GI in overweight/obese females (e.g., HR 1.25–1.37 for T2DM in US/Asian cohorts) and protective effects of cereal fiber mitigated by high GL but not GI. Sensitivity analyses confirmed robustness, with no direction changes upon excluding studies.

Dose-Response Analyses for Whole Grains

Each of the lead meta-analyses identified also conducted rigorous dose response analysis for whole grains. Collectively, these studies showed that a 30g/day increase in whole grain consumption would be associated with a 6% reduction in all-cause mortality, 8% reduction in cardiovascular disease, 7% reduction in obesity, 6% reduction in colorectal cancer and 24% reduction in type 2 diabetes (findings for colorectal cancer and type 2 diabetes were reported per 15g whole grains but since these associations were also linear we are reporting here per 30g whole grains to be consistent).

Dose-Response Analyses and Optimal Intakes for Total Dietary Fibers

Dose-response relationships for total dietary fibers were reported in approximately 60% of included meta-analyses, providing critical insights into optimal intake levels for

reducing disease risk across the different outcomes examined. Findings are summarized on **Table 3** and described below:

Mortality: Mirrafiei et al (11) reported an inverse nonlinear dose–response for total dietary fiber and all-cause mortality (HR: 0.83 [95% CI: 0.78–0.88] for highest vs. lowest intake). The risk reduction was steepest at lower intakes, flattening at higher levels without a clear plateau threshold. These benefits may relate to fiber's effects on blood pressure, insulin sensitivity, and gut fermentation, though mechanisms for the curve shape are not detailed. Subgroup analyses showed no significant differences by sex, region, or other participant/study characteristics (high heterogeneity I^2=83%, potentially due to varying fiber sources like cereal vs. fruit/vegetable). GRADE certainty was moderate, downgraded for inconsistency and risk of bias but supported by a dose-response gradient across consistent observational data from 21 cohorts.

Colorectal Cancer: Reynolds et al (7) identified a linear dose–response between dietary fiber intake and colorectal cancer incidence. Comparing highest versus lowest intake, the pooled relative risk (RR) was 0.84 (95% CI: 0.78–0.89). The largest relative risk reductions (15–20% lower risk compared with <15 g/day) were observed at 25–29 g/day intake, beyond which benefits plateaued. For colorectal cancer, each 8 g/day increment in fiber was associated with an RR of 0.92 (95% CI: 0.89–0.95). Heterogeneity for this association was very low (I^2=19%), indicating robust consistency across cohorts. Complementary randomized controlled trials within the meta-analysis showed fiber intake significantly reduced body weight (mean difference [MD]: –0.37 kg) and systolic blood pressure (MD: –1.27 mmHg), both mechanistically relevant surrogates for cancer risk via reduced obesity and inflammation. According to GRADE assessment, the certainty of evidence was rated moderate, downgraded for reliance on observational data but upgraded due to the presence of a strong dose–response gradient.

Cardiovascular Disease (CVD): Hardy et al (17) did not evaluate dose-response for stroke or CHD due to insufficient primary studies reporting incremental data (fewer than 10 HRs per category). Overall associations were null or weak (stroke HR: 0.96 [95% CI: 0.89–1.04]; CHD HR: 0.80 [95% CI: 0.61–1.04]), with low certainty due to imprecision (wide CIs) and limited evidence; heterogeneity was low but dose–response modeling was not feasible. Subgroup analyses suggested protective effects for cereal fiber vs. total dietary fiber in US cohorts for CHD, but no per-increment linear trend was reported. Notably, high GL nullified fiber’s protective effects in some cohorts, whereas high GI did not for cereal fiber sources, suggesting source-specific interactions. The lack of dose-response data limits conclusions for CVD subtypes.

Type 2 Diabetes (T2D): Hardy et al (17) reported a linear dose–response for total dietary fiber and incidence of type 2 diabetes in US cohorts (HR: 0.92 [95% CI: 0.88–0.96] for highest vs. lowest intake). Per 5 g/day increase, the HR was 0.94 (95% CI: 0.92–0.97) for total fiber, with a stronger effect for cereal fiber (HR: 0.67 [95% CI: 0.60–0.74]). Protective associations were strongest in US cohorts, with high heterogeneity partly reflecting differences in dietary staples; sex-specific patterns were less consistent

for fiber than for GI/GL. Heterogeneity for total and cereal fiber analyses was high ($I^2 \approx$ 78–83%), partly reflecting differences in study populations and dietary staples (e.g., rice-based diets in Asia, bread in the US, potatoes in Europe). Dose–response models showed no evidence of nonlinearity, indicating consistent benefits with increasing intake. According to GRADE, overall certainty was rated moderate: downgraded for reliance on observational designs and heterogeneity, but upgraded for the presence of strong dose–response relationships.

Across outcomes, dose–response evidence supports an optimal fiber intake around 25–29 g/day for maximal risk reduction, with ~15–20% lower risk for mortality, colorectal cancer, and type 2 diabetes, and up to ~30% in specific subgroups or for cereal fiber. Complementary directions of effect in randomized trials on body weight, systolic blood pressure, and cholesterol strengthen causal inference (Reynolds et al., 2019). Nonlinear patterns for mortality suggest targeting populations with low intake (<15–20 g/day) for greatest impact, while largely linear trends for type 2 diabetes and colorectal cancer imply benefits from incremental increases at least up to ~30 g/day. Cereal fiber shows superior efficacy for type 2 diabetes (e.g., 33% lower risk per 5 g/day in US cohorts), and this protection is resistant to high glycemic index but attenuated by high glycemic load. Key limitations include reliance on observational data and sparse event-level RCTs for CVD; priorities include diverse populations and fiber subtype analyses.

Evidence-to-Decision (EtD) Framework

Criterion	Description
Problem & importance	Carbohydrate quality is a major determinant of chronic disease risk. Refined grains and high-GI diets are prevalent in the U.S. and globally, and fiber intake in the population is consistently lower than recommendations (<15 g/day for most adults). These factors have been shown to contribute to obesity, type 2 diabetes, cardiovascular disease, and colorectal cancer, whereas evidence suggests that whole grain carbohydrates and dietary fiber are protective. The purpose of this umbrella review was to conduct an update systematic analysis of the effects of these different carbohydrate exposures on clinical outcomes in order to identify optimal recommendations for health related to carbohydrate quality.
Certainty of evidence (per outcome)	**For whole grains**: 17% lower risk for Mortality & 15% lower risk of cardiovascular disease at High GRADE; 13% lower risk of colorectal cancer at Moderate GRADE; 15% lower risk of Obesity and 33% lower risk of T2D at Low GRADE. Dose response analysis indicates that a 30g/day increase in whole grain consumption would be associated with a 6% reduction in all-cause mortality, 8% reduction in cardiovascular disease, 7% reduction in obesity, 6% reduction in colorectal cancer and 24% reduction in type 2 diabetes **For total dietary fiber**: 17% lower risk for Mortality,16% lower risk of colorectal cancer and 8% lower risk of type 2 diabetes at Moderate GRADE; 20% lower risk of coronary heart disease at Low GRADE. Optimal fiber intake was indicated at ~25–29 g/day. **For refined grains**: 12% higher risk for all-cause mortality and 10% higher risk for cardiovascular disease, both not significant and at Low GRADE. **For Glycemic Index**: 8% higher risk for Mortality and 5% higher risk of diabetes-related cancers at Very Low GRADE; 15% higher risk of cardiovascular disease and 27% higher risk of type 2 diabetes at Low GRADE
Benefits vs harms	Whole grains and fiber were consistently associated with reduced risk of mortality, CVD, T2D, obesity, and CRC (15–33% lower risk) in a dose-responsive fashion. Refined grains show null/weak associations. High-GI diets increase risks (5–27%). No harms for higher fiber or whole grains are evident and no harms for lower refined grains or GI were evident.

Criterion	Description
Implementation considerations	Dietary guidelines emphasizing whole grains, fiber-rich food, fruits, vegetables, and legumes is entirely feasible and acceptability is likely. Barriers could include cost, availability, and individual dietary preferences. Substitution of refined grains and high GI foods with whole grains is practical and aligns with current policy frameworks. Even small changes will likely be beneficial as dose-response analysis indicated significant risk reduction for 30g per day of increase in whole grains (6% for all-cause mortality, 8% for cardiovascular disease, 6% for colorectal cancer, 24% for type 2 diabetes and 7% for obesity. Front of package food labeling could be helpful for consumers to easily identify foods meeting criteria aligned with recommendations.
Preliminary recommendation statement	Collectively, the evidence supports a **strong recommendation** to promote the replacement of refined grains and high-GI foods with whole grains and other fiber-rich carbohydrate sources to reduce mortality, CVD, T2D, obesity and colorectal cancer.

Statement of Findings

Higher intake of whole grains and total dietary fiber is consistently associated with lower risk of all-cause mortality, cardiovascular disease, type 2 diabetes, obesity, and colorectal cancer (Moderate–High certainty, depending on outcome), with optimal benefits observed at ~25–29 g/day of fiber. Refined grains show null or weak associations with mortality and CVD, with Low certainty. Diets with higher glycemic index increase risk of type 2 diabetes, cardiovascular disease, and mortality (Low–Very Low certainty). No evidence of harm from higher whole grain or fiber intake was evident. Overall, the evidence supports a **Strong recommendation** to promote whole grain and high fiber foods and replace refined grains and high-GI foods with whole grains and other fiber-rich carbohydrate sources to improve cardiometabolic and cancer outcomes.

Discussion

This umbrella review systematically synthesized evidence from 19 meta-analyses and identified 6 lead meta-analysis to evaluate the associations between whole grains and related carbohydrate quality indicators—refined grains, total dietary fibers, and glycemic index (GI)—with clinical health outcomes, including all-cause mortality, cardiovascular disease (CVD; encompassing coronary heart disease [CHD] and stroke), colorectal cancer, obesity, and type 2 diabetes mellitus (T2DM). Our findings, grounded in high-quality meta-analyses, provide robust evidence that higher consumption of whole grains and total dietary fibers is associated with significant reductions in risk (15–33%) for mortality, T2DM, obesity, CVD, and colorectal cancer, with optimal fiber intakes at 25–29 g/day (4,7,11,17). In contrast, refined grains showed null or weak positive associations with these outcomes, often with low certainty due to imprecision and

heterogeneity (4). Higher GI diets were consistently linked to increased risks (5–27%) for T2DM, CVD, diabetes-related cancers, and mortality, though certainty was low to very low due to observational data and inconsistency (8).

Summary of Evidence

The protective effects of whole grain carbohydrates and total fiber intake was consistent with prior syntheses, reinforcing their role in mitigating metabolic and inflammatory pathways (7,11,18). Whole grains were associated with a 17% lower risk of all-cause mortality, 15% lower risk of cardiovascular disease, 13% lower risk of colorectal cancer, 15% lower risk of obesity and 33% lower risk of T2D with Low to High GRADE certainty. Dose response meta-analysis from lead studies indicated that a 30g/day increase in whole grains would be associated with a 6% reduction in all-cause mortality, 8% reduction in cardiovascular disease, 6% reduction in colorectal cancer, 24% reduction in T2D and 7% reduction on obesity. Total fibers showed similar benefits with low to moderate GRADE certainty (17% lower risk of all-cause mortality, 16% lower risk of colorectal cancer, 20% lower risk for cardiovascular disease and 8% lower risk for T2D), with cereal fiber exhibiting stronger effects (11,17). Dose-response analyses for fibers indicated an optimal dose of 25–29 g/day for mortality, colorectal cancer, and T2DM, supported by both observational and RCT data including reductions in body weight and HbA1c (11,17). Linear trends for T2DM and cancer imply sustained benefits with incremental increases, especially for cereal fibers, which resist high-GI confounding but not high GL (17). Findings for refined grains showed no significant associations.GI was associated with an 8% risk of all-cause mortality, 27% risk of T2D and a 15% risk of cardiovascular disease although findings for GI were rate as Low or Very Low certainty. These findings are mechanistically plausible: fibers slow glucose absorption, reduce postprandial insulin spikes, and enhance gut microbiota diversity, thereby attenuating insulin resistance and systemic inflammation (19–21).

Strengths and Limitations

The strengths of this umbrella review include its comprehensive scope, rigorous methodology (PRISMA-guided, ROBIS and GRADE assessments), and prioritization of clinical endpoints, ensuring relevance to public health (4,13,15). The inclusion of 19 meta-analyses, covering over 135 million person-years, enhances generalizability across diverse populations (US, Europe, Asia). The selection of high-quality lead meta-analyses (90% ROBIS high rating) and consistent dose-response findings bolster confidence in protective effects of whole grains and fibers (4,7,11).

Several limitations should also be considered. Most of the evidence was derived from observational cohorts, limiting causal inference due to residual confounding (e.g., lifestyle factors like physical activity) (8). High heterogeneity (I^2 up to 83%) in some analyses, particularly for GI and refined grains, reflects variability in dietary assessment (e.g., FFQs), regional diets (e.g., rice-based in Asia), and adjustment models (4,8). FFQs were widely used in the included meta-analysis, and the validation evidence for refined grains and GI is limited – therefore measurement error may have attenuated

associations for these exposures. While numerous RCTs compare low- versus high-carbohydrate diets and demonstrate short-term improvements in cardiometabolic risk factors (e.g., lipids, weight, insulin resistance, etc.), data on hard clinical CVD endpoints (e.g., events, mortality) remain sparse, particularly for refined grain-specific interventions that isolate processing effects. Subgroup analyses, while valuable (e.g., stronger GI risks in obese females), were inconsistently reported, limiting exploration of effect modifiers like sex or ethnicity (8,17). Publication bias was not evident in lead meta-analyses, but smaller reviews may be underpowered, potentially underestimating risks (4). Another limitation is that most included studies did not distinguish between refined and fortified grain products. As a result, the potential contribution of fortified grains to increase micronutrient adequacy (eg folate, iron, and B-vitamins) could not be evaluated. This distinction is important, as fortified refined grains play a recognized role in meeting nutrient requirements, as emphasized in the 2025 DGAC report. However, any potential nutrient benefits from refined grains that have been fortified would likely be offset by recommendations to increase whole grain consumption, which naturally retain intrinsic micronutrients and fiber, thereby providing a more nutrient-dense carbohydrate source. Thus dietary guidance should balance both micro-nutrient adequacy and chronic disease prevention considerations.

Comparison with Other Studies and Prior Dietary Guidelines

Our findings align with prior umbrella reviews on carbohydrate quality, which consistently report protective effects of fiber-rich carbohydrates (12,18). For instance, Aune et al (18) found comparable risk reductions (20–30%) for T2DM and CVD with whole grain intake, supporting our dose-response conclusions. However, the lower or null findings for refined grains contrast with some cohort studies suggesting modest risks, possibly due to meta-analyses pooling heterogeneous populations or inadequate comparator groups (4,16).

The findings from this umbrella review strongly reinforce prior dietary guideline recommendations, which have consistently emphasized the consumption of whole grains and dietary fiber while advising limits on refined grains and high glycemic index foods. The U.S. Dietary Guidelines for Americans (DGAs) 2020–2025, for example, recommended that at least half of total grain intake be whole grains and highlighted low fiber consumption as a concern. Similarly, international guidelines from the WHO and European Food Safety Authority have endorsed higher intake of whole grains and fiber-rich foods to reduce cardiometabolic and cancer risk. However, past guidelines have generally relied on evidence graded as moderate or limited, and often lacked integration of umbrella-level syntheses. Our findings provide an updated and more comprehensive and robust evidence base, particularly with the high-certainty associations between whole-grain and mortality and cardiovascular disease, that strengthens the rationale behind these existing recommendations and underscores the importance of carbohydrate quality in overall dietary patterns.

Implications for Public Health and Future Research

The evidence supports dietary guidelines that advocate for replacement of refined grains with fiber-rich whole-grain sources to reduce risks of mortality, T2DM, obesity, CVD, and colorectal cancer. The stronger efficacy of cereal fiber suggests prioritizing sources like oats and barley, particularly in high-GI diets. Public health strategies should target populations with low fiber intake (<15 g/day), where benefits are most pronounced. In addition, the strong evidence for whole grains underscores the need for consideration of policies that would address issues such as front-of-pack labeling, procurement policies, and food industry standards to prioritize minimally processed, fiber-rich whole grain products. The consistent evidence for optimal benefits of fiber at 25–29 g/day supports setting clearer intake thresholds in guidelines, such as school meal standards, and food labeling policies which has been shown to be effective for improving dietary choices (22). Together, these results argue for strengthening global nutrition policies that elevate carbohydrate quality, by promoting whole grains and fibers, while discouraging refined grains and high-GI foods, as a central strategy for preventing obesity, diabetes, cardiovascular disease, and colorectal cancer. Research gaps identified include the need for RCTs on refined grains and CVD, given null findings in observational data. Mechanistic trials exploring fiber subtypes (e.g., soluble vs. insoluble) and their interaction with GI/GL could clarify differential effects. Subgroup analyses by sex, obesity status, and ethnicity should be standardized to address disparities, as seen in stronger GI risks among obese females (8,17). In addition there is a strong need for prospective and intervention studies in children and across life-course.

Conclusion

This umbrella review provides robust and updated evidence that carbohydrate quality profoundly influences cardiometabolic health outcomes as well as colorectal cancer and all-cause mortality. Higher intakes of fiber-rich whole-grain carbohydrates and total dietary fiber are both associated with significant reductions in risk for all-cause mortality (17%), type 2 diabetes mellitus (T2DM; 33%), cardiovascular disease (CVD; 15%), obesity (15%) and colorectal cancer (13%), with optimal fiber intakes of 25–29 g/day (4,7,11). These protective effects, often rated moderate to high certainty by GRADE in the lead reviews, are mechanistically consistent with attenuated postprandial glycemia and trial evidence of lower body weight and systolic blood pressure with higher fiber intake (7). High glycemic index (GI) diets were associated with increased risks of T2DM (27%), CVD (15%), diabetes-related cancers (5%), and mortality (8%) in large cohort meta-analyses, though certainty for GI findings was rated as Low or Very Low Certainty (8,17).

These findings advocate for dietary strategies prioritizing whole grains and fibers over refined grains and other carbohydrates to mitigate the global burden of chronic and cardiometabolic related disease outcomes in the population. Public health guidelines should emphasize achieving 25–29 g/day of dietary fiber, with cereal fiber showing particularly strong inverse associations with T2DM risk (7,17). The lower or null effects

of refined grains underscore replacing them with nutrient-dense alternatives rather than indiscriminate carbohydrate restriction (4). Future research should prioritize randomized trials (e.g. whole grain substitution for refined grains and cardiometabolic outcomes) and under-represented populations including children and critical stages of development.

Table 1: Outcome Summary Table

Exposure	Outcome	Lead Meta-Analysis	Effect Size (RR/HR, 95% CI)	Heterogeneity (I^2)	Quality Rating
Whole Grains	Mortality	Hu et al., 2023	RR: 0.83 (0.78–0.89)	83%	High
Whole Grains	Cancer (colorectal only)	Reynolds et al., 2019	RR: 0.87 (0.79–0.96)	52%	High
Whole Grains	Cardiovascular (all)	Hu et al., 2023	RR: 0.85 (0.80–0.91)	52%	High
Whole Grains	Obesity (adults)	Schlesinger et al., 2019	RR: 0.85 (0.79–0.91)	0%	High
Whole Grains	Type 2 Diabetes	Reynolds et al., 2019	RR: 0.67 (0.58–0.78)	82%	High
Refined Grains	Mortality	Hu et al., 2023	RR: 1.12 (0.95–1.31)	71%	High
Refined Grains	Cardiovascular (all)	Hu et al., 2023	RR: 1.10 (0.91–1.34)	81%	High
Total Fibers	Mortality	Mirrafiei et al., 2023	HR: 0.83 (0.78–0.88)	83%	High
Total Fibers	Cancer (colorectal)	Reynolds et al., 2019	RR: 0.84 (0.78–0.89)	0%	High
Total Fibers	Cardiovascular (stroke)	Hardy et al., 2020	HR: 0.96 (0.89–1.04)	0%	High
Total Fibers	Cardiovascular (CHD)	Hardy et al., 2020	HR: 0.80 (0.61–1.04)	0%	High
Total Fibers	Type 2 Diabetes	Hardy et al., 2020	HR: 0.92 (0.88–0.96)	78%	High
Glycemic Index	Mortality	Jenkins et al., 2024	RR: 1.08 (1.05–1.12)	90%	High
Glycemic Index	Cancer (diabetes-related)	Jenkins et al., 2024	RR: 1.05 (1.02–1.08)	23%	High
Glycemic Index	Cardiovascular (all)	Jenkins et al., 2024	RR: 1.15 (1.11–1.19)	35%	High
Glycemic Index	Type 2 Diabetes	Jenkins et al., 2024	RR: 1.27 (1.21–1.34)	71%	High

Table 2: Summary of Findings (SoF) Table

Exposure	Outcome	Lead Meta-Analysis	Effect Size (RR/HR, 95% CI)	Dose-Response Analysis	GRADE Rating	Rationale (GRADE)
Whole Grains	Mortality	Hu et al., 2023	RR: 0.83 (0.78–0.89)	Non-linear	High	Reported as such in the MA; low risk of bias, consistent evidence
Whole Grains	Cancer (colorectal only)	Reynolds et al., 2019	RR: 0.87 (0.79–0.96)	Linear	Moderate	Reported as such in the MA; some inconsistency in primary studies
Whole Grains	Cardiovascular (all)	Hu et al., 2023	RR: 0.85 (0.80–0.91)	Non-linear	High	Reported as such in the MA; precise estimates, no serious imprecision
Whole Grains	Obesity (adults)	Schlesinger et al., 2019	RR: 0.85 (0.79–0.91)	Linear (no evidence of non-linearity)	Low	Reported as such in the MA; downgraded for indirectness and imprecision
Whole Grains	Type 2 Diabetes	Reynolds et al., 2019	RR: 0.67 (0.58–0.78)	Linear	Low	Reported as such in the MA; downgraded for inconsistency (high I^2)
Refined Grains	Mortality	Hu et al., 2023	RR: 1.12 (0.95–1.31)	Linear	Low	Reported as such in the MA; wide CI, imprecision
Refined Grains	Cardiovascular (all)	Hu et al., 2023	RR: 1.10 (0.91–1.34)	Linear	Low	Reported as such in the MA; high heterogeneity, inconsistency
Total Fibers	Mortality	Mirrafiei et al., 2023	HR: 0.83 (0.78–0.88)	Non-linear	Moderate	Reported as such in the MA; some publication bias suspected
Total Fibers	Cancer (colorectal)	Reynolds et al., 2019	RR: 0.84 (0.78–0.89)	Linear; greatest at 25–29 g/day	Moderate	Reported as such in the MA; consistent but observational data
Total Fibers	Cardiovascular (stroke)	Hardy et al., 2020	HR: 0.96 (0.89–1.04)	Not evaluated	Low	Reported as such in the MA; null effect, imprecision
Total Fibers	Cardiovascular (CHD)	Hardy et al., 2020	HR: 0.80 (0.61–1.04)	Not evaluated	Low	Reported as such in the MA; wide CI, low events
Total Fibers	Type 2 Diabetes	Hardy et al., 2020	HR: 0.92 (0.88–0.96)	Linear	Moderate	Reported as such in the MA; supported by RCTs on surrogates
Glycemic Index	Mortality	Jenkins et al., 2024	RR: 1.08 (1.05–1.12)	Non-linear	Very Low	Reported as such in the MA; downgraded for inconsistency and indirectness
Glycemic Index	Cancer (diabetes-related)	Jenkins et al., 2024	RR: 1.05 (1.02–1.08)	Not evaluated	Very Low	Reported as such in the MA; low events, publication bias
Glycemic Index	Cardiovascular (all)	Jenkins et al., 2024	RR: 1.15 (1.11–1.19)	Not evaluated	Low	Reported as such in the MA; observational, moderate inconsistency
Glycemic Index	Type 2 Diabetes	Jenkins et al., 2024	RR: 1.27 (1.21–1.34)	Linear	Low	Reported as such in the MA; high heterogeneity but consistent direction

Table 3: Detailed Dose-Response for Total Fibers by Outcome (Per Increment)

Outcome	Lead Meta-Analysis	Increment	Effect Size (HR/RR, 95% CI)	Type	Notes
Mortality	Mirrafiei et al., 2023	Per 10 g/day	HR: 0.85 (0.81–0.88)	Nonlinear	Steepest <25 g/day; plateaus >30 g/day; no major subgroup differences
Colorectal Cancer	Reynolds et al., 2019	Per 8 g/day	RR: 0.92 (0.89–0.95)	Linear	Greatest at 25–29 g/day; supported by RCTs on weight, BP
CVD (Stroke)	Hardy et al., 2020	N/A	Insufficient fiber-specific data	N/A	Insufficient data; some associations by GI/GL, not consistent for fiber alone
CVD (CHD)	Hardy et al., 2020	Per 5 g/day (cereal fiber)	HR: 0.83 (0.77–0.90)	Potential linear	Evidence mainly from US subgroups; total fiber often null; effect attenuated by GL
T2DM	Hardy et al., 2020	Per 5 g/day (total)	HR: 0.94 (0.92–0.97)	Linear	Cereal fiber stronger (HR: 0.67 [0.60–0.74]); amplified in obese females; attenuated by GL

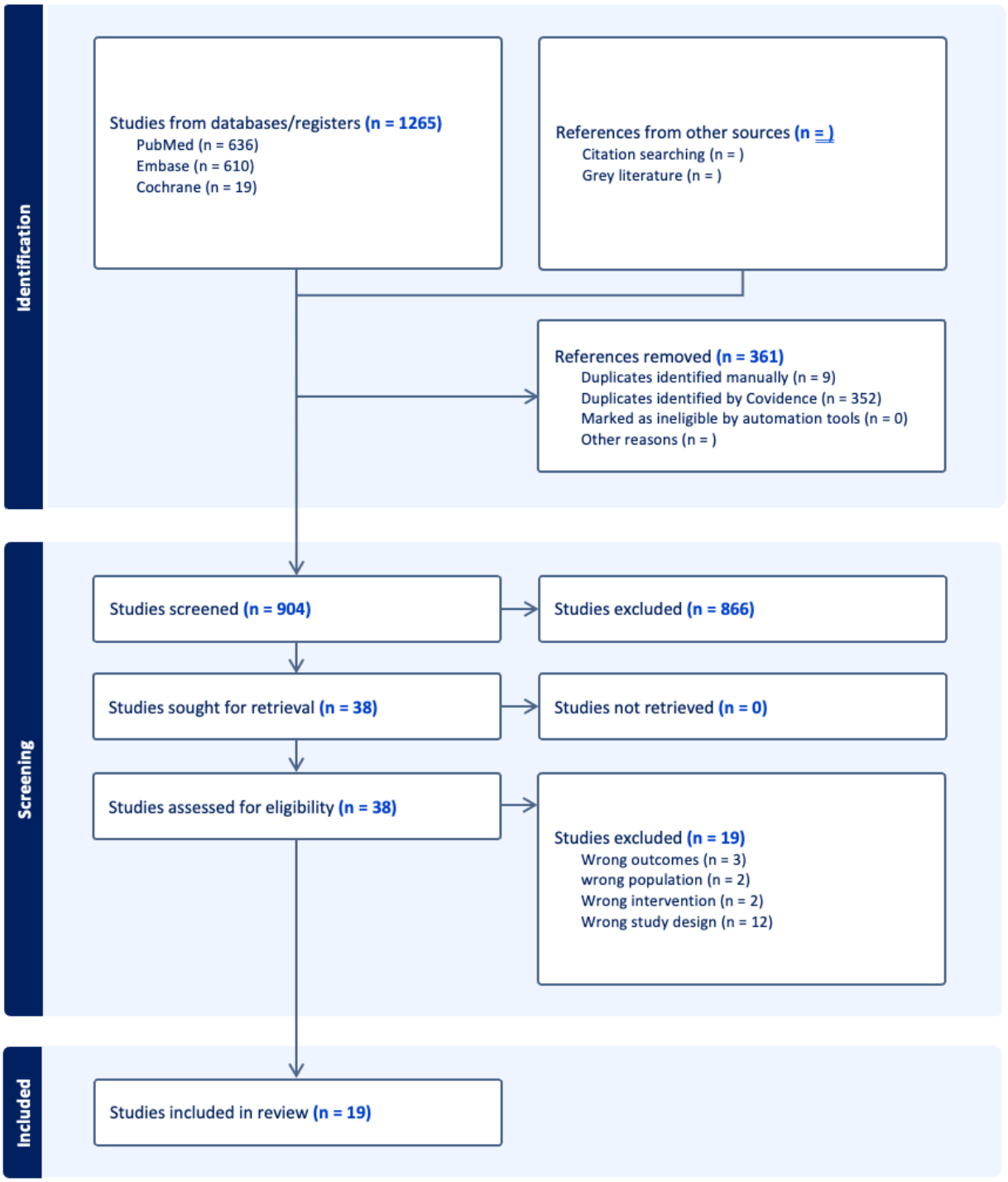

Figure 1: Flow Diagram Showing Selection of Papers at Various Stages of the Process

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Appendix: Database Search Strategy

Database: PubMed/MEDLINE
Platform: National Library of Medicine
Date Searched: 9/27/2025
Results: 636

	Concept	Search Strategy	Results
#1	Dietary carbohydrate	"Dietary Carbohydrates"[Majr] OR "dietary carbohydrate*"[Title/Abstract] OR "fibre*"[Title/Abstract] OR "fiber*"[Title/Abstract] OR "fibre*"[Title/Abstract] OR "Glycemic Index"[Majr] OR "glycemic index*"[Title/Abstract] OR "glycaemic index*"[Title/Abstract] OR "glycemic load*"[Title/Abstract] OR "glycaemic load*"[Title/Abstract] OR "Whole Grains"[Majr] OR "whole grain*"[Title/Abstract] OR "wholegrain*"[Title/Abstract]	507,808
#2	Diabetes/ Cardiovascular / Obesity/ Mortality	"Diabetes Mellitus, Type 2"[Majr] OR "type two diabet*"[Title/Abstract] OR "type II diabet*"[Title/Abstract] OR "type 2 diabet*"[Title/Abstract] OR "T2D"[Title/Abstract] OR "T2DM"[Title/Abstract] OR "Cardiovascular Diseases"[Mesh:NoExp] OR "cardiovascular*"[Title/Abstract] OR "CVD"[Title/Abstract] OR "Myocardial Ischemia"[Majr] OR "myocardial ischemia*"[Title/Abstract] OR "myocardial infraction*"[Title/Abstract] OR "Cerebrovascular Disorders"[Majr] OR "Peripheral Arterial Disease"[Majr] OR "peripheral arterial disease*"[Title/Abstract] OR "cardiomyopath*"[Title/Abstract] OR "Heart Failure"[Majr] OR "heart failure*"[Title/Abstract] OR "Coronary Disease"[Majr] OR "coronary"[Title/Abstract] OR "CHD"[Title/Abstract] OR "Obesity"[Majr] OR "obes*"[Title/Abstract] OR "Mortality"[Majr] OR "mortalit*"[Title/Abstract]	3,345,457
#3	Systematic reviews/ Meta-analysis	"Systematic Review"[Publication Type] OR "Systematic Reviews as Topic"[Mesh] OR "systematic review"[Title/Abstract:~2] OR "Meta-Analysis" [Publication Type] OR "Meta-Analysis as Topic"[Mesh] OR "meta-analysis"[Title/Abstract] OR "meta-analyses"[Title/Abstract]	591,874

	Concept	Search Strategy	Results
#4	Combined Concepts	(#1 AND #2 AND #3)	1,129
#5	Limits	((#1 AND #2 AND #3) NOT ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh])) NOT ("Models, Animal"[Mesh] OR "Mice"[Mesh] OR "Rats"[Mesh] OR "animal model*"[Title/Abstract] OR "rat"[Title] OR "rats"[Title] OR "mouse"[Title/Abstract] OR "mouse"[Title/Abstract] OR "mice"[Title/Abstract] OR "Letter"[Publication Type] OR "Editorial"[Publication Type] OR "Comment"[Publication Type] OR "News"[Publication Type] OR "Congress"[Publication Type] OR "Consensus Development Conference"[Publication Type] OR "protocol*"[Title] "symposium*"[Title] OR "proceeding*"[Title]) Filters: from 2018/1/1 - 2025/12/31	636

Appendix 4.5. Low-Carbohydrate Diets for Weight & Type 2 Diabetes

A REVIEW OF LOW-CARBOHYDRATE DIETS FOR WEIGHT LOSS, METABOLIC SYNDROME AND TYPE 2 DIABETES

A Narrative Umbrella Review

Jeff Volek, PhD, RD
Department of Human Sciences
The Ohio State University

Introduction

The majority of adults in the United States are suffering from some form of impaired or suboptimal metabolic health, which may be as high as 88% of Americans (1). For many Americans, this involves an excessive accumulation of adipose tissue that presents a risk to health. The prevalence of obesity (BMI ≥30) has more than tripled since the 1960s and now represents more than 4 in 10 adults (2) with 7 in 10 adults overweight or obese (BMI ≥25). Obesity is estimated to reach close to half of Americans by 2030 (3), and has already surpassed that level in Black women (2). This puts the number of adults in the United States with overweight and obesity at an estimated 172 million people (4).

Obesity is strongly associated with impaired glucose metabolism and insulin resistance, a condition that manifests in metabolic syndrome, prediabetes, and type 2 diabetes (T2D). Metabolic syndrome is defined as having at least three of the following markers: high triglycerides, low HDL-cholesterol, high fasting plasma glucose, high blood pressure, and high waist circumference. Metabolic syndrome increases risk of developing T2D and cardiovascular disease and is estimated to affect more than one-third of adults in the United States (5). Prediabetes and T2D have both increased dramatically over the last several decades, and now affect over half of Americans or more than 130 million adults (6). The total healthcare costs attributed to managing T2D exceed $400 billion per year (7).

While the root causes driving the surge in impaired metabolic health are heavily debated, the sharp rise in the prevalence of obesity, metabolic syndrome and T2D over the last half century has occurred in the backdrop of a population dietary shift characterized by less fat and more carbohydrate consumption (8). Dietary carbohydrate is the primary driver of insulin secretion, a hormone with dominant regulatory control over adipose tissue storage/release (i.e., insulin promptly and potently inhibits breakdown and oxidation of fat). Given that insulin resistance is associated with hyperinsulinemia, the potential benefits of restricting carbohydrate to a level that a person can metabolize in a healthy manner without exacerbating hyperinsulinemia represents a reasonable hypothesis. Indeed, a wide range of researchers in many different disciplines have examined the effects of low-carbohydrate diet studies making it one of the most well-studied eating patterns over the last 25 years.

Because many Americans are overweight/obese with some type of metabolic impairment, it is important we understand how diets varying in carbohydrate may influence weight loss and metabolic health. The primary objective of this narrative umbrella review is to address the question “**What is the evidence to support lower carbohydrate diets**?” This narrative umbrella review is limited to discussing the scientific rationale and evidence for low-carbohydrate diets relevant to weight loss, metabolic syndrome, and T2D outcomes. Given the large number of individual studies published in the last two decades, the focus is on examining meta-analyses of randomized clinical trials (RCTs) that included low-carbohydrates diets.

Methods

This rapid narrative umbrella review summarizes evidence from numerous previously published quantitative meta-analyses on the consumption of low-carbohydrate diets relative to diets higher in carbohydrate on weight loss, metabolic syndrome and T2D outcomes.

Rather than simply repeating the work of others, this report focuses on a qualitative analysis in order to provide a high-level perspective of the breadth of low-carbohydrate diet research and consistency of findings across meta-analyses that assessed RCTs.

PICO(T)

Population (P): Adults aged ≥18 years with overweight, obesity, type 2 diabetes, metabolic syndrome, or general adult populations. Pregnant or lactating women, critically ill patients, hospitalized patients, people with other serious diseases (e.g., epilepsy, cancer) and pediatric populations were excluded.

Intervention (I): Low-carbohydrate diets defined broadly defined as ≤45% of total daily calories from carbohydrates or explicitly described as "low carbohydrate" or "ketogenic" by study authors.

Comparison (C): Diets higher in carbohydrate (>45% carbohydrate) including low-fat diets, usual care, other dietary interventions.

Outcomes (O): Primary outcomes included body weight change and hemoglobin A1c in diabetic populations. Secondary outcomes included body mass index, body fat percentage, triglycerides, HDL-C, LDL-C, blood pressure, fasting glucose, diabetes remission, and adverse events.

Time (T): Short-term (≤6 months), intermediate (6-12 months), long-term (>12 months)

Eligibility Criteria

Previously published meta-analyses and systematic reviews of randomized controlled trials (RCTs) were included. Primary studies were excluded from analysis. We limited the search to peer-reviewed publications in English with no date restriction. We only included studies that examined the effects of low-carbohydrate diets on weight loss, metabolic syndrome, and T2D in humans. Studies that involved other clinical conditions such as epilepsy or cancer were excluded. Meta-analyses that included RCTs where low-carbohydrate diets were compared to low-fat or some other comparison dietary pattern were prioritized. We excluded very low-calorie or semi-starvation diets (<800 kcal/day). As a complementary analysis to make inferences on safety, efficacy, and sustainability, low-carbohydrate diet intervention studies were considered in the Discussion section that were not randomized but included either short-term highly controlled feeding interventions or single arm low-carbohydrate diet interventions over long periods of time.

Definition of Low-Carbohydrate Diets

Historically, authors of low-carbohydrate diets studies have not used a standardized definition nor followed a specific formulation and implementation framework. A common theme across studies is that dietary carbohydrate was restricted relative to a person’s habitual diet and a comparison diet, often emphasizing head-to-head comparison with a low-fat diet. More recently, definitions of carbohydrate diets have emerged (9,10), which helps to describe and interpret the heterogeneous nature of low-carbohydrate diet studies. Since many low-carbohydrate diet studies involve some type of caloric restriction, and therefore variable total caloric intake, it is most appropriate to define low-carbohydrate diets based on the absolute

amount of carbohydrate in grams per day. For purposes of this review the following terms are used to describe diets relative to their carbohydrate content (**Table 1**).

Table 1. Definitions of dietary patterns based on their carbohydrate content.

Diet	Carbohydrate (g/day)	Percent of Energy from Carbohydrate (%)*
Carbohydrate-restricted dietary pattern		
Ketogenic diet (very low-carbohydrate, high-fat)	20-50	<10%
Low-carbohydrate (non-ketogenic) diet	51-129	10-25%
Noncarbohydrate-restricted dietary pattern		
Moderate-carbohydrate diet**	130-224	26-44%
High-carbohydrate diet	225-324	45-64%
Very-high-carbohydrate diet	≥325	≥65%

**Based on a 2,000 kcal/day diet.*

***Considered as a "low-carbohydrate diet" for purposes of this umbrella review since many study authors defined a low-carbohydrate diet as less than 45% energy intake from carbohydrate.*

Based on these definitions, a low-carbohydrate diet consists of fewer than 130 grams per day. Because low-carbohydrate diets are limited in carbohydrate and moderate in protein, the majority of other calories are derived from dietary fat. Thus, low-carbohydrate diets are often referred to as low-carbohydrate/high-fat (LCHF) diets. While lower than the typical amount of carbohydrate in the standard American diet, intakes between 130-224 grams per day (26-44%) would be consider "moderate-carbohydrate" diets and those with >224 grams per day (>45%) as "high-carbohydrate" diets. It is acknowledged that some of the studies described as low-carbohydrate included in meta-analyses would be considered moderate-carbohydrate using this terminology.

Ketogenic diets are a subset of low-carbohydrate diets that usually consist of 20-50 grams carbohydrate per day with the goal of elevating circulating ketone bodies into a range referred to as physiological or nutritional ketosis. Nutritional ketosis is distinct from keto-acidosis. Ketogenic diets are adequate but not excessive protein, and consist of varying amounts of fat depending on the intended body weight goals. At these lower levels of carbohydrate intake, the production of ketone bodies in the liver is upregulated, which are then exported to extrahepatic tissues where they serve as an efficient alternative metabolic fuel and signaling molecule. Ketone bodies, notably beta-hydroxybutyrate – the primary circulating ketone body – exert physiologic effects on energy yielding and regulatory pathways (11,12).

Search Strategy

A literature search limited to “meta-analyses” was conducted in PubMed, Scopus, Web of Science, and Cochrane Database of Systematic Reviews using the terms “low-carbohydrate diet”, “ketogenic diet”, “low-carbohydrate/high-fat diet”, “very low-carbohydrate diet”, “carbohydrate-restricted diet”, and “Atkins Diet” in September 2025. Retrieved articles were screened for the predefined inclusion and exclusion criteria. Only meta-analyses that reported weight loss, metabolic syndrome or T2D outcomes were selected. Additional searches were conducted in the references listed in all identified reviews.

Data Extraction and Synthesis

Full-text articles meeting criteria were downloaded and reviewed. Aggregated results from each meta-analyses were extracted and summarized, but not the individual study-level data. Key details summarized were the study population, number of trials and total number of participants (n size) examined, the definition of a low-carbohydrate diet and the comparator diet, key outcomes, and takeaway conclusions. This also included classifying each meta-analysis into one of three categories regarding the effect of low-carbohydrate diets relative to the comparator diet on weight loss, metabolic syndrome markers, and T2D outcomes over any duration: less effective (↓), neutral (↔), or more effective (↑).

Results

A total of 34 meta-analyses (13-46) were identified as meeting the inclusion and exclusion criteria, which are summarized in **Table 2**. The articles were published between 2008 and 2025, and all performed a meta-analysis examining the consumption of low-carbohydrate diets with reported outcomes on weight loss, metabolic syndrome markers, and/or T2D management in adults. The articles were published across a wide range of independent, peer-reviewed journals and included trials conducted in multiple countries.

The exact definition of a low-carbohydrate diet varied, but no meta-analysis included low-carbohydrate diets that were above 45% of energy from carbohydrate. Most articles defined low-carbohydrate diets as described in **Table 1** as <130 g/day or <26% of energy from carbohydrate, although many included higher amounts of carbohydrate including up to 45% of energy (which would be considered moderate-carbohydrate). Several articles either distinguished or focused on very low-carbohydrate ketogenic diets, defined as <50 g/day or <10% of energy from carbohydrate. Although these definitions represent a wide intake of carbohydrate, it is fair to say in all cases the low-carbohydrate diets evaluated were intended to have less carbohydrate relative to the comparison diets.

The populations studied were mostly overweight and obese adults with or without T2D. Several meta-analyses (n=14) focused on individual with a diagnosis of T2D and reported on glucose control and other parameters related to T2D management (e.g., reversal or remission). The duration of follow-up varied widely from short-term (a few weeks) to as long as 2 years. Most meta-analyses reported on outcomes at 3, 6, and 12-months. The number of individual RCTs assessing long-term follow-up at 2-years is relatively small and there were credible concerns with compliance and adherence to the assigned diets as it is known that this is challenging with any dietary intervention.

Overall, the vast majority of meta-analyses (n=28/34 or 82%) reported significant benefits of low-carbohydrate diets over higher-carbohydrate comparators on one or more of the outcomes in the short-term. In no cases were low-carbohydrate diets shown to be inferior to higher-carbohydrate diets. In general, the benefits of low-carbohydrate diets on weight loss and T2D were strongest in the short-term and attenuated when examining moderate- to long-term differences with higher-carbohydrate diets. Thus, there is universal agreement across these meta-analyses that low-carbohydrate diets do at least as well as higher-carbohydrate diets, and they tend to outperform them in the short-term. Put in terms of an inferiority analysis, there was no evidence from a single meta-analysis that low-carbohydrate diets were inferior to low-fat diets. In the articles that distinguished ketogenic diets, the benefits compared to higher-carbohydrate diets were more consistent and of higher magnitude (19,21,27,31,37,40,43,44).

In the short-term (≤6 months), compared to higher-carbohydrate diets, low-carbohydrate diets were associated with moderately greater weight loss, decreased wait circumference and fat mass, better glycemic control (decreased HbA1c or fasting blood glucose), and improved dyslipidemia (decreased triglycerides and increased HDL-C). Low-carbohydrate diets were shown to decrease blood pressure and increase LDL-C in some meta-analyses although the results were modest and variable across studies.

In the intermediate-term (6-12 months) studies, the differences between low-carbohydrate and higher-carbohydrate diets were lessened with some studies showing continued benefits of low-carbohydrate diets, especially ketogenic diets. In long-term (>12 months) studies there is little confidence in the findings due to limited individual studies and adherence concerns.

Two of the meta-analyses focused specifically on metabolic syndrome outcomes (30,45) and both reported that low-carbohydrate diets improved all metabolic syndrome-related markers including weight, waist circumference, systolic and diastolic blood pressure, blood glucose, HbA1c, HDL-C, and triglycerides.

Fourteen meta-analyses focused on individuals with T2D (13,23-26,31,32,34-37,40,42,46). In the short-term (≤6 months) low-carbohydrate, and especially ketogenic diets, produced consistently lower HbA1c, blood glucose, triglycerides, increased HDL, and reduced weight. These benefits tended to wane or disappear at 12 months with adherence consistently cited as an important issue. The trials that focused on ketogenic diets produced the most consistent improvements in glycemic control, weight loss, and improvements in dyslipidemia (31,37,40).

Discussion

There has been a great deal of interest in low-carbohydrate diets over the last two decades as reflected by the relatively large number of meta-analyses performed contrasting this eating pattern to diets higher in carbohydrate. The majority of these studies have focused on outcomes related to weight loss, metabolic syndrome markers, and T2D management. In the 34 meta-analyses reviewed here, despite all the differences across articles and the individual studies selected, there are some common findings that warrant calling out. Taking the most conservative view, there was uniformity across all meta-analyses that low-carbohydrate diets performed at least as well as low-fat diets (i.e., no studies showed that higher carbohydrate,

low-fat diets were associated with greater weight loss, improved markers of metabolic syndrome, or better management of T2D). Nearly all meta-analyses showed benefit of low-carbohydrate diets during the short-term. This is likely due in part to there being more studies that compared low-carbohydrate and low-fat diets <6 months and the better adherence achieved in short-term studies. Nearly all meta-analysis were consistent in showing any benefits of low-carbohydrate diets were attenuated after 6 months. This is likely due to fewer available studies to analyze and the deteriorating adherence to assigned dietary protocols that are known to be a major challenge in long-term diet studies.

Weight Loss Considerations

Most of the meta-analysis did not report on the composition of weight loss in terms of fat mass and lean mass. Systematic reviews and meta-analyses have shown that low-carbohydrate diets beyond a few weeks show a similar or greater loss of body fat compared to a low-fat diet (21,47-49).

In many studies, individuals assigned to the low-carbohydrate diet were encouraged to restrict carbohydrate but not provided with explicit instructions to decrease caloric intake as was the case for those assigned to the low-fat diet. This protocol difference would tend to work against the low-carbohydrate diet for weight loss, yet this was not evident in the results.

It can be difficult for people with excess adiposity to make the behavioral modification necessary to sustain a low-carbohydrate diet, or any dietary pattern for that matter, in a free-living environment. Although with proper education and support it is possible. In a non-randomized study of a low-carbohydrate ketogenic diet in individuals with T2D provided frequent coaching, there was clinically significant weight loss at 1 year (-12%) and 2 years (-10%) that was sustained at 5 years (-7.6%)(50).

Although not a focus of this narrative umbrella review, studies have reported that individuals who are insulin sensitive tend to respond similarly to either low-fat or low-carbohydrate diets, but those with insulin resistance lose significantly more weight on the latter (51,52). Thus, the degree of insulin resistance a person has before adopting a diet may be one factor that determines how they respond to diets varying in carbohydrate.

Metabolic Syndrome Considerations

The findings from this narrative umbrella review that low-carbohydrates improve markers of metabolic syndrome mimic the responses observed in short-term highly controlled feeding trials where low-carbohydrate diets consistently improve dyslipidemia (53). They are also consistent with a meta-analysis (54) that was not included because it examined observational studies. They reported a linear association between increasing carbohydrate consumption and metabolic syndrome markers. For every 5% increase in energy from carbohydrate there was a 2.6% increase in the risk of metabolic syndrome. This is consistent with the findings from controlled feeding studies that demonstrate it is the carbohydrate restriction, not weight loss per se, that is the primary driver of improvement in metabolic syndrome markers (55).

Although LDC-C is not considered in the diagnosis of metabolic syndrome, it was assessed in many studies. Low-carbohydrates diets tend to raise LDL-C compared to low-fat diets, although the effect is quite variable. In studies where LDL-C is increased, it is accompanied by decreased triglycerides and increased HDL-C (53). There is also consistent evidence

demonstrating that low-carbohydrate diets change the LDL-C profile by decreasing smaller particles, which are believed to be more highly associated with CVD risk (53). The increase in LDL-C on a low-carbohydrate diet is mostly attributed to larger more buoyant LDL particles that are generally not associated with increased atherogenic risk (56).

Type 2 Diabetes Considerations

In the context of T2D, remission is defined as HbA1c <6.5% achieved with at least 3 months without glucose-lowering medications and T2D reversal is defined as HbA1c <6.5% achieved without glucose-lowering medications or only with metformin. Remission from T2D is infrequent without intervention (1-2%). There are few nutritional approaches that demonstrate T2D reversal or remission is possible. Formula total diet replacement with very low-calories (<1,000 kcal/day), which by their very low-calorie nature are lower in carbohydrate, are associated with an approximate 50% remission after 1 year and 10% remission after 5 years (57-61). These were not included in the meta-analyses because of the severe caloric restriction.

Long-term studies of low-carbohydrate ketogenic diets show similar potential for T2D reversal and remission, although they were not included in the meta-analyses because they were not RCTs. In 262 adults who had T2D for an average of 8.4 years (46% on insulin) and received telemedicine counseling on a ketogenic diet by a health coach and physician-guided medication management team, over half of the participants reversed their T2D after 1 year (62), where T2D reversal was defined as having a HbA1c below 6.5% while taking no diabetes medication or only metformin. Subjects also successfully reduced body weight, by an average of 12%, improved most of their cardiovascular risk factors, and 94% of subjects eliminated or reduced use of insulin medication (63,64). The majority of participants in this trial remained engaged in the program with patient retention of 83% at 1-year and 74% at 2-years (64). After 5 years 33% of completers demonstrated T2D reversal with sustained weight loss and improvements in triglycerides, HDL-C, and inflammatory markers, with no significant changes in LDL-C and total cholesterol (50). In a similar longitudinal study using this telemedicine approach over 2-years, 96 patients with pre-diabetes experienced a 52% reversal of their pre-diabetes diagnoses (65).

In a real-world general clinical practice that prescribed a low-carbohydrate diet to individuals with T2D over a period of 8 years, remission was achieved in 51% of the cohort (66). In those with T2D less than 1 year, remission was 77% and in those with T2D >15 years remission was 20%. There were significant improvements in blood lipids and blood pressure, as well as reductions in the cost of care.

It should be noted that when low-carbohydrate diets reverse T2D it is often in the context of reducing glucose-lowering and blood pressure medications. Because of the concern for hypoglycemia, the use of a low-carbohydrate diet or ketogenic diet for T2D should be done in conjunction with a medical team experienced in the de-prescription of these medications.

Based in large part on the findings from these trials demonstrating safety and efficacy of low-carbohydrate diets for T2D, the American Diabetes Association (ADA) updated its nutrition recommendations to allow for more flexibility. Starting with their 2019 standards of care for patients with diabetes, the ADA stated that “Low-carbohydrate eating patterns, especially

very low-carbohydrate (VLC) eating patterns, have been shown to reduce A1C and the need for antihyperglycemic medications. These eating patterns are among the most studied eating patterns for type 2 diabetes." (67,68). Other countries have adopted a similar position acknowledging low-carbohydrate diets effective in management of T2D (69,70).

Low-Carbohydrate Nutrition

- Well-constructed, nutrient-dense, low-carbohydrate dietary patterns are adequate and comparable in diet quality to existing DGA menu models. They can include a wide range of whole foods.
- The formulation of safe, effective, palatable, and sustainable low-carbohydrate diets entails relatively simple adjustments in conventional diets, focused primarily on replacing sugar- and carbohydrate-dense foods with un-processed, low-carbohydrate/high-fat foods.
- Proper formulation entails restriction of carbohydrate and intake of adequate—but not high—protein and sufficient minerals to offset the natriuretic effect of ketosis and lower insulin levels. Counting calories is usually not necessary.
- Adding a low-carbohydrate dietary pattern to the DGA is consistent with improved nutrition security and health equity.
- Long-term adherence with low-carbohydrate diets may be achievable and comparable to that of other healthy dietary patterns, given adequate education, resources, and support.
- Current eating patterns in the DGA do not reflect an adequate range of macronutrient distribution that could benefit metabolically vulnerable subpopulations such as Black and Hispanic populations that are at greater risk for impaired glucose/insulin dynamics.
- There is substantial evidence that allowing macronutrient flexibility, including a low-carbohydrate dietary pattern, within the DGA could help address health disparities and advance health equity by providing culturally tailored dietary options that address common metabolic issues in historically marginalized communities.
- Low-carbohydrate diets can be adequately adapted to diverse ways of eating including plant-based diets and culturally-relevant foodways.

Limitations And Gaps

This narrative umbrella review of meta-analyses of RCTs identified several limitations and gaps for future research to address. This review of meta-analyses was not quantitative and thus did not follow all the requirements typical of quantitative umbrella reviews. Given that the 34 meta-analyses were published over a relatively short time period, it is likely that the same original research studies were included in different meta-analyses. While such overlap of original research across meta-analyses would contribute to the observed uniformity of reporting the same findings, it also demonstrates consistency across many different authors and research groups from around the World, which provides a certain level of protection from bias. All the meta-analyses examined intermediate biomarkers (e.g., weight loss, lipids, glucose) and did not address hard endpoints. Adherence was acknowledged as a limitation in most articles, owing to the challenges in maintaining long-term compliance with diet regimens. There were inconsistencies in defining low-carbohydrate diets and how they were

formulated (e.g., high-fat versus higher protein) and implemented (e.g., varying degrees of education and support). There is also a lack of any objective biomarkers to verify compliance with diets, with the exception of ketogenic diets that often measure circulating ketones. Most diet intervention studies involved relatively small samples sizes, at least compared to pharmaceutical trials. Side effects were not adequately addressed in the meta-analyses, but there were no reports of serious adverse effects for any of the diets examined. Nutrient adequacy was not assessed in the studies. There is however compelling evidence that both low-fat and low-carbohydrate diets can be formulated with nutrient-dense food in a manner that achieves adequate essential macronutrient and micronutrient intakes.

Conclusion

This narrative umbrella review of 34 meta-analyses found that existing evidence from RCTs supports either a neutral or beneficial effect of low-carbohydrate diets on weight loss, metabolic syndrome, and T2D. None of the meta-analyses showed an inferior effect of low-carbohydrate diets relative to a higher carbohydrate comparison diet. While a majority of studies show short-term benefits of low-carbohydrate, especially ketogenic diets, as the intervention duration is extended beyond 6 months there is an increasing number of null findings. Based on the available evidence, including a low-carbohydrate dietary pattern as one option for people who are overweight or obese with metabolic syndrome or T2D is scientifically justified.

Table 2. Meta-analyses of low-carbohydrate diet studies on weight loss, metabolic syndrome, and T2D.

Study	Population / Trials	LC Definitions	Outcomes / Key Findings	Takeaway Conclusion	LC v HC Effectiveness
Kirk 2008 (13)	T2D, 13 studies (9RCTs) (n=263)	Carb-restricted ≤45%, 1-26wk	LCD improved HbA1c, FPG, TG, HDL ↑. LDL mixed.	LC beneficial in T2D for glycemia and lipid profile	↑
Hu 2012 (14)	Overweight/obese adults, 23 RCTs (n=2788)	LC ≤45% (mean 23%), 3–24 mo	Both reduced weight; no significant difference	LC ↑HDL, ↓TG; LF better for LDL/TC	↔
Santos 2012 (15)	Adults, 17 trials (n=1141) (within LC arm)	LC 3-36mo	LC arms: −7.0 kg weight, −4.0 kg fat, −7.4 cm waist LC ↑HDL, ↓TG, small ↑LDL	LC favorable for weight loss & CVD risk factors	↑
Bueno 2013 (16)	Overweight/obese, 13 RCTs (n=1415)	LC <130g/d or VLC <10%, ≥12 wk v LF	KD > LF (−0.9 kg at 1 y) LC ↑HDL, ↓TG; ↑LDL. ↓DBP	LC better for weight loss and dyslipidemia	↑
Johnston 2014 (17)	Overweight/obese, 48 RCTs (n=7286) (network)	LC (≤40%), LF, Atkins, Zone, 6-12 mo	Both LC & LF lost 7–9 kg; differences negligible	Minimal differences between diets	↔
Naudé 2014 (18)	Overweight/obese, 19 RCTs (n=1745)	LC (<45%) v balanced 3–24 mo	No clinically meaningful weight difference	Short- and long-term differences small	↔
Sackner-Bernstein 2015 (19)	Adults, 17 RCTs (n=1797)	LC ≤120g/d, 8 wk-24 mo	↓ weight (Δ=−2.0kg v LF), ↓ CV risk LC ↓TG, ↑HDL, small ↑LDL	KD better for weight loss and reducing CVD risk	↑
Tobias 2015 (20)	Adults, 53 RCTs (n=68128)	LF v other diets (incl. LC) ≥12 mo	LC > LF by −1.15 kg	LC better than LF for weight loss	↑
Hashimoto 2016 (21)	Obese, 8RCTs (n=1416)	VLC 50g/d or 10%; mild LC ~40%	↓ weight (−0.70kg) and fat mass; not significant >12 months; best results with KD	LCD, especially KD, better for decreased fat mass	↑
Mansoor 2016 (22)	Overweight/obese, 11 RCTs (n=1369)	LC (<20% CHO) v LF ≥6 mo	↓ weight (−2.17kg v LF), ↑HDL, ↑LDL	LC better for weight loss and dyslipidemia	↑
Meng 2017 (23)	T2D, 9 RCTs, (n=734)	LC <130g/d or <26%, 3-24 mo	HbA1c ↓0.44%, TG ↓, HDL ↑; weight −1.18 kg. LDL/TC no effect.	LC better for glucose control and dyslipidemia, but weight loss minimal	↑
Huntriss 2018(24)	T2D, 18 RCTs (7 in MA; n=2204)	LC <50-130g/d, 12 wk-1 yr	HbA1c ↓0.28%, TG ↓, HDL ↑, SBP ↓. No diff LDL, TC, weight.	LC better for glucose control and dyslipidemia, but weight loss minimal	↑

Study	Population / Trials	LC Definitions	Outcomes / Key Findings	Takeaway Conclusion	LC v HC Effectiveness
Sainsbury 2018 (25)	T2D, 25 RCTs (n=2412)	LC ≤45%, 3-24 mo	LC (<26%) ↓ HbA1c 3 and 6 mo, but not 12 mo, no weight benefit v controls. TG ↓, HDL ↑ early	LC but not MC better for glucose control and dyslipidemia, but weight loss minimal	↑
McArdle 2019 (26)	T2D, 25 RCTs (n=2132)	LC <50g/d, 50-130g/d, 138-293g/d, ≥8 wk	No overall HbA1c effect. Subgroup (≤6 mo, 50–130 g/day) → HbA1c ↓0.49%.	LC better for glucose control out to 6 mo	↑
Castellana 2020 (27)	Overweight/obese, 12 trials not all RCT (n=801)	KD v LF ≤12 mo	KD highly effective for weight loss and improving dyslipidemia out to 2 years	KD safe and effective long-term	↑
Smith 2020 (28)	Adults, 25 RCTs (n=3340)	LC (<150 g/d) v LF, 3–24 mo	Response heterogeneity between LC and LF similar	No diff LC v LF	↔
Chawla 2020 (29)	Adults, 38 RCTs (n=6499)	LC (≤40%) v LF	LC greater weight loss LC ↑HDL, ↓TG, ↑LDL	LC better for weight loss and dyslipidemia	↑
Willems 2020 (30)	Obese, 12 RCTs (n=1457)	LC (<40% to <20% CHO) 6-24mo	LC improved weight, waist circumference, TG, HDL-C. Smaller reductions in BP and FPG.	LC more effective for managing MetS, especially for obesity and dyslipidemia.	↑
Yuan 2020 (31)	T2D, 13 studies (n= 567)	KD 1-56wk	HbA1c ↓1.07%, FPG ↓1.29 mM, TG ↓0.72, TC ↓0.33, HDL ↑0.14, weight −8.7 kg.	Strong effects of KD on glucose control, weight, and dyslipidemia out to 1 year.	↑
Goldenberg 2021 (32)	T2D, 23 RCTs (n=1357)	LC <130g/d or VLC <10%, ≥12 wk	At 6 mo: HbA1c ↓0.41%, remission ↑ (RR 1.47), weight −3 kg. By 12 mo: no sig. diff.	LCD effective for short-term remission	↑
López-Espinoza 2021 (33)	Obese adults, 10 RCTs n=943)	KD/ VLC <10% 4 wk-2 yr	No significant benefit v balanced diet	No diff LC v LF	↔
Jayedi 2022 (34)	T2D, 50 RCTs, (n=4291) (dose–response)	Carb-restricted ≤45%, 6-12mo	Each −10% carbs → HbA1c ↓0.20%, FPG ↓0.34, weight ↓1.44 kg. LDL showed U-shaped response.	Carb restriction reduced levels of CVD risk in a linear fashion in T2D	↑
Apekey 2022 (35)	T2D, 22 RCTs (n=1391)	LC <130g/d or <26%, 3-24 mo	LC improved HbA1c, weight, TG at 3 mo; effects waned thereafter.	Short-term efficacy of LC but differences with LF are minimal long-term.	↑
Parry-Strong 2022 (36)	Prediabetes or T2D, 8 RCTs (n=606) (NZ trials)	VLC/KD ≤50g/d or ≤10%, 3-24 mo	LCD/KD produced early HbA1c and weight benefits but not superior at 12 mo.	Short-term efficacy of LC but differences with LF are minimal long-term.	↑

Study	Population / Trials	LC Definitions	Outcomes / Key Findings	Takeaway Conclusion	LC v HC Effectiveness
Zhou 2022 (37)	Overweight T2D, 8 RCTs (n=611)	KD <50g/d, 3 mo-2 yr	HbA1c ↓0.38, TG ↓0.36, HDL ↑0.28, weight −5.6 kg, waist −2.3 cm. No change in LDL/TC.	KD effective for overweight T2D for glucose control, weight loss, & dyslipidemia;	↑
Naudé 2022 (38)	Overweight/obese Adults, 19 RCTs (n=3209)	LC (<45%) v balanced CHO 3-24mo	Little to no difference between diets (<1 kg) Similar LDL, HbA1c, BP	LC no diff than LF for weight loss and lipids	↔
Silverii 2022 (39)	Obese, 26 RCTs	LC mild 26-45%, VLC <26% or <130g/d, 3-30 mo	LC advantage at 3–8 mo (≈−2.6 kg), none at 10–30mo LC ↓TG, ↑HDL	LC better short-term weight loss & long-term effects on CVD risk factors risk factors	↑
Jing 2023 (40)	T2D, 42 RCTs (n=4,809); 10 diet types	10 dietary approaches, ≥6 mo	LCD ↓HbA1c (−0.69%); KD ↓(−0.73%). Mediterranean & low-GI also effective.	KD most effective for glucose control	↑
Akbari 2024 (41)	Overweight/obese, 7 RCTs (n=1004)	Mediterranean, LC, LF 3-12mo	LC better than LF and Mediterranean diet for short-term weight and fat loss	LC associated with greater weight loss compared to other diets	↑
Hironaka 2024 (42)	T2D, 6 RCTs (n=400) 3-18mo	Not defined	HbA1c ↓0.25%, weight & TG ↓, HDL ↑; LDL NS.	LC better than control diets for weight loss and improving HbA1c	↑
Liao 2024 (43)	Overweight/obese adults, 17 RCTs (n=5802)(network)	KD, LF, LC, Mediterranean up to 24mo	Ranking: KD > LF > LC > Mediterranean	KD better than other diets for weight loss	↑
Leung 2025 (44)	Overweight/obese adults, 33 RCTs (n=2821)	KD/LC ≤100 g/d v LF 1-24mo	LC significantly ↓BW, BMI, fat %; strongest ≤1 mo, <50 g CHO/day best results	LC, especially KD, results in greater weight loss	↑
Zheng 2025 (45)	Adults, 30 RCTs (n=3,806)	LC = 50–130 g/day or 10–40%; subgroup VLCD <25% energy ≥12wk	LC better for weight loss, WC, BP, FBG, & dyslipidemia	LC more effective on all markers of metabolic syndrome	↑
Badrooj 2025 (46)	T2D, 80 RCTs (n=9232)	VLC, high-protein, calorie-restricted, 4-192 wk	VLC most effective for improving HbA1c, weight, TG at 6–12 mo	VLC more effective for glucose control and weight loss	↑

CVD=cardiovascular disease; LC=low-carbohydrate diet; DBP=diastolic blood pressure; HbA1c=hemoglobin A1c; LF=low-fat diet; FPG=fasting plasma glucose; GI=glycemic index; HC=high-carbohydrate diet; KD=ketogenic diet; MA=meta-analysis; MetS=metabolic syndrome; MC=moderate-carbohydrate diet; NZ=New Zealand; RCT=randomized clinical trial; RR=relative risk; SBP=systolic blood pressure; TC=total cholesterol; TG=triglycerides; T2D=type 2 diabetes; VLC=very low-carbohydrate diet.

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Appendix 4.6. Reducing Saturated Fat Below 10% of Energy and Risk of Coronary Heart Disease

CAUSAL EVIDENCE ON REDUCING SATURATED FAT BELOW 10% OF ENERGY AND RISK OF CORONARY HEART DISEASE AND MORTALITY

A Systematic Review

Daisy Zamora, PhD
Department of Physical Medicine and Rehabilitation
The University of North Carolina at Chapel Hill

Michael Goran, PhD
Professor and Vice Chair for Research
Department of Pediatrics, Keck School of Medicine
Children's Hospital of Los Angeles
University of Southern California

Abstract

Background

For decades, U.S. dietary guidelines have advised limiting saturated fatty acids (SFA) to <10% of energy to prevent coronary heart disease (CHD), reflecting the traditional diet-heart hypothesis that replacing SFA with polyunsaturated fatty acids (PUFA) would lower CHD risk through reduction in serum cholesterol. However, it remains unclear whether findings from randomized controlled trials (RCTs) demonstrate that limiting SFA intake lowers CHD or mortality.

Objective

The purpose of this systematic review is to identify and synthesize existing systematic reviews and meta-analyses of RCTs on SFA modification and to evaluate whether these studies provide evidence that reducing SFA below 10% of energy lowers coronary heart disease or all-cause mortality.

Methods

Searches of PubMed/MEDLINE, Embase, and the Cochrane Database of Systematic Reviews (January 2010 - August 2025; adults; English) identified systematic reviews/meta-analyses of RCTs that modified or replaced SFA and reported coronary heart disease (CHD) endpoints or all-cause mortality. Two reviewers screened and extracted data independently. Included reviews were classified as estimating causal substitution of SFA (e.g., trials of targeted replacement with a prespecified macronutrient) or not estimating causal substitution (e.g., studies with large between-group differences in multiple dietary components). Methodological quality was assessed with the Risk of Bias in Systematic Reviews (ROBIS) tool. Findings were synthesized narratively without new quantitative pooling. Certainty of evidence for each clinical outcome was graded using the GRADE framework.

Results

Nine reviews that met criteria for inclusion were identified. Only three reviews were classified as estimating causal substitution of SFA; all three examined the effect of replacing SFA with omega-6 PUFA (mainly linoleic acid from vegetable oils). Pooled estimates showed no reduction in all-cause mortality (moderate certainty) or CHD mortality (moderate certainty) and no consistent effect on CHD events (very low certainty). Apparent benefits identified in other reviews were attributable to inclusion of non-randomized or multicomponent trials. Evidence for SFA replacement with monounsaturated fats, protein, or carbohydrate was absent or insufficient.

Conclusions

Causal evidence from RCTs does not demonstrate that reducing SFA to <10% of energy—particularly through replacement with linoleic acid rich vegetable oils—lowers CHD or all-cause mortality. Because existing trials provide little information on other potential replacements and cannot isolate effects of saturated fat apart from the

nutrients that replace it, strong conclusions about the health effects of SFA intake cannot be drawn until modern, substitution-specific trials test clearly defined replacements and assess their clinical effects.

Introduction

Since the early 1960s, dietary guidance in the United States has consistently advised limiting saturated fat (SFA) intake to reduce coronary heart disease (CHD).[1-4] These recommendations were incorporated into the first Dietary Guidelines for Americans (DGA) in 1980 [5] and have persisted for over four decades. In the most recent 2020-2025 DGA, this guidance remains explicit: "For those two years and older, intake of saturated fat should be limited to less than 10 percent of calories per day by replacing them with unsaturated fats, particularly polyunsaturated fats."[6] The consistency of this recommendation reflects long-standing confidence in the traditional diet-heart hypothesis, which posits that the serum cholesterol lowering effects of replacing SFA with vegetable oil rich in linoleic acid (LA, an omega-6 PUFA) will slow progression of atherosclerosis, reduce CHD events, and improve survival.

The mechanistic rationale for reducing SFA arose from controlled feeding studies showing that isocaloric replacement of SFA with LA lowers total and LDL-cholesterol concentrations.[7,8] Because statins—agents that reduce LDL-C via inhibition of cholesterol synthesis—were subsequently proven in RCTs to reduce CHD morbidity and mortality,[9] it was inferred that dietary lowering of LDL-C would have a similar benefit. However, whether LDL-C reduction achieved through dietary modification produces the same causal effect on CHD events as pharmacologic LDL-C reduction has yet to be proven.[10]

To test this hypothesis, a series of large dietary RCTs were conducted from the 1960s through the 1980s, comparing usual diets of the time versus diets lower in SFA and higher in LA and/or other nutrients. However, despite decades of consistent guidance, the strength and certainty of the evidence linking reduced SFA intake—particularly below 10% of energy—to lower CHD or mortality risk remains uncertain.[11,12] Concerns have persisted regarding the design, conduct, and interpretation of early trials, as well as the quality and relevance of later syntheses that combined heterogeneous dietary interventions and study designs.

Why Causal Evidence Matters

When formulating dietary recommendations, distinguishing between association and causation is crucial.[13-15] Observational studies can identify correlations between SFA intake and cardiovascular outcomes, but they cannot adequately control for confounding or isolate the effects of specific macronutrient substitutions. Causal inference typically requires randomized allocation to interventions that differ only in the variable of interest—here, the nutrient replacing SFA—while holding other dietary and lifestyle factors constant. Under the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework,[16] RCTs are favored as high-certainty evidence for

causal effects because randomization minimizes confounding and establishes temporality between exposure and outcome. Reliance on causal evidence helps ensure that public health recommendations achieve their intended effects and avoid unintended outcomes.[13] A recent example comes from allergy prevention. For many years, guidelines recommended delaying peanut introduction,[17,18] during which peanut allergy prevalence in children markedly increased.[19,20] High-quality RCTs later showed that introducing peanut-containing foods at 4-6 months reduces allergy risk by 70-80%,[21,22] prompting a global reversal of guidelines and demonstrating how untested advice can inadvertently cause harm.[18]

Defining Causal Evidence in Nutrition

In the context of dietary fats, causal evidence refers to findings from RCTs that estimate the clinical effects of reducing SFA while maintaining total energy intake through reasonably controlled replacement with a prespecified macronutrient. A rigorous RCT testing whether reducing SFA below 10% of total energy improves cardiovascular outcomes should therefore (1) replace SFA with a predefined nutrient—such as LA-rich PUFA or oleic-rich monounsaturated fat (MUFA), in an isocaloric fashion; (2) maintain equivalence in other dietary components so that factors such as carbohydrate quality, intake of added sugars, fiber, vegetables, fish or long-chain n-3 fatty acids, and trans-fat exposure do not differ meaningfully between groups. Without these controls, observed effects cannot be attributed to SFA reduction itself. Equally important are design safeguards: blinding or treatment concealment (e.g., neutral-packaged oils or spreads), equivalent participant contact time across groups, and blinded endpoint adjudication. Trials should also be long enough and powered to detect differences in clinical rather than surrogate endpoints because changing biomarkers that might be indicators of risk is not the same as changing the endpoints of interest, such as reduced incidence or mortality.

Objective of This Review

Given the persistent public-health emphasis on limiting SFA to <10%E and the evolving understanding of dietary lipid mechanisms, it is essential to critically evaluate the evidence underlying this guidance. The purpose of this systematic review is to identify and synthesize existing systematic reviews and meta-analyses of RCTs that tested SFA modification in relation to CHD and mortality outcomes. Since reducing one macronutrient inevitably increases another in isocaloric and eucaloric diets, interventions to “reduce SFA” cannot be interpreted without specifying what replaces it. For example, replacing SFA with refined carbohydrate may differ fundamentally from replacing SFA with MUFA, both in metabolic consequences and potential cardiovascular risk. Thus, we specifically sought reviews that isolated the nutrient-substitution contrast necessary to infer causality—RCTs that replaced SFA with a prespecified macronutrient (PUFA, MUFA, carbohydrate, or protein) while avoiding multifactorial interventions that confound dietary effects (e.g., simultaneous increases in

fruits, vegetables, fiber, or fish, or behavioral components such as weight loss and smoking cessation).

Methods

The review protocol prespecified the objectives, eligibility criteria, and analytical framework (see Supplement). Comprehensive searches were conducted in PubMed/MEDLINE, Embase, and the Cochrane Database of Systematic Reviews for publications between January 2010 and August 2025. Search strategies combined controlled vocabulary and text words for SFA, CHD, and mortality outcomes, and systematic review/meta-analysis filters. Full search strings for each database are presented in Supplement. Searches were limited to human studies published in English. Two independent reviewers screened all records. Duplicates were removed programmatically, and titles and abstracts were screened for relevance. Full texts were retrieved for all potentially eligible records or when inclusion status was uncertain. Discrepancies at any stage were resolved by consensus.

Eligible reviews met the following inclusion criteria: (1) self-identified as a systematic review and meta-analysis of RCTs in adults (≥18 years); (2) examined replacement of dietary SFA; and (3) reported at least one of the following primary outcomes: CHD events (fatal/nonfatal), CHD mortality, or all-cause mortality. We excluded reviews without reproducible systematic methods; reviews published before 2010; and reviews including only observational studies or mixed designs that did not report separable RCT results. We excluded reviews that reported only surrogate outcomes such as serum cholesterol or blood pressure. Reviews that included only multifactorial dietary interventions (e.g., “heart-healthy,” “Mediterranean,” “DASH,” or “prudent” diets) were excluded when multiple nutrients or food groups were modified simultaneously (e.g., fruits, vegetables, fish, or fiber), because these designs preclude isolating the effects of SFA reduction.

Data extraction was performed independently by two reviewers with disagreements resolved by consensus. When reviews included mixed study designs, only RCT-derived estimates were extracted. We verified the list of RCTs included in each meta-analysis to confirm study design, dietary interventions, and eligibility. To clarify overlap across reviews, we constructed a citation matrix listing all RCTs included in each systematic review. This mapping enabled quantification of overlap, identification of unique versus duplicated trials, and assessment of whether differences in results was explained by differences in trial inclusion.

The methodological quality of each included review was assessed using the Risk of Bias in Systematic Reviews (ROBIS) tool,[23] which evaluates four domains: study eligibility criteria, identification and selection of studies, data collection and appraisal of included studies, and synthesis and findings. Findings were synthesized narratively; no new quantitative meta-analyses were performed. Each included review was classified by causal focus as either (1) a causal substitution review—those explicitly analyzing replacement of SFA with a prespecified macronutrient (e.g., LA-rich PUFA, MUFA,

carbohydrate)—or (2) a non-causal review—those including trials with heterogeneous or multicomponent dietary interventions. ROBIS results were used, in combination with each review's causal focus, to identify the "anchor", or lead review, for each outcome (operationalized as those that explicitly assessed the causal effect of SFA replacement and were rated at the lowest overall risk of bias). When reviews included dose-response analyses, we summarized their approaches and findings to assess whether greater SFA reductions (<10% of total energy) were associated with larger effects on CHD or mortality outcomes. The certainty of the evidence was evaluated in GRADEpro GDT using the GRADE framework.[16]

Results

Overview of Included Reviews

Database searches identified 794 unique records from PubMed, Embase, and the Cochrane Library. Of those, 177 were duplicates and 617 were screened. Following title and abstract screening, 14 articles were assessed in full text, and nine reviews met inclusion criteria and were included in the synthesis (see PRISMA Flow Diagram, detailed study characteristics, and ROBIS quality ratings in the Supplement). The nine included reviews were published between 2010 and 2025 and collectively evaluated evidence from 17 primary studies addressing dietary SFA reduction or modification in adults, with varying levels of specificity regarding replacement nutrient, intervention design, and clinical endpoints (**Table 1)**. Some reviews focused specifically on replacement of SFA with n-6 PUFA, primarily LA-rich vegetable oils such as corn, safflower, or soybean oil. Others included trials involving mixed fat replacement (n-6 PUFA, n-3 EPA+DHA, and MUFA), carbohydrate, trans-fat reduction, or broader changes in dietary patterns. Comparator diets generally reflected higher-SFA intake or "usual" control diets consistent with prevailing population patterns at the time of each trial. We had planned to classify reviews by substitution pattern (e.g., SFA→PUFA, SFA→MUFA, SFA→carbohydrate). However, no RCT-based reviews were found for SFA→MUFA or SFA→carbohydrate replacement; all identified reviews addressed SFA→PUFA or mixed dietary modifications. Methodological quality as assessed by the ROBIS tool ranged from low to high. The most common sources of bias involved inclusion of multicomponent dietary trials, misclassification of non-randomized studies, and insufficient reporting of concurrent dietary changes, which obscured causal interpretation of SFA replacement effects.

Table 1. Characteristics of Included Reviews

Review Name [a]	Intervention	RCTs (participants)	Outcomes	Effect Estimates	I^2	Randomized Controlled Trials [b]	ROBIS Risk of Bias
Mozaffarian 2010 [24]	n-6 + n-3 PUFA, multicomponent	7 (13,614)	MI or CHD deaths	RR 0.81 (0.70-0.95)	37%	DART, **FMHS**, LAV, MCE, MRC, **ODHS**, STARS	High
Ramsden 2010 [25]	n-6 PUFA	3 (9,569)	All-cause mortality	RR 1.16 (0.95-1.42)	NR	MCE, RCOT, SDHS	High
		2 (9,111)	CHD deaths	RR 1.17 (0.82-1.68)	NR	MCE, RCOT	
		2 (9,111)	Nonfatal MI + CHD deaths	RR 1.13 (0.84-1.53)	NR	MCE, RCOT	
		2 (9,111)	Nonfatal MI	RR 1.03 (0.62-1.73)	NR	MCE, RCOT	
	Sensitivity analysis including n-3 EPA+DHA and multicomponent interventions	7 (11,275)	All-cause mortality	RR 0.92 (0.80-1.06)	NR	LAV, MCE, MRC, **ODHS**, RCOT, SDHS, STARS	
		6 (10,817)	CHD deaths	RR 0.81 (0.64-1.03)	NR	LAV, MCE, MRC, **ODHS**, RCOT, STARS	
		6 (10,817)	Nonfatal MI + CHD deaths	RR 0.78 (0.65-0.93)	NR	LAV, MCE, MRC, **ODHS**, RCOT, STARS	
		6 (10,817)	Nonfatal MI	RR 0.73 (0.54-0.99)	NR	LAV, MCE, MRC, **ODHS**, RCOT, STARS	
Chowdhury 2014 [26]	n-6 + n-3 PUFA, multicomponent	8 (14,476)	MI or CHD deaths	RR 0.86 (0.69-1.07)	59%	DART, **FMHS**, LAV, MCE, MRC, **ODHS**, SDHS, STARS	High
		7 (14,018)	MI or CHD deaths	RR 0.81 (0.68-0.98)	NR	DART, **FMHS**, LAV, MCE, MRC, **ODHS**, STARS	
Schwingshackl 2014 [27]	n-6 + n-3 PUFA, multicomponent	6 (3,405)	All-cause mortality	RR 0.99 (0.68-1.25)	44%	DART, MRC, **ODHS**, RCOT, SDHS, STARS	High
		6 (3,405)	CVD deaths	RR 1.05 (0.76-1.44)	51%	DART, MRC, **ODHS**, RCOT, SDHS, STARS	
		6 (3,405)	MI	RR 0.91 (0.65-1.29)	54%	DART, MRC, **ODHS**, RCOT, SDHS, STARS	
		6 (3,405)	CVD events	RR 0.85 (0.65-1.34)	61%	DART, MRC, **ODHS**, RCOT, SDHS, STARS	
Ramsden 2016 [10]	n-6 PUFA	5 (10,808)	All-cause mortality	HR 1.07 (0.90-1.27)	39%	LAV, MCE, MRC, RCOT, SDHS	Low
		5 (10,808)	CHD deaths	HR 1.13 (0.83-1.54)	45%	LAV, MCE, MRC, RCOT, SDHS	
	Sensitivity analysis including n-3 EPA+DHA and multicomponent interventions	8 (13,308)	All-cause mortality	HR 1.00 (0.87-1.15)	34%	DART, LAV, MCE, MRC, **ODHS**, RCOT, SDHS, STARS	
		8 (13,308)	CHD deaths	HR 1.00 (0.81-1.24)	38%	DART, LAV, MCE, MRC, **ODHS**, RCOT, SDHS, STARS	
Hooper 2018 [28]	n-6 PUFA	6 (4,154)	All-cause mortality	RR 1.00 (0.88-1.15)	3%	Amrita, DART, LAV, MRC, NDHS, SDHS	High
		5 (3,832)	CVD deaths	RR 1.04 (0.71-1.52)	71%	DART, Houtsmuller, LAV, MRC, SDHS	
		5 (4,441)	MI	RR 0.87 (0.75-1.01)	0%	DART, Houtsmuller, LAV, MRC, NDHS	
		5 (3,832)	CHD events	RR 0.85 (0.61-1.17)	80%	DART, Houtsmuller, LAV, MRC, SDHS	
		5 (4,797)	CVD events	RR 0.95 (0.78-1.16)	58%	DART, LAV, MRC, NDHS, SDHS	
		2 (2,879)	MACCEs	RR 0.84 (0.59-1.20)	79%	DART, LAV	
		4 (3,730)	Stroke	RR 1.36 (0.45-4.11)	56%	DART, LAV, MRC, SDHS	
Hooper 2020 [29]	n-6 + n-3 PUFA, MUFA, CHO, protein, multicomponent	11 (55,858)	All-cause mortality	RR 0.96 (0.90-1.03)	2%	Black, DART, LAV, Ley, MRC, **ODHS**, Rose, SDHS, STARS, WHI, WINS	High

Review Name [a]	Intervention	RCTs (participants)	Outcomes	Effect Estimates	I^2	Randomized Controlled Trials [b]	ROBIS Risk of Bias
		8 (53,159)	CHD deaths	RR 0.97 (0.82-1.16)	28%	DART, Houtsmuller, LAV, MRC, **ODHS**, Rose, SDHS, WHI	
		10 (53,421)	CVD deaths	RR 0.94 (0.78-1.13)	36%	Black, DART, LAV, Ley, MRC, **ODHS**, Rose, SDHS, STARS, WHI	
		10 (53,167)	Fatal MI	RR 0.90 (0.80-1.01)	10%	DART, Houtsmuller, LAV, Ley, MRC, Moy, **ODHS**, Rose, STARS, WHI	
		10 (53,199)	CHD events	RR 0.83 (0.68-1.01)	62%	DART, Houtsmuller, LAV, Ley, MRC, Moy, **ODHS**, Rose, STARS, WHI	
		12 (53,758)	CVD events	RR 0.83 (0.70-0.98)	67%	Black, DART, Houtsmuller, LAV, Ley, MRC, Moy, **ODHS**, Rose, SDHS, STARS, WHI	
		7 (52,834)	Nonfatal MI	RR 0.97 (0.87-1.07)	0%	DART, LAV, MRC, Moy, **ODHS**, Rose, WHI	
		7 (50,952)	Stroke	RR 0.92 (0.68-1.25)	9%	LAV, Ley, MRC, Moy, **ODHS**, STARS, WHI	
Jayedi 2024 [30]	n-6 PUFA	4 (11,602)	CHD events	RR 1.14 (0.87-1.49)	53%	DART, MCE, RCOT, SDHS	High
	n-6 + n-3 PUFA, multicomponent	5 (2,524)	CHD events	RR 0.71 (0.61-0.84)	0%	**FMHS**, LAV, MRC, **ODHS**, STARS	
Yamada 2025 [31]	n-6 + n-3 PUFA, multicomponent	9 (13,532)	All-cause mortality	OR 1.01 (0.89-1.14)	13%	Amrita, DART, LAV, MCE, MRC, **ODHS**, RCOT, SDHS, STARS	Low
		9 (13,532)	CVD deaths	OR 0.94 (0.75-1.19)	44%	Amrita, DART, LAV, MCE, MRC, **ODHS**, RCOT, SDHS, STARS	
		6 (3,962)	MI	OR 0.85 (0.71-1.02)	24%	Amrita, DART, LAV, MRC, **ODHS**, RCOT	
		9 (13,532)	CVD events	OR 0.85 (0.65-1.11)	55%	Amrita, DART, LAV, MCE, MRC, **ODHS**, RCOT, SDHS, STARS	

[a] For simplicity, we name the systematic reviews by the last name of the first author and the year it was published.
[b] Bolded trials (ODHS, FMHS) indicate studies not suitable for estimating SFA replacement effects: ODHS because of major co-interventions, and FMHS because it was not randomized.
Acronyms: Amrita=Amrita Institute of Medical Sciences, Black=Black (1994),[32] DART=Diet and Reinfarction Trial, FMHS=Finnish Mental Hospital Study, Houtsmuller=Houtsmuller (1979),[33] LAV=Los Angeles Veterans Administration Trial, Ley=Ley (2004),[34] MCE=Minnesota Coronary Experiment, Moy=Moy (2001),[35] MRC=Medical Research Council Soya-Bean Oil Trial, NDHS=National Diet-Heart Study, ODHS=Oslo Diet-Heart Study, RCOT=Rose Corn Oil Trial, Rose=Same as RCOT with additional intervention of olive oil, SDHS=Sydney Diet-Heart Study, STARS=St Thomas Atherosclerosis Regression Study, WHI=Women's Health Initiative, WINS=Women's Intervention Nutrition Study.
Abbreviations: CHD=coronary heart disease, CHO=carbohydrates, CVD=cardiovascular disease, HR=hazard ratio, MACCEs=major adverse cardiac and cerebrovascular events, MI=myocardial infarct, MUFA=monounsaturated fat, NR=not recorded, OR=odds ratio, PUFA=polyunsaturated fat, RR=risk ratio.

Overview of Principal RCT Evidence

Among the classic studies that form the foundation for evaluating the causal effects of dietary SFA reduction or replacement (**Supplementary Table 1**), a critical distinction emerges between multicomponent diet trials and those that tested nutrient substitution under controlled conditions (**Table 2**).

Trials Not Specifically Testing SFA Replacement

The Oslo Diet-Heart Study [36] is often cited as supporting SFA reduction (risk ratio for combined cardiovascular events= 0.71; 95% CI 0.55-0.92), yet the intervention involved major co-interventions unrelated to SFA substitution. Experimental dieters were advised

to replace meats and eggs with fish, shellfish, and “whale beef” and were supplied “considerable quantities of Norwegian sardines canned in cod-liver oil” providing approximately 5 grams per day of EPA + DHA (~30 times normal intake) alongside soybean oil delivering ~15.6% energy as LA and 2.7% as α-linolenic acid (ALA). The intervention group (but not the control group) also increased fruits, vegetables, and whole grains, while industrial trans-fat-rich margarines were eliminated from the diet. Thus, the ODHS intervention substantially increased n-3 PUFA, vitamin D, and other cardioprotective factors while simultaneously decreasing trans-fat exposure. The control diet, in contrast, provided an estimated 9.6% energy as trans-fat from partially hydrogenated fish oil (PHFO) margarines. This control group consumed a remarkable ~25%E from PHFO and partially hydrogenated vegetable oil margarines. Given these extensive between-group differences, the reduction in coronary events observed after five years cannot be attributed to SFA replacement. Notably, the ODHS was included in every meta-analysis that reported reduced risk of CHD events or deaths from SFA replacement (**Table 1**).

The Finnish Mental Hospital Study [37] is often cited as supporting SFA reduction (risk ratio for combined CHD events=0.59; 95% CI 0.46-0.75), yet it is not an RCT. Instead, two state hospitals alternated between a high-PUFA “cholesterol-lowering” diet and a conventional diet in a 12-year crossover design, during which the patient cohorts were “rejuvenated” midway by replacement of older patients with new admissions. This design was not only not randomized, but it was also biased towards the “SFA lowering” intervention group: the cardiotoxic antipsychotic thioridazine was disproportionately used more in the control group, trans-fat intake was restricted in the intervention group, and psychiatric comorbidities and medication patterns were unevenly distributed between the groups across the two hospitals. Moreover, thioridazine use is associated with sudden cardiac death (via drug induced arrhythmia) and alters electrocardiographic in a way that can mimic myocardial infarction. This is an example of why random allocation is essential to causal inference—to distribute known and unknown confounders evenly and prevent systematic bias that can create the illusion of benefit.

Trials Testing SFA Replacement

Only a small number of RCTs have replaced SFA with a predefined macronutrient (unsaturated fats) in a mostly controlled manner. These trials are the Minnesota Coronary Experiment,[38] Los Angeles Veterans Study,[39] Medical Research Council (MRC) Soy Oil Trial,[40] Rose Corn Oil Trial,[41] and Sydney Diet-Heart Study.[42] Collectively, these trials provided corn, safflower, or soybean oils (rich in LA, 50-75% of fatty acids) in place of animal fats, as well as shortenings and margarines containing SFA and industrial trans-fat.[25]

- The Minnesota Coronary Experiment (1968-73) was the largest (n=9,057) double blinded dietary RCT testing SFA replacement.10,38 Participants (institutionalized men and women with and without CHD) in six Minnesota state hospitals and a nursing home received a serum-cholesterol-lowering diet in which SFA was

halved (18.5 → 9% energy) and LA increased nearly three-fold (3.4 → 13% energy) using corn oil and corn-oil margarine. Despite an average between-group 13.8% reduction in serum cholesterol, there was no reduction in mortality: the hazard ratio for CHD death was 1.13 (95% CI 0.83-1.54) and for all-cause death 1.07 (0.90-1.27). Autopsy data showed no difference in coronary atherosclerosis and a higher incidence of myocardial infarcts in the intervention arm (41% vs 22%). Each 30 mg/dL fall in serum cholesterol was paradoxically associated with a 22% higher risk of death (HR 1.22; 95% CI 1.14-1.32).

- The Minnesota Coronary Experiment was explicitly designed to test whether replacing SFA with LA (from corn oil) reduced serum cholesterol and coronary events. Trans-fat rich margarines in the intervention diet would have undermined the cholesterol-lowering effects of LA—as per research by one of the principal investigators (Ancel Keys).43 By contrast, the control group consumed common margarines and shortenings—known sources of trans-fat and SFA. It has been suggested that although the intervention likely reduced total trans-fat intake relative to the control diet,44 the special corn-oil margarine used might have been higher in trans-linoleic acid specifically.3 However industrial partial hydrogenation of vegetable oils mainly converts LA (18:2 n-6) to trans-18:1 isomers, with trans-18:2 as only a trace component.45 Thus, even if lightly hydrogenated, the intervention margarine would have contained far less trans-fat overall (including trans-18:2) than the common margarines and shortenings used in the control diet. This means that any residual trans-fat exposure would have biased results in favor of the intervention, exaggerating the likelihood of detecting a benefit. The absence of a mortality reduction despite this potential bias further reinforces the conclusion that replacement of SFA with LA-rich oils did not reduce coronary or all-cause mortality in this trial.
- The Los Angeles Veterans Study (1959-67) randomized 846 male veterans to institutional feeding with corn/soybean-oil diets versus mixed-fat controls. The intervention achieved large differences in unsaturated fat intake (LA ~15% vs 5% energy) and reduced serum cholesterol ~12%. However, after eight years, there was no mortality benefit: HR 0.82; 95% CI 0.56-1.21 for CHD deaths and HR 0.97; 95% CI 0.83-1.14 for all-cause deaths.
- The MRC Soy Oil Trial (UK, 1960-67) replaced butter and animal fat with soybean oil (~16% energy LA, 2% ALA) among 393 men after myocardial infarction. After 2-7 years, the combined endpoint of non-fatal MI + CHD death did not differ between groups (RR 0.86; 95% CI 0.61-1.22).
- The Rose Corn Oil Trial (1962-65) randomized ambulatory men with CHD to either corn oil intervention (provided 64 g/day of corn oil [~15% energy LA in lieu of SFA]), olive oil intervention (provided 58 grams per day of olive oil [~19% energy MUFA in lieu of SFA]) or control group (no dietary fat advice or oil provided). The corn intervention group had four-fold higher mortality (RR 4.64; 95% CI 0.58-37.2) and investigators concluded that “corn oil cannot be recommended in the treatment of ischemic heart disease”.

- The Sydney Diet-Heart Study (1966-73) randomized 458 men (post-MI or CHD) to a safflower-oil and safflower-margarine diet versus usual diet containing butter and hard margarines. The intervention increased LA to ~14% energy, decreased SFA to 9.3% energy, eliminated n-3 PUFA, and reduced trans-fat exposure. Recovered data show 62% higher all-cause mortality (HR 1.62; CI 1.00 to 2.64) and 74% higher CHD mortality (HR 1.74; CI 1.04 to 2.92) in the intervention group compared to the control group.

Table 2. Diet-Heart Randomized Controlled Trials

Study	Years Active	Blinding	Between group differences in diet		Between-group differences in cholesterol reduction	Summary of major between-group confounders	Other limitations
			Dietary LA	Trans fat from partially hydrogenated oils			
Diet and Reinfarction Trial (n=2,033) [46]	~1983-1987	Single	Intervention: Unspecified Control: Unspecified	Intervention: restricted Control: ~2%E	-4.0%	Minimal changes and detailed information about diets not available.	Advice only-no foods/oil provided
Los Angeles Veterans Admin. Trial (n=846)	1959-1967	Double	Intervention: 14.8%E from corn and soybean oil Control: 4.8%E	Intervention: restricted Control: ~2%E	-12.7%	(1) PHVOs restricted in intervention group but provided estimated 7-8%E in control group; (2) Control group consumed extremely low <0.1% of energy from n-3 ALA (likely due to hydrogenation of control oils). Intervention increased to 0.7%E.	
Medical Research Council Soya-Bean Oil Trial (n=393)	1960-1967	Single	Intervention: 16.3%E from soybean oil Control: unspecified	Intervention: restricted Control: ~1.6%E	-13.3%	PHVOs restricted in intervention but not control.	Control group ate habitual diets so had less intensive intervention.
Minnesota Coronary Experiment (n=9,057)	1968-1973	Double	Intervention: 14.5%E from corn oil Control: 4.8%E	Intervention: restricted Control: ~2%E	-13.8%	Tightly controlled, double-blinded with no major between-group diet confounders.	Substantial censoring (average exposure slightly longer than one year)
Oslo Diet-Heart Study (n=412) [a]	1956-1964	Single	Intervention: 15.6%E from soybean oil Control: 2.6%E	Intervention: restricted Control: ~9-10%E	-13.9%	(1) Intervention provided very large dose of EPA+DHA (~5g per person daily); (2) PHFO/PHVOs provided remarkable 25%E in control group; (3) Intervention group ate less sugar and refined grains and more fruits, vegetables, and nuts	Controls ate habitual diets so had less intensive intervention

Study	Years Active	Blinding	Between group differences in diet		Between-group differences in cholesterol reduction	Summary of major between-group confounders	Other limitations
			Dietary LA	Trans fat from partially hydrogenated oils			
Rose Corn and Olive Oil Trial (n=80)	~1962-1965	Single	Intervention 1: 14.9% from corn oil Intervention 2: 19.3%E (as MUFA) from olive oil Control: unspecified	Intervention 1: restricted Intervention 2: restricted Control: ~1.6%E	-11.8% +4.6%	None noted	Small study
St Thomas Atherosclerosis Regression Study (n=55) [47] [b]	~1982-1990	Single	Intervention: 5.6%E Control: 4.0%E	Intervention: ~1.8%E Control: ~1.1%E	-12.2%	(1) Processed food and PHVOs restricted in intervention group (2) Fiber 53% higher in intervention group; (3) EPA+DHA doubled in intervention group; (4) Total fat 27% lower in intervention group.	Control group ate habitual diets so had less intensive intervention; SFA reduction unclear
Sydney Diet-Heart Study (n=458)	1966-1973	Single	Intervention: Unspecified Control: PUFA 8.4%E unspecified	Intervention: restricted Control: ~1.3%E	-7.8%	No known between-group diet confounders. PHVOs consumed by both groups.	Controls ate habitual diets so had less intensive intervention.

Since the Finnish Mental Hospital Study lacked randomization, it was not included in this table.
[a] Intervention group was provided sardines canned in cod liver oil (5g of EPA+DHA per day; 30 times the average US intake). They were also instructed to eat more fruits and vegetables and to restrict intake of refined grains and sugar.
[b] Intervention and control groups consumed 210mg per day and 100mg per day, respectively, of n-3 EPA+DHA. The intervention group was also instructed to eat less processed food and more fiber (increased 53%).
Abbreviations: %E=percent of energy, LA=linoleic acid, MUFA=monounsaturated fat, PHFO=partially hydrogenated fish oil, PHVO=partially hydrogenated vegetable oil, PUFA=polyunsaturated fatty acids, RCT=randomized controlled trial

Overlap of RCTs in the Included Systematic Reviews

The trials described above formed the foundation of nearly all subsequent meta-analyses examining the relationship between SFA intake, serum lipids, and CHD outcomes. The degree of overlap among reviews was quantified by constructing a citation matrix of all studies (see **Supplementary Table 1**). This mapping revealed that a small number of trials were repeatedly included across multiple reviews, many differing in their handling of the same set of studies. For example, MRC Soy Oil appeared in every review, Los Angeles Veterans and Rose trials appeared in nearly every review. Conversely, studies that cannot be considered tests of SFA replacement were included in the main analyses of six of nine reviews: the Oslo Diet-Heart Study (which combined SFA reduction with drastic reduction of trans-fat intake and ~40 times higher EPA+DHA intake in the intervention) and the Finnish Mental Hospital Study (which was not randomized).

The inclusion of multicomponent trials—those involving major between-group differences dietary components (e.g., increased fish, fruits, and/or vegetables;

decreased trans-fat intake), introduced variability in how reviews defined and interpreted "SFA reduction." This contributed to notable heterogeneity in reported results across analyses. One recent review expanded inclusion criteria to encompass larger but less specific interventions, such as the Women's Health Initiative (WHI) [48] and the St Thomas' Atherosclerosis Regression Study (STARS),[47] which further broadened the range of dietary contrasts represented.

Overall, the overlap analysis highlighted that the current evidence base for RCTs on SFA reduction and cardiovascular outcomes remains anchored in a relatively small set of historical RCTs. Differences among reviews primarily reflected variation in trial inclusion, treatment of comparator fat types, and handling of studies that involved partially hydrogenated oils or multifactorial dietary interventions.

Reviews Focused on the Causal Effect of SFA Replacement

Of the nine reviews we identified, only three—Ramsden 2010, Ramsden 2016, and Hooper 2018—isolated the effect of replacing SFA with another nutrient, and all three specifically examined replacement with LA-rich n-6 PUFA (**Table 3**). These reviews reported no benefit from this substitution for mortality or major cardiovascular outcomes. Hooper 2018 conducted a meta-analysis that assessed RCTs of increasing n-6 PUFA in place of SFA and found little or no effect on all-cause or cardiovascular mortality. Ramsden 2010 conducted a meta-analysis of trials replacing SFA with vegetable oils rich in LA and similarly observed no reduction in coronary events or deaths. Ramsden 2016 re-analyzed recovered data from the Minnesota Coronary Experiment (the largest RCT on the topic) and pooled it with other LA-specific trials (Los Angeles Veterans, MRC Soy Oil, Sydney Diet-Heart, and Rose Corn Oil). Despite large cholesterol reductions, no mortality benefit was observed. Of these three, only Ramsden 2016 was rated low risk of bias on ROBIS, whereas the other two were rated at high risk of bias (see Supplement for detailed ratings).

Table 3. Summary of Reviews Focused on the Causal Effect of SFA Replacement*

Review	Outcomes	Key Findings	Risk of Bias (ROBIS)
Hooper 2018	All-cause mortality, CVD mortality, CHD events	Increasing n-6 PUFA in place of SFA showed **no effect** on mortality or CHD events, even after applying post-hoc ≥12-month "continuous involvement" rule excluding MCE.	High
Ramsden 2010	CHD events, CHD mortality	Explicit SFA→ linoleic acid analysis found **no overall benefit** when SFA replaced vegetable oils rich in LA	High
Ramsden 2016	All-cause and CVD mortality, CHD events	Meta-analysis of LA-specific trials (Los Angeles Veterans, MRC Soy Oil, Sydney Diet-Heart, Rose Corn Oil, MCE). Despite large cholesterol reductions, **no mortality benefit** was observed; greater cholesterol lowering correlated with *higher* mortality.	Low

*Reviews that focused on RCTs replacing SFA with any nutrient, excluding overtly confounded trials (i.e., Oslo Diet Heart Trial) and any non-randomized trials (i.e., Finnish Mental Hospital Study).

Reviews Not Focused on the Causal Effect of SFA Replacement

The remaining six reviews—Mozaffarian 2010, Chowdhury 2014, Schwingshackl 2014, Hooper 2020, Jayedi 2024, and Yamada 2025—all used methods that were inconsistent with determining causal effects of SFA replacement (summarized on **Table 4**). All were rated high risk of bias, except for Yamada 2025, which was low risk (see Supplement for detailed ratings). Four (Chowdhury 2014, Schwingshackl 2014, Hooper 2020, and Yamada 2025) reported no benefit for all-cause, cardiovascular, or coronary mortality. Mozaffarian 2010 and Jayedi 2024 reported apparent benefit, however, both of their pooled estimates included non-randomized and multicomponent trials, notably the Oslo Diet-Heart Study and the Finnish Mental Hospital Study, which confound SFA reduction with trans-fat elimination and extremely high intake of marine n-3 PUFA (EPA + DHA). Their results therefore cannot be interpreted as the effect of replacing dietary SFA.

Table 4. Reviews *not* focused on the causal effect of SFA replacement*

Review	Outcomes	Key findings / interpretation	Risk of bias (ROBIS)
Mozaffarian 2010	CHD events, CHD mortality, all-cause mortality	Meta-analysis of 7 RCTs increasing PUFA intake; pooled benefit (19% lower CHD events) driven by studies not testing SFA replacement, i.e., **ODHS** (major co-interventions + extreme trans-fat control diet) and non-randomized **FMHS**.	**High**
Chowdhury 2014	CHD events, CHD mortality	Pooled 8 "PUFA-for-SFA" RCTs (same as Mozaffarian 2010 with added SDHS); null for n-6 PUFA (RR 0.86, 95% CI 0.69-1.07). Also included studies not testing SFA replacement, i.e., **ODHS** (major co-interventions + extreme trans-fat control diet) and non-randomized **FMHS.**	**High**
Schwingshackl 2014	All-cause and CVD mortality, CHD events, myocardial infarction	Secondary-prevention RCTs comparing reduced/modified-fat diets. No significant differences for mortality or CHD outcomes. Included **ODHS** (major co-interventions + extreme trans-fat control diet) and other multicomponent trials (STARS).	**High**
Hooper 2020	All-cause and CVD mortality, CHD mortality/events, combined CVD events	No effect on any "hard" endpoints (e.g., mortality or CHD events); small benefit only for "combined CVD events," a composite including soft outcomes (angina, revascularisation). Included **ODHS** (major co-interventions + extreme trans-fat control diet) and other multicomponent trials (WHI, STARS). Applied post-hoc ≥24-month rule excluding MCE.	**High**
Jayedi 2024	"Coronary events" (fatal + non-fatal + angina)	Reported lower risk with higher PUFA intake. Included studies not testing SFA replacement, i.e., **ODHS** (major co-interventions + extreme trans-fat control diet) and non-randomized **FMHS**.	**High**
Yamada 2025	All-cause and CVD mortality, CHD events	RCTs of SFA restriction regardless of replacement nutrient. Found no reduction in mortality or CHD outcomes. Included multicomponent/confounded interventions (**ODHS**, STARS).	**Low**

*Reviews that included overtly multifactorial trials (i.e., Oslo Diet Heart Trial) and/or non-randomized trials (i.e., Finnish Mental Hospital Study).
Abbreviations: CHD=coronary heart disease, CVD=cardiovascular disease, FMHS=Finnish Mental Hospital Study, MCE=Minnesota Coronary Experiment, ODHS=Oslo Diet-Heart Study, SDHS=Sydney Diet-Heart Study; PUFA=polyunsaturated fatty acid, SFA=saturated fatty acid.

Hooper 2020 found no effect of SFA replacement on all-cause or cardiovascular mortality, or on coronary events.[29] A nominal benefit was reported only for "combined cardiovascular events". However, this review incorporated multicomponent dietary trials, meaning that its pooled estimate reflects the effect of being assigned to an intervention in which SFA happened to decrease, rather than the effect of SFA reduction itself. For example, in the Women's Health Initiative trial, participants in the low-fat intervention arm were advised to increase fruits, vegetables, and whole grains while reducing total fat intake; the modest reduction in SFA intake (from ~12% to 9% of energy) occurred alongside substantial increases in carbohydrate, fiber, and micronutrient intake and greater weight loss compared with controls. Similarly, in the Oslo Diet-Heart Study, the

intervention combined SFA reduction with large increases in fish (extreme increase in omega-3 EPA+ DHA) and vegetable intake and elimination of trans-fat-rich margarines. Thus, the modest risk reduction of combined CVD events reported in Hooper 2020 reflects the cumulative influence of multiple concurrent dietary and behavioral changes, rather than an isolated causal effect of lowering SFA.

In Hooper 2020 the authors performed two trial-level dose-response analyses of SFA reduction and cardiovascular outcomes. Notably, greater reductions in SFA were not significantly associated with larger cardiovascular benefits. In a meta-regression of 8 RCTs, the authors calculated associations between changes in saturated fat and "cardiovascular events"; there was no significant association, with a coefficient of 0.05 (95% CI -0.03-0.13) for change in SFA as % of energy. A threshold analysis was also conducted to evaluate whether achieving specific saturated fat targets in the intervention arm yielded different outcomes when the control arm remained above those thresholds (e.g., <10% relative to >10% of energy). It is not clear how many studies were pooled for this analysis. For the 10% of energy SFA threshold, risk ratios were approximately 0.99 (95% CI 0.90-1.09) for all-cause mortality, 1.05 (0.77-1.43) for CHD mortality, and 0.82 (0.60-1.13) for CHD events.

Summary of Findings

Table 5 presents our Summary of Findings. The lead RCT reviews were Ramsden 2016 [10] for CHD and all-cause mortality and Hooper 2018 [28] for CHD events. Together these syntheses encompassed 12,937 participants from diet-heart trials comparing replacement of SFA with vegetable oils. Across outcomes, no statistically significant effect of SFA reduction/replacement was observed. In Ramsden et al. 2016, the pooled hazard ratio for CHD mortality was 1.13 (95% CI 0.83-1.54; I^2 = 45%), and for all-cause mortality 1.07 (0.90-1.27; I^2 = 39%); certainty of evidence was moderate and downgraded for imprecision. In Hooper 2018, the pooled hazard ratio for CHD events was 0.85 (0.61-1.17); certainty of evidence was very low due to downgrades for imprecision, indirectness and inconsistency (large unexplained heterogeneity in the pooled estimate).

Table 5. Summary of Findings

Outcome	Lead Review (Year)	Effect (95% CI)	Trials / n Events	I²	Certainty (GRADE)	Notes
Randomized controlled trials						
All-cause mortality	Ramsden 2016	HR 1.07 (0.90-1.27)	LAV, MCE, MRC, RCOT, SDHS / 1,001 deaths	I²=39%	Moderate	Downgrade for imprecision [a]
CHD mortality	Ramsden 2016	HR 1.13 (0.83-1.54)	LAV, MCE, MRC, RCOT, SDHS / 324 deaths	I²=45%	Moderate	Downgrade for imprecision [a]
CHD events	Hooper 2018	RR 0.85 (0.61-1.17)	DART, Houtsmuller, LAV, MRC, SDHS / 1,037 events	I²=80%	Very Low	Downgrade for imprecision,[a] indirectness,[b] and inconsistency [c]

[a] Confidence intervals include both clinically important benefit and harm.
[b] Meta-analysis included the Diet and Re-Infarction Trial (DART), which did not test a defined substitution of SFA with another nutrient.
[c] I^2 =80%; Downgraded for inconsistency.
Abbreviations: MCE, Minnesota Coronary Experiment; SDHS, Sydney Diet-Heart Study; RCOT, Rose Corn Oil Trial; LAV, Los
Angeles Veterans Trial; MRC, Medical Research Council Soy Oil Trial; DART, Diet and Reinfarction Trial.

Discussion

This systematic review evaluated the causal evidence from RCTs for the long-standing dietary recommendation to reduce SFA to below 10% of total energy for the prevention of CHD and mortality. Among nine included systematic reviews, only three—Ramsden 2010, Ramsden 2016, and Hooper 2018— were designed to isolate the causal effect of replacing SFA with a specified macronutrient, and all three focused on substitution with LA-rich n-6 PUFA. Across these analyses, replacing SFA with n-6 PUFA showed no reduction in CHD or all-cause mortality, and no consistent effect on CHD events. The certainty of evidence was rated moderate for mortality outcomes and very low for CHD events. Together, these findings indicate that the current RCT evidence does not demonstrate benefit of dietary replacement of SFA as a means to prevent CHD or reduce mortality.

Historical Context

The persistence of SFA restriction in dietary policy reflects the enduring influence of the traditional diet-heart hypothesis: that replacing SFA with LA-rich oils lowers LDL-cholesterol (LDL-C) and thereby reduces CHD risk. This reasoning became embedded in American Heart Association advice in 1961 [2] and in the first DGA (1980). However, the five major RCTs designed to test this hypothesis—the Minnesota Coronary Experiment, Los Angeles Veterans Study, Sydney Diet-Heart Study, and Medical Research Council Soy Oil Trial —were all conducted in the 1960s and 1970s. Collectively, these studies achieved large and sustained reductions in serum cholesterol through targeted replacement of SFA with vegetable oils rich in LA, yet none demonstrated a significant benefit in terms of reducing CHD mortality or all-cause mortality. In the Minnesota Coronary Experiment, for example, each 30 mg/dL reduction

in cholesterol was associated with a 22% higher, rather than lower, risk of death. Similarly, the Sydney Diet-Heart Study—the only trial to use safflower oil, nearly devoid of n-3 PUFA—showed a 62% higher all-cause mortality in the intervention group.[49]

These counterintuitive findings were not widely disseminated, and in some cases not fully published, until decades later, meaning early guidelines were developed before the full body of trial evidence was available to scientists—including those who formulated the original US dietary guidelines—and the public.

Misinterpretation of Non-Causal Evidence

One reason for continued belief in the benefits of SFA reduction/replacement is that many meta-analyses of RCTs purporting to address this question included the non-randomized Finnish Mental Hospital Study and the Oslo Diet-Heart Study.[3,25,26] The Finnish Mental Hospital Study, for example, is sometimes cited as an RCT, yet patients were assigned by hospital in a 12-year crossover design with major imbalances in medications, psychiatric profiles, and trans-fat exposure; it therefore cannot support causal inference. Likewise, the Oslo Diet-Heart Study combined SFA reduction with a suite of co-interventions—including replacement of meat with fish, elimination and replacement of trans fat margarines with sardines canned in cod liver oil providing ~5 g/day of marine EPA + DHA, increased fruits and vegetables, and elimination of trans-fat margarines—making it impossible to attribute benefits to SFA replacement. As a simple analogy, this is akin to testing whether drinking less soda improves health while simultaneously providing participants with fruits and vegetables, and advising them to exercise and sleep better: any observed improvement could result from any of those changes rather than soda reduction itself. Since both the Finnish Mental Hospital Study and the Oslo Diet-Heart Study found that the intervention group had less events than the control group, their frequent inclusion in meta-analyses has led to the erroneous interpretation of “SFA reduction” as the active factor driving benefit. Moreover, much of the SFA in these historical control diets came from partially hydrogenated oils rather than natural foods.[25] Consequently, extrapolating these findings to naturally occurring sources of SFA—such as dairy, meat, or coconut fat—is not scientifically justified.

Another reason for continued belief in the benefits of SFA reduction—particularly replacement with LA—is apparent support from longitudinal cohort studies.[30,50-52] When observational studies and RCTs reach different conclusions, greater weight should be given to RCTs because they directly test causality by randomly assigning exposures and minimizing confounding.[13] In contrast, cohort studies can only observe associations that may be distorted by factors such as unmeasured or residual confounding, selection bias, reverse causation, or correlated health behaviors. Large prospective cohorts such as the Nurses’ Health Study, Health Professionals Follow-Up Study, Atherosclerosis Risk in Communities, and Iowa Women's Health Study were launched after public-health campaigns had already encouraged the use of vegetable oils and discouraged animal fats. Thus, in these populations, higher LA intake might indicate adherence to prevailing "heart-healthy" advice rather than an independent biological effect. Although

statistical models in these cohorts adjust for multiple variables—including smoking, body-mass index, physical activity, alcohol use, and dietary pattern scores—residual confounding is inevitable. Even if perfectly measured, observational estimates are indirect with respect to the specific intervention tested in RCTs.[14,15] "PUFA-for-SFA" substitution models in cohorts are statistical constructs that infer what would happen if calories from SFA were replaced by PUFA. Because the underlying foods contain combinations of n-6 and n-3 fatty acids and other nutrients, such models cannot isolate the independent effect of LA. They represent participant's selection of dietary patterns, as opposed to nutrient replacements, and are therefore hypothesis-generating rather than hypothesis-confirming.

Selection Bias and Protocol Drift in Systematic Reviews

Even within modern systematic reviews, selection bias introduced by protocol deviations has influenced pooled estimates. The 2014 protocol for Hooper 2018 specified a ≥6-month duration criterion. The 2018 update,[28] however, applied a post-hoc undisclosed protocol change to 12 months of "continuous involvement", which resulted in excluding the Minnesota Coronary Experiment. Similarly, the Hooper 2020 review introduced a new ≥24-month minimum follow-up rule, also post-hoc, which again excluded the Minnesota Coronary Experiment. The Cochrane Handbook explicitly warns that post-hoc modification of eligibility criteria—particularly when "made on the basis of the findings of the studies or the synthesis"—can introduce bias.[53] While these changes may have been made to improve consistency, their cumulative effect was to down-weight large trials (with null findings) and up-weight multifactorial ones, thereby shifting the summary evidence base.

Understanding Different Biochemical Effects of Unsaturated Fatty-Acids

Although public-health recommendations often refer to "unsaturated fats" as a single category, the biological effects of individual fatty acids differ markedly. MUFAs and PUFA differ by double bond number and ω position, which governs metabolism and signaling.[54] LA (18:2 omega-6) is metabolized to arachidonic acid (20:4 omega-6), the precursor to some pro-inflammatory eicosanoids such as prostaglandins and leukotrienes; LA and its oxidized derivatives (OXLAMs) can also influence pain signaling, oxidative stress, and endothelial function.[55,56] In contrast, ALA (18:3 omega-3) competes for the same enzymes and is partly converted to eicosapentaenoic acid (EPA; 20:5 omega-3) and docosahexaenoic acid (DHA; 22:6 omega-3), which give rise to anti-inflammatory metabolites.[54] Consequently, the health effects of dietary PUFA depend not only on total unsaturation but also on the relative balance and competition between omega-6 and omega-3 species for enzymatic conversion and incorporation into cell membranes. MUFAs such as oleic acid (18:1 omega-9) are less prone to oxidation and do not directly participate in eicosanoid synthesis.[57] Because these fatty acids interact and compete within shared metabolic pathways and cell membranes, the health effects of "unsaturated fat" depend on their relative proportions and overall dietary context.

Thus, trials that combined LA with omega-3 fatty acids and other nutrient changes tested a fundamentally different exposure than LA alone—consistent with the subgroup differences observed in the randomized evidence. Specifically, in Ramsden, et al. 2016, the LA-only subgroup (Minnesota Coronary Experiment, Sydney Diet-Heart Study, Rose Corn Oil Trial) yielded a pooled HR for CHD mortality of 1.33 (95% CI 0.99-1.79), whereas trials that combined LA with omega-3 fatty acids and (including extreme increases in n-3 EPA and DHA and other dietary changes) were neutral or modestly protective—demonstrating why causal claims must rest on trials that specify the replacement fat or other nutrient.

Moreover, biochemical evidence provides plausible mechanisms for harm in LA-only interventions. Although LA effectively lowers LDL-C by enhancing hepatic LDL receptor activity,[58] its high degree of unsaturation renders it prone to peroxidation.[57] Increased dietary LA elevates tissue levels of oxidized LA metabolites (OXLAMs)[59] and reactive aldehydes such as 4-hydroxynonenal,[60] which damage lipids, proteins, and DNA. Incerased dietary LA also promotes inflammatory and atherogenic signaling.[61,62] Oxidative modification of LDL particles by OXLAMs produces oxidized LDL, a driver of atherosclerosis and endothelial dysfunction. High LA intake may also reduce the bioavailability of omega-3 fatty acids within cell membranes by competing for desaturase and elongase enzymes thereby limiting the synthesis of anti-inflammatory mediators.[63,64] These mechanisms together may provide an explanation for why LA-only interventions could yield biochemical improvements yet fail to reduce, or even increase, CHD risk—however, more research is needed to determine their effect.

Interpretation of Hooper 2020 and Related Meta-Analyses

The Hooper 2020 review on SFA reduction exemplifies these interpretive challenges. It found no effect on all-cause or cardiovascular mortality and a nominal benefit only for a composite “combined cardiovascular events” endpoint—a construct heavily influenced by soft outcomes such as angina and revascularization that are sensitive to subjective reporting and clinical discretion. Moreover, the review pooled trials where SFA reduction occurred alongside multiple other dietary changes. For example, in the Women’s Health Initiative, participants in the low-fat intervention arm increased fruit, vegetable, and whole-grain intake while reducing total fat and modestly lowering SFA (~12% → 9%E), accompanied by greater weight loss and higher fiber intake compared with controls. Similarly, the Oslo Diet-Heart Study combined SFA reduction with a major reduction in trans fat intake and large increases in marine n-3 fats and other dietary modifications. As such, the modest benefit—restricted to “combined CVD events”—reported in Hooper 2020 may reflect the combined influence of these co-interventions rather than the isolated effect of SFA reduction itself. Recognizing this distinction is essential to avoid attributing causal meaning to what are, in effect, complex multicomponent dietary interventions.

Strengths and Limitations

Our review provides an updated synthesis aligned with GRADE evidence rating and ROBIS risk of bias frameworks, emphasizing causality and methodological transparency. Strengths include a prespecified protocol, duplicate screening and extraction, and explicit causal classification of reviews. The principal limitation lies in the historical and narrow RCT corpus—limited to n-6 PUFA replacements in mid-20th-century settings, and with only one study (the Minnesota Coronary Experiment) including women. The diets and populations of those trials differ from contemporary contexts. Nonetheless, these are the only RCTs capable of addressing the causal question directly, and they consistently show no mortality benefit. Another limitation is the scarcity of trials testing SFA→MUFA or SFA→carbohydrate substitution, leaving these potential replacements untested at the event level.

Future Research

Nutrition science—and cardiovascular nutrition in particular—needs a methodological reset to move policy from inference to causation. For decades, population guidance on SFA has leaned on observational associations and surrogate markers (e.g., LDL cholesterol) even as event level randomized trials failed to show benefit for targeted SFA replacement. Before continuing any recommendations to limit SFA, new trials should use isocaloric, substitution specific designs that prespecify what replaces SFA (e.g., MUFA from olive oil, mixed fat foods, or defined, high quality carbohydrate), maintain energy balance, and minimize co-interventions, so the contrast tests SFA per se rather than a lifestyle bundle. Key safeguards include treatment concealment, balanced participant contact, and blinded endpoint adjudication. Because mechanism matters, trials should incorporate objective adherence biomarkers (e.g., erythrocyte/plasma fatty acids) and oxidative/peroxidation metrics (e.g., OXLAMs, 4 HNE adducts) to test whether different replacements have distinct redox effects—relevant because LA is peroxidation-prone whereas SFA is comparatively peroxidation-resistant.

Since the current evidence base is somewhat outdated, the overall design, analysis and synthesis of trials needs to utilize modern approaches such as adequate sample size/duration, intention-to-treat analyses with per-protocol sensitivity analyses, explicit reporting of achieved intakes, and pre-registered protocols and analysis plans. The field would benefit from a coordinated portfolio of large, simple, substitution explicit trials testing SFA→MUFA, SFA→high-quality carbohydrates, and food-based replacements (e.g., nuts, dairy matrices), with embedded mechanistic substudies. Without renewed methodological rigor, future dietary guidance risks perpetuating conclusions based on indirect evidence rather than verified causal outcomes.

Policy Implications

Given moderate certainty of no benefit for the replacement of SFA with LA-rich vegetable oils on CHD and all-cause mortality—and very low certainty for CHD events—we judge the causal evidence insufficient to support a population-wide <10%

energy SFA cap. The lack of demonstrated benefit for this specific replacement should not be interpreted as evidence that SFA intake is protective as the current evidence base provides very limited evidence on other potential replacements. Because the health impact of any nutrient depends on its dietary context—including the replacement nutrient and food sources—strong conclusions about SFA intake cannot be drawn until modern, substitution-specific trials test clearly defined replacements and assess their clinical effects.

Conclusion

After more than half a century of investigation, the totality of evidence from RCTs shows that reducing dietary SFA to below 10% of energy—particularly through replacement with LA-rich vegetable oils—lowers serum cholesterol but does not reduce CHD or all-cause mortality. The apparent benefits reported in some meta-analyses arise from inclusion of non-randomized, multifactorial, or confounded studies rather than from true causal effects. We did not identify systematic reviews evaluating the causal effect of SFA replacement for MUFA or carbohydrate (i.e., interventions that were not multicomponent). These findings call for re-evaluation of the <10% of energy SFA target within the DGA, emphasizing replacement nutrient specificity, transparency in evidence grading, and the need for new, modern RCTs before strong population-wide recommendations can be justified.

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Supplement 1 – Protocol

Evidence from Randomized Controlled Trials on the Effects of Reducing Saturated Fat Below 10% of Energy on Cardiovascular and Mortality Outcomes: Protocol For a Comprehensive Review

1) Objective

The objective of this comprehensive umbrella-style review is to evaluate whether reducing dietary saturated fat (SFA) below ~10% of total energy, through isocaloric replacement with any other macronutrient, improves clinical cardiovascular outcomes in adults. We will identify and synthesize systematic reviews and meta-analyses of randomized controlled trials (RCTs) that report hard clinical endpoints (i.e., coronary heart disease [CHD] events, CHD mortality, and all-cause mortality). A secondary objective is to examine dose-response evidence within RCT syntheses (where available), assessing whether lower SFA targets (including <10% energy) are associated with greater benefit. Because historical interventions sometimes involved partially hydrogenated oils (PHOs; industrial trans fats) as comparators or replacements, we will explicitly identify and account for PHO exposure in intervention and control diets when interpreting effects for modern policy relevance.

2) Question & Scope (PICO(T))

Primary Question: What is the evidence from RCTs that reducing dietary saturated fat to below approximately 10% of total energy — through replacement with any other macronutrient — reduces coronary heart disease (CHD) mortality, all-cause mortality, or CHD events in adults?

Population (P): Adults (≥18 years) in both primary and secondary prevention settings.

Intervention/Exposure (I): Reduction of dietary saturated fat intake through isocaloric replacement by another macronutrient (e.g., PUFA, MUFA, or carbohydrate). Eligible interventions must primarily target SFA reduction and describe the intended or achieved change in SFA (% of energy). We will flag PHO/trans-fat content in replacement oils/foods to support sensitivity/interpretation.

Comparator (C): Higher SFA intake or usual diet. We will flag PHO/trans-fat content in control foods (e.g., hard margarines/shortenings) to support sensitivity/interpretation.

Outcomes (O): Coronary heart disease (CHD) mortality; All-cause mortality; CHD events (fatal and nonfatal, as defined by included reviews).

Timing (T): Minimum of ≥1 year follow-up

3) Eligibility Criteria

Inclusion criteria:

- Self-identified systematic review and meta-analyses of RCTs with reproducible methods (documented search strategy, explicit eligibility criteria, and data extraction process).
- Must report at least one of the following outcomes: CHD mortality, all-cause mortality, CHD events
- Publication window: Published between January 2010 and August 2025.
- Language: English.
- Population: Adults (≥18 years) in either primary or secondary prevention settings.

- Intervention: Reduction of dietary SFA through isocaloric replacement with another macronutrient (e.g., carbohydrate, monounsaturated fat, or polyunsaturated fat).
- Comparator: Higher SFA intake or usual diet.

Exclusion criteria:

- Narrative, scoping, or umbrella reviews without reproducible systematic methods.
- Reviews published before 2010.
- Reviews including observational studies only or mixed designs that do not report separable RCT results.
- Multicomponent dietary interventions (e.g., “heart-healthy,” “Mediterranean,” “DASH,” or “prudent” diets) that simultaneously modify multiple nutrients or food groups such as fruits, vegetables, fish, or fiber—since the effect of SFA reduction cannot be isolated.
- Reviews where **SFA replacement** cannot be determined (e.g., “low-fat” diets without specifying replacement macronutrient).
- Reviews that report only intermediate or surrogate outcomes (e.g., serum cholesterol, triglycerides, blood pressure) without mortality or CHD events.
- Pediatric (<18 years) or pregnant populations.
- Non-English publications.

4) Information Sources & Search

- Databases: PubMed (MEDLINE), Cochrane Database of Systematic Reviews, Embase.
- Limits: English; January 2010 to August 2025.
- Search strings: see Appendix A.

A librarian will run all searches and document the exact search strings used for each database. Results will be exported to Covidence for de-duplication and screening.

5) Screening

Title/Abstract Screening
Reviews will be screened in duplicate, independently by two reviewers, with discrepancies resolved by consensus. Articles must meet three criteria. First, they must be systematic reviews and meta-analyses of RCTs; narrative reviews, scoping reviews, single primary studies, or animal studies will be excluded. Second, they must specifically evaluate the modification or substitution of saturated fatty acids. Third, they must report on at least one of the prespecified primary outcomes—CHD events, CHD mortality, or all-cause mortality—in adults aged 18 years and older. Articles that meet all three criteria will be included for full-text screening. Disagreements between reviewers will be resolved through discussion. When eligibility cannot be determined from the abstract alone, the full text will be retrieved for clarification.

Full-Text Screening
Full-text screening will be conducted independently by two reviewers, with reasons for exclusion documented. Discrepancies will be resolved by consensus.

6) Data Extraction

Process
Data will be extracted by two independent reviewers using a standardized template. Discrepancies will be resolved by a third party.

Data items

For each included review, we will extract:

- Citation details (author, year)
- Number of included RCTs
- Intervention details
- Comparator details
- Outcomes reported
- Effect estimates (RR/HR with 95% CI)
- Events and participants (by outcome, if reported)
- Heterogeneity statistics (I^2, τ^2, prediction interval if available)
- Key conclusions

7) Risk of Bias/ Quality Appraisal of Reviews

The methodological quality of included reviews will be assessed using the ROBIS tool (Risk of Bias in Systematic Reviews). ROBIS evaluates four domains (eligibility criteria, identification and selection of studies, data collection and appraisal of included studies, and synthesis and findings) and provides an overall rating of risk of bias. All quality appraisals will be conducted by one reviewer, with a second reviewer verifying a random 20% sample for consistency. Disagreements will be resolved by consensus.

8) Synthesis Plan

We will conduct a narrative synthesis of findings from all eligible reviews. Because this umbrella review summarizes previously aggregated evidence, no new quantitative meta-analysis will be performed.

Findings from the ROBIS appraisal (Section 7) will be considered alongside a detailed mapping of primary RCTs to evaluate both methodological quality and causal focus. While ROBIS assesses risk of bias in review conduct (e.g., search, data extraction, synthesis methods), it does not directly capture whether a review isolates the causal effect of SFA replacement. Therefore, we will construct a citation matrix of all primary RCTs included across reviews to quantify overlap and identify the nature of each review's evidence base. This mapping will help distinguish reviews that primarily incorporate trials focused on isocaloric nutrient modification from those that include multifactorial interventions, thereby informing the selection of the anchor (lead) review for each outcome. We will compare direction and magnitude of effects across overlapping reviews and assess whether differences can be explained by factors like eligibility criteria or treatment of trans-fat and other exposures in comparator or intervention diets.

Findings will be classified according to macronutrient substitution pattern (e.g., SFA to PUFA, SFA to MUFA, SFA to carbohydrate, mixed/unclear), using anchor reviews as the primary reference and other reviews to highlight inconsistencies or mixed substitutions. Where dose-response analyses are available, we will summarize their methods and results, noting whether lower achieved SFA levels (< 10 % of energy) were associated with greater benefit.

9) Certainty Summaries

Certainty of evidence for each primary outcome will be evaluated using the GRADE framework. GRADE rates confidence in effect estimates across five domains: risk of bias, inconsistency, indirectness, imprecision, and publication bias. A Summary-of-Findings (SoF) table will be prepared

for each outcome, presenting the effect estimate (RR or HR with 95 % CI) and GRADE certainty with concise reasons for downgrading or upgrading.

10) Evidence-to-Decision (EtD) Framework

Evidence from this review will be organized using a GRADE-based Evidence-to-Decision (EtD) framework to support dietary guideline development. The framework will summarize the burden of disease, benefits and harms of SFA reduction/replacement, certainty of the evidence, and key implementation considerations such as feasibility, and acceptability. Judgments will be informed by the Summary of Findings and contextual factors, with recommendations categorized as strong, conditional, or none.

Evidence-to-Decision Framework Template

Criterion	Evidence Summary	Committee Judgment
Problem & importance	[Describe burden: e.g., high prevalence of SSB consumption, linked to obesity/T2D]	[Is the problem a priority: Yes/No/Uncertain]
Certainty of evidence (per outcome)	use GRADE ratings from SoF table. [Eg. toal mortality = High, HbA1c = Moderate, T2D incidence = Low]	[Accept as is?]
Benefits vs harms	[How substantial are the desirable anticipated effects? How substantial are the undesirable anticipated effects?]	[Benefits outweigh harms? Yes/No/Uncertain]
Implementation considerations/feasibility		[Feasible? Acceptable?]
Recommendation strength		[Strong / Conditional / No recommendation]

Supplement 2 – Database Search Strategies

Database: PubMed/MEDLINE
Platform: National Library of Medicine

	Concept	Search Strategy	Results
#1	Saturated Fat	"Fatty Acids"[Mesh:NoExp] OR "saturated fatty acid*"[Text Word] OR "saturated fat*"[Text Word] OR "SFA"[Title/Abstract]	123,895
#2	Cardiovascular Disease/ mortality	"Cardiovascular Diseases"[Mesh:NoExp] OR "cardiovascular disease*"[Text Word] OR "coronary disease"[Title/Abstract:~2] OR "coronary diseases"[Title/Abstract:~2] OR "heart disease*"[Text Word] OR "myocardial infarction*"[Text Word] OR "Myocardial Ischemia"[Mesh] OR "myocardial ischemia*"[Text Word] OR "angina"[Text Word] OR "heart attack*"[Text Word] OR "Mortality"[Mesh] OR "mortality" [Subheading] OR "mortality"[Text Word] OR "death*"[Text Word]	3,445,603
#3	Study Design	"Systematic Review"[Publication Type] OR "Systematic Reviews as Topic"[Mesh] OR "systematic review"[Title/Abstract:~2] OR "Meta-Analysis" [Publication Type] OR "Meta-Analysis as Topic"[Mesh] OR "meta-analysis"[tw] OR "meta-analyses"[tw]	594,281
#4	Combined Concepts	#1 AND #2 AND #3	345
#5	Limits	((#1 AND #2 AND #3 AND #4) NOT ("Animals"[Mesh] NOT ("Animals"[Mesh] AND "Humans"[Mesh]))) NOT ("Congress"[Publication Type] OR "Consensus Development Conference"[Publication Type] OR "proceeding*"[Title]) AND ("2010/01/01"[Date - Publication] : "2025/08/31"[Date - Publication]) AND English[lang]	277

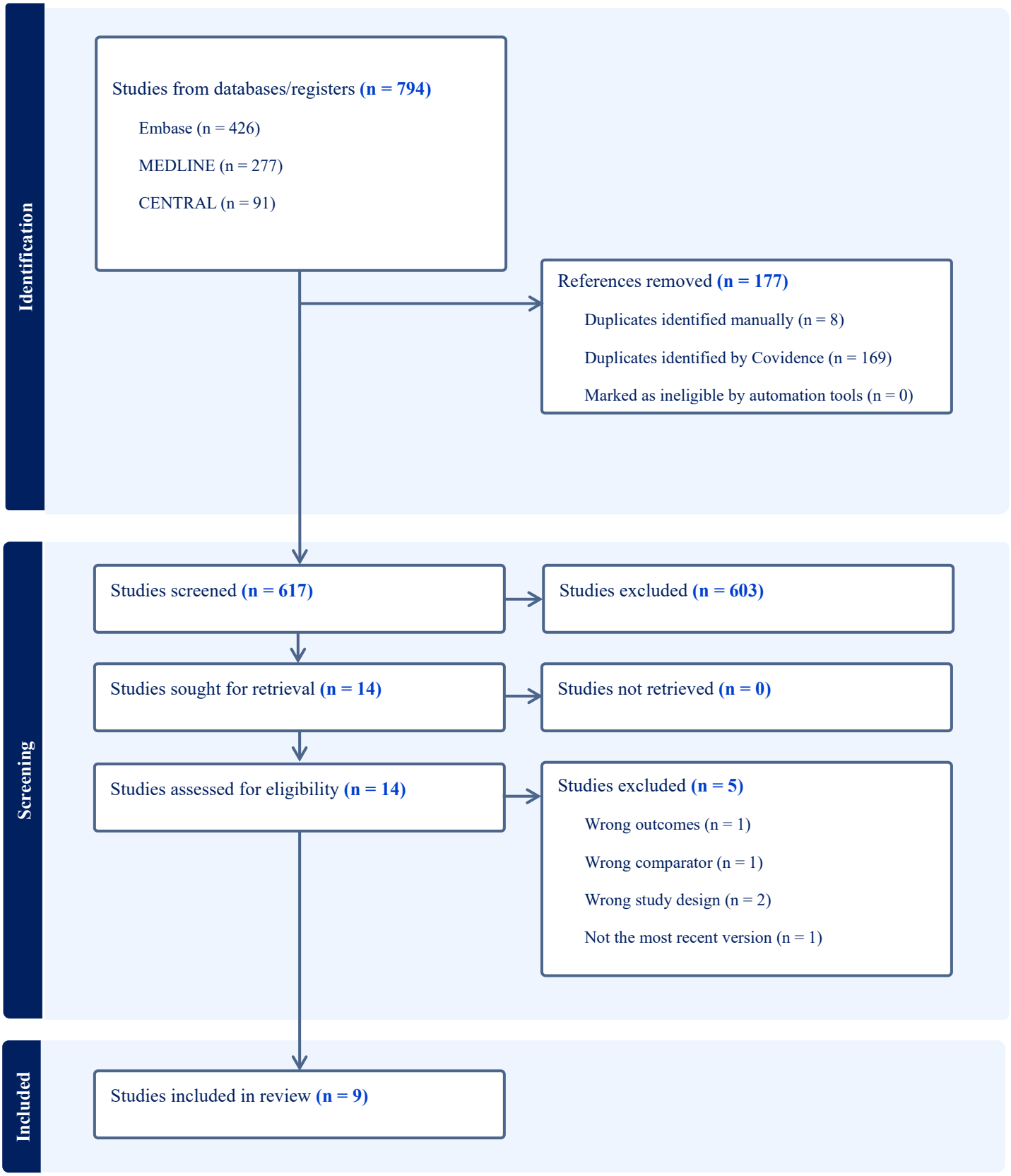
Database: Cochrane Library
Platform: Wiley & Sons

	Concept	Search Strategy	Results
#1	Saturated Fat	[mh ^"Fatty Acids"] OR (saturated NEXT fat*) OR "SFA"	5,423
#2	Cardiovascular Disease/ mortality	[mh ^"Cardiovascular Diseases"] OR (cardiovascular NEXT disease*) OR (coronary NEXT/2 disease*) OR (myocardial NEAR infarction*) OR (heart NEXT disease*) OR (myocardial NEXT infarction*) OR [mh "Myocardial Ischemia"] OR (myocardial NEAR ischemia*) OR angina OR (heart NEXT attack*) OR [mh "Mortality"] OR mortality OR death	276,683
#4	Study Design	#1 AND #2 (with Cochrane Library publication date from Jan 2010 to Dec 2025, in Cochrane Reviews)	91

Database: Embase
Platform: Elsevier

	Concept	Search Strategy	Results
#1	Saturated Fat	'fatty acid'/de OR 'saturated fat*':ti,ab,kw OR 'sfa':ti,ab,kw	193,485
#2	Cardiovascular Disease/ mortality	'cardiovascular disease'/de OR 'cardiovascular disease*':ti,ab,kw,de,dn,df,mn,tn OR (('coronary' NEAR/3 'disease'):ti,ab,kw) OR (('coronary' NEAR/3 'diseases'):ti,ab,kw) OR 'heart disease*':ti,ab,kw OR 'myocardial infarction*':ti,ab,kw OR 'heart muscle ischemia'/de OR 'myocardial ischemia*':ti,ab,kw OR 'angina':ti,ab,kw OR 'heart attack*':ti,ab,kw OR 'mortality'/mj OR 'mortality':ti,ab,kw OR 'death*':ti,ab,kw	4,162,749
#3	Study Design	'systematic review':it OR 'systematic review (topic)'/exp OR (('systematic' NEAR/3 'review'):ti,ab,kw) OR 'meta-analysis':it OR 'meta analysis (topic)'/exp OR 'meta-analysis':ti,ab,kw OR 'meta-analyses':ti,ab,kw	735,933
#4	Combined Concepts	#1 AND #2 AND #3	571
#5	Limits	#1 AND #2 AND #3 NOT ('animal'/exp NOT ('animal'/exp AND 'human'/exp)) NOT ('congress':it OR 'consensus development conference':it OR 'proceeding*':ti) AND [english]/lim AND [embase]/lim AND [2010-2025]/py	401

Supplement 3 – PRISMA Flow Diagram



Supplement 4 – Inclusion Matrix of Studies Across Systematic Reviews

Studies	Mozaffarian (2010)	Ramsden (2010)	Chowdhury (2014)	Schwingshackl (2014)	Ramsden (2016)	Hooper (2018)	Hooper (2020)	Jayedi (2024)	Yamada (2025)
SDHS		✓	✓	✓	✓	✓	✓	✓	✓
MRC	✓	✓ *	✓	✓	✓	✓	✓	✓	✓
DART	✓		✓	✓	✓ *	✓	✓	✓	✓
LAV	✓	✓ *	✓		✓	✓	✓	✓	✓
MCE	✓	✓	✓		✓			✓	✓
RCOT		✓		✓	✓		✓	✓	✓
ODHS	✓	✓ *	✓	✓	✓ *		✓	✓	✓
STARS	✓	✓ *	✓	✓	✓ *		✓	✓	✓
FMHS	✓		✓					✓	
Amrita						✓			✓
Houtsmuller						✓	✓		
Black							✓		
Ley							✓		
Moy							✓		
NDHS						✓			
Rose Olive †							✓		
WHI							✓		
WINS							✓		

* Included in sensitivity analysis.
† This is a separate arm of the Rose Corn Oil Trial (RCOT).[1]
Abbreviations: Amrita=Amrita Institute of Medical Sciences, Black=Black (1994),[2] DART=Diet and Reinfarction Trial, FMHS=Finnish Mental Hospital Study, Houtsmuller=Houtsmuller (1979),[3] LAV=Los Angeles Veterans Administration Trial, Ley=Ley (2004),[4] MCE=Minnesota Coronary Experiment, Moy=Moy (2001),[5] MRC=Medical Research Council Soya-Bean Oil Trial, NDHS=National Diet-Heart Study, ODHS=Oslo Diet-Heart Study, RCOT=Rose Corn Oil Trial, SDHS=Sydney Diet-Heart Study, STARS=St Thomas Atherosclerosis Regression Study, WHI=Women's Health Initiative, WINS=Women's Intervention Nutrition Study.

1. Rose GA, Thomson WB, Williams RT. Corn Oil in Treatment of Ischaemic Heart Disease. *Br Med J*. Jun 12 1965;1(5449):1531-3. doi:10.1136/bmj.1.5449.1531
2. Black HS, Herd JA, Goldberg LH, et al. Effect of a low-fat diet on the incidence of actinic keratosis. *N Engl J Med*. May 5 1994;330(18):1272-5. doi:10.1056/NEJM199405053301804
3. Houtsmuller AJ, Zahn KJ, Henkes HE. Unsaturated fats and progression of diabetic retinopathy. *Doc Ophthalmol*. Apr 15 1980;48(2):363-71. doi:10.1007/BF00141465
4. Ley SJ, Metcalf PA, Scragg RK, Swinburn BA. Long-term effects of a reduced fat diet intervention on cardiovascular disease risk factors in individuals with glucose intolerance. *Diabetes Res Clin Pract*. Feb 2004;63(2):103-12. doi:10.1016/j.diabres.2003.09.001
5. Moy TF, Yanek LR, Raqueno JV, et al. Dietary Counseling for High Blood Cholesterol in Families at Risk of Coronary Disease. *Prev Cardiol*. Autumn 2001;4(4):158-164. doi:10.1111/j.1520-037x.2001.00543.x

Supplement 5, Part 1 – Risk of Bias Summary for Included Reviews (ROBIS Tool)

	Mozaffarian 2010	Ramsden 2010	Chowdhury 2014	Schwingshackl 2014	Ramsden 2016
Domain 1-Study eligibility					
1. Pre-defined objectives/eligibility?	Y	N	Y	N	Y
2. Eligibility criteria appropriate?	PN	Y	Y	PN	Y
3. Eligibility criteria unambiguous?	PY	PY	PY	N	PY
4. Restrictions on study characteristics appropriate?	Y	Y	Y	Y	Y
5. Restrictions on sources of info appropriate?	Y	PN	Y	Y	PY
Concern	Low	High	Low	High	Low
Rationale	Pre-specified, eligibility was broad	No evidence of pre-specification; limited database scope.	Protocol stated, MOOSE &PRISMA	No evidence of pre-specification; eligibility was broad	PRISMA; clear eligibility; minor language restriction.
Domain 2-Study selection					
1. Search included appropriate databases?	Y	PN	Y	Y	Y
2. Additional methods used to identify reports?	Y	Y	Y	Y	Y
3. Search strategy terms/structure appropriate?	Y	PN	Y	Y	Y
4. Restrictions on date/format/language appropriate?	Y	NI	Y	Y	PY
5. Efforts to minimise error in selection?	Y	NI	Y	Y	Y
Concern	Low	High	Low	Low	Low
Rationale	Multiple databases + grey sources; duplicate screening.	Medline/ISI only; search date not reported; unclear dual screening.	Comprehensive, reproducible search with dual independent selection.	Multiple databases + grey sources; duplicate screening.	Multiple databases + grey sources; duplicate screening.
Domain 3-Data collection					
1. Efforts to minimise error in data collection?	Y	PN	Y	Y	Y
2. Sufficient study characteristics available?	N	Y	N	N	Y
3. All relevant results collected for synthesis?	Y	Y	Y	Y	Y
4. RoB/quality formally assessed appropriately?	PN	N	N	PY	Y
5. Efforts to minimise error in RoB assessment?	Y	N	PN	PY	Y
Concern	High	High	High	High	Low
Rationale	Missing essential dietary and methodological details (e.g. unacknowledged non-randomization in FMHS or multicomponent interventions) prevents evaluation of internal validity and confounding.	No duplicate extraction; no formal trial RoB tool; narrative appraisal only.	FMHS misclassified as RCT and rated low bias. Missing essential dietary and methodological details (multicomponent interventions) prevents evaluation of internal validity and confounding.	Dietary descriptions, particularly the concurrent food or nutrient changes (e.g., fish, fruit, vegetable, or fiber intake), were not systematically summarized. Although stated that they used the Cochrane risk-of-bias tool, they did not present individual or summary ratings. The absence of reported RoB tables or figures prevents verification of how rigorously or consistently the tool was applied, representing an unclear risk of bias in the appraisal process.	Duplicate extraction; formal trial RoB.

	Mozaffarian 2010	Ramsden 2010	Chowdhury 2014	Schwingshackl 2014	Ramsden 2016
Domain 4-Synthesis					
1. Synthesis included all eligible studies?	Y	Y	PY	Y	Y
2. All pre-defined analyses reported?	Y	Y	Y	Y	Y
3. Synthesis appropriate to Q/designs/outcomes?	N	Y	PN	Y	Y
4. Heterogeneity minimal or addressed?	Y	PY	N	Y	Y
5. Findings robust (funnel plots/sensitivity)?	Y	PY	PY	Y	Y
6. Bias in primary studies addressed?	N	PY	N	N	Y
Concern	High	Some	High	Low	Low
Rationale	Main pooled estimate derived from a set of trials not all truly randomized and not comparable in intervention content.	Appropriate MA but small/old trials; some selective assumptions (e.g., SDHS CHD death imputation).	FMHS inclusion inflates ω-6 effects; multicomponent trials (OSLO and STARS) increase conceptual noise but are within scope	Appropriate statistical models; transparent null reporting	Random-effects MA; heterogeneity & sensitivity; addressed TFA/confounding.
Overall					
Risk of bias	High	High	High	High	Low
Rationale	(1) Inclusion of a non-randomized, confounded trial (FMHS), (2) inclusion of multifactorial trials (Oslo DHS), and (3) inadequate reporting of concurrent dietary exposures.	High overall risk from search/selection and lack of formal RoB.	Driven by Domain 3 (appraisal) and Domain 4 (synthesis) for the specific analysis of SFA to n-6 PUFA substitution	Strong methodology; concern on dietary-detail reporting and inclusion of multicomponent dietary trials, as well as risk of bias assessements for individual trials	Overall low RoB with pre-specified, transparent synthesis.
Phase 3-Judging risk of bias					
A. Did interpretation address concerns in Domains 1–4?	N	N	N	N	Y
B. Was study relevance appropriately considered?	N	Y	N	PY	Y
C. Did reviewers avoid selective emphasis on significance?	PN	Y	Y	Y	Y

Supplement 5, Part 2 – Risk of Bias Summary for Included Reviews (ROBIS Tool)

	Hooper 2018	Hooper 2020	Jayedi 2024	Yamada 2025
Domain 1-Study eligibility				
1. Pre-defined objectives/eligibility?	N	N	Y	Y
2. Eligibility criteria appropriate?	PN	PN	PN	Y
3. Eligibility criteria unambiguous?	PY	PY	PN	PN
4. Restrictions on study characteristics appropriate?	N	N	Y	PY
5. Restrictions on sources of info appropriate?	Y	Y	Y	Y
Concern	High	High	Low	Low
Rationale	The eligibility criteria were generally appropriate but applied differently from the 2014 protocol, which required ≥6 months' duration. The 2018 review instead used a stricter "≥ 12-month continuous involvement" rule, excluding at least one study despite up to four years of follow-up. This undeclared/unjustified protocol change, based on study-specific features, may have introduced selection bias.	Selective inclusion; trial duration threshold of 24 months was not justified; inconsistent with protocol rule of 6 months. The 24-month threshold aligns exactly with an earlier subgroup analysis that showed benefit in trials >24months (Hooper 2000).	PROSPERO registered. Inclusion criteria were broad and appropriate for mixed designs, but not pre-specified for RCT substitution contrasts (no definition of SFA replacement or comparator diet).	Pre-specified, eligibility was broad
Domain 2-Study selection				
1. Search included appropriate databases?	Y	Y	Y	Y
2. Additional methods used to identify reports?	Y	Y	Y	Y
3. Search strategy terms/structure appropriate?	Y	Y	Y	Y
4. Restrictions on date/format/language appropriate?	Y	Y	Y	Y
5. Efforts to minimise error in selection?	Y	Y	PY	Y
Concern	Low	Low	Low	Low
Rationale	Comprehensive search and dual-reviewer screening		Five databases; dual screening; ref checks.	
Domain 3-Data collection				
1. Efforts to minimise error in data collection?	Y	Y	Y	Y
2. Sufficient study characteristics available?	Y	N	N	N
3. All relevant results collected for synthesis?	Y	Y	Y	PY
4. RoB/quality formally assessed appropriately?	Y	PN	N	Y
5. Efforts to minimise error in RoB assessment?	Y	Y	Y	Y
Concern	Low	High	High	High
Rationale	Cochrane RoB tool application.	Missing essential dietary and methodological details (multicomponent interventions) prevents evaluation of internal validity and confounding.	Dual extraction; ROBINS-I and RoB2 applied. RCT methods were briefly described; intervention details (con-interventions, achieved intakes) largely missing; Missclassified FMHS as RCT and Oslo Diet Heart Study as LA+ALA intervention	Robust appraisal workflow, but dietary/methodological details are insufficient to evaluate confounding from co-changes; outcome harvesting from trials with non-CVD primary endpoints adds ambiguity.
Domain 4-Synthesis				
1. Synthesis included all eligible studies?	N	PN	Y	Y

	Hooper 2018	Hooper 2020	Jayedi 2024	Yamada 2025
2. All pre-defined analyses reported?	Y	N	N	Y
3. Synthesis appropriate to Q/designs/outcomes?	Y	N	Y	PY
4. Heterogeneity minimal or addressed?	Y	Y	N	PY
5. Findings robust (funnel plots/sensitivity)?	PY	Y	PN	PY
6. Bias in primary studies addressed?	N	N	N	N
Concern	High	High	High	Low
Rationale	Appropriate meta-analytic methods; clear sensitivity analyses.	Exclusion of trials >6months and <24months, trials likely inflated effect, as per Hooper 2000. Synthesis does not isolate SFA to macronutrient substitution effect; combined partially confounded studies.	Random-effects and dose-response; heterogeneity + GRADE. But, bias in LA+ALA RCTs not addressed	Appropriate statistics and transparency, but causal attribution to SFA reduction is blurred by inclusion of multicomponent dietary trials and no stratification by replacement nutrient.
Overall				
Risk of bias	High	High	High	Low
Rationale	Robust Cochrane methodology throughout; however, non-standard application of eligibility criteria and selective exclusion of MCE inconsistent with Cochrane Handbook guidance.	Transparent methods, duplicate screening, and Cochrane RoB, appropriate meta-analytic techniques. However, **high** RoB due to post-hoc eligibility change, missing critical methodological detail, and inclusion of multifactorial trials (e.g., **Oslo**) that preclude isolating the causal effect of saturated-fat reduction.	Robust search, dual review, structured RoB tools; however, the RCT component was secondary and under-specified; e.g., the table of study characteristics does not have the information on the concurrent interventions	Methodologically stronger than many prior reviews (registered, RoB2, excludes FMHS), but still mixes multicomponent dietary RCTs and lacks replacement-nutrient stratification, so under ROBIS it rates Moderate risk of bias for isolating the causal effect of SFA reduction on clinical CVD outcomes.
Phase 3-Judging risk of bias				
A. Did interpretation address concerns in Domains 1–4?	N	N	N	PY
B. Was study relevance appropriately considered?	Y	N	PN	Y
C. Did reviewers avoid selective emphasis on significance?	Y	PN	Y	Y

Appendix 4.7. Saturated Fat Intake, Mortality & Cardiovascular Disease

SATURATED FAT INTAKE EFFECTS ON TOTAL MORTALITY AND CARDIOVASCULAR OUTCOMES

A Systematic Review

J. Thomas Brenna, PhD
Departments of Nutrition, of Chemistry, and of Pediatrics
Dell Pediatric Research Institute
University of Texas at Austin

Abstract

Objective. The Dietary Guidelines for Americans consistently recommend reducing saturated fat (SF) intake, currently limiting it to 10% of total calories.

Methods. Using Bayesian methods, we synthesized findings from meta-analyses and systematic reviews purporting to examine whether SF intake influences all-cause mortality, cardiovascular disease (CVD) risk, including stroke. Screening yielded 26 studies (2001-2025): nine synthesized randomized controlled trials (RCTs) and 17 used observational/prospective cohort trials (PCS). These provided 65 discrete risk assessments—29 for disease incidence and 36 for mortality outcomes.

Results. Universal Confounding by Partially Hydrogenated Oil (PHO; *trans* fat). Nearly all reviews ascribed combined saturated fat and *trans* fat (SF+PHO) effects exclusively to saturated fat. US *trans* fat intake (1999-2002) was 5-7.8 g/day from >20 g/day of PHO; in Europe, the intake was estimated as high as 50 g/d of *partially hydrogenated whale and fish oil*. This uncontrolled covariate invalidates all purported saturated fat meta-analytic and umbrella review conclusions, as the evidence base cannot distinguish saturated fat effects from those of *trans* fatty acids. We analyzed these studies as combined SF+PHO studies.

Most analyses (67%) reported null findings/no relationship between SF+PHO intake and cardiovascular outcomes. We found:

- **High certainty**: No effect on total mortality or CHD mortality; beneficial effect on stroke incidence
- **Moderate certainty**: Mild benefit for stroke mortality; mild harm for CHD and CVD incidence
- **Low certainty**: Equivocal effect on CVD mortality

Conclusions. Current recommendations to limit saturated fat to 10% of energy lack evidentiary support because all studies conflate saturated fat with atherogenic *trans* fat (PHO). Even within the data, we find strong evidence that limiting SF+PHO is not justified for mortality outcomes. Because the harmful component (PHO) is known and accounts for the observed associations with disease incidence, we conclude that future studies examining natural saturated fat without PHO will likely demonstrate it to be benign or beneficial for total mortality.

Saturated Fat

Evidence-to-Decision (EtD) Framework

Criterion	Description
Problem & importance	Natural saturated fat (SF) is a major nutrient in diets globally and a key human metabolite. We examined whether restricting SF intake is justified for reducing total mortality and cardiovascular disease, based on the weight of existing evidence from randomized controlled trials and prospective cohort studies. Critical to our analysis was whether studies adequately distinguished SF from partially hydrogenated oils (PHO) containing non-natural fatty acids such as industrially produced trans fats.
Certainty of evidence (per outcome)	No RCT and few prospective cohort studies previously cited in reviews as examining SF were free from confounding by PHO exposure; consequently, little unambiguous evidence exists for SF in isolation. For mixed SF+PHO exposures: Total mortality, CHD mortality, and stroke incidence = **High** certainty. CHD incidence, CVD incidence, and stroke mortality = **Moderate** certainty. CVD mortality = **Low** certainty.
Benefits vs harms	SF+PHO has a neutral effect on total mortality and on CHD mortality. SF-CHO has a mildly harmful effect on CVD mortality, CHD incidence, and CVD incidence. SF+PHO has a mildly beneficial effect on stroke mortality and incidence.
Implementation considerations	Natural saturated fat from animal source foods and from minimally processed plant oils does not diminish the overall nutritional quality of foods. Recommendations for these foods can be made confidently without restriction beyond standard guidance to avoid excess total calories, including during life stages with elevated nutrient demands, such as pregnancy, lactation, and childhood.
Preliminary recommendation statement	Strong evidence indicates that SF does not increase total mortality when naturally present in dairy, beef, pork, and other highly nutrient-dense foods, which should be emphasized regardless of their natural levels of saturated fat. The prominent display of saturated fat on the Nutrition Facts label warrants reconsideration.

Statement of Findings

Higher intake of SF+PHO shows neutral associations with total mortality and CHD mortality (High certainty), with mildly beneficial effects on stroke mortality and incidence (Moderate certainty). Overall, the evidence supports a Strong recommendation to emphasize nutrient-dense foods regardless of their natural saturated fat content, and to reconsider dietary restrictions and labeling prominence of saturated fat. Future trials should rigorously distinguish natural saturated fat from industrially produced trans fats and PHO, include hard clinical endpoints, and examine effects across critical life stages, including pregnancy, lactation, and growth.

Introduction

Saturated fat is widely considered to limit lifespan via atherogenicity, the major cause of mortality in the US. Previous analyses of the effects of saturated fat have been in two forms, randomized controlled trials (RCTs) and prospective cohort studies (PCSs). Studies have been conducted since at least the 1960s through the 2020s, with most studies prior to the labeling of trans fat from partially hydrogenated oils (PHO) in the US in 2006. Numerous meta-analyses have appeared to integrate the overall data. Because of the large number of studies of saturated fat intake, meta-analyses and systematic reviews can focus on various aspects, for instance, on intake of saturated fat per se, intake of saturated fat-rich foods such as dairy, and supplementation of unsaturated oils to implicitly reduce intake of saturated fat.

Our objective here is to identify meta-analyses of hard clinical endpoints, in contrast to metabolic biomarkers, that specifically address changes in saturated fat intake to isolate their effects. We then perform an umbrella review on RCTs and separately on PCSs. In two isolated cases, we collected data from the original underlying studies and conducted a *de novo* meta-analysis as a check.

To our knowledge, only two umbrella reviews on this topic exist[1,2] and only one on hard endpoints,[2] which reports only a narrative summary without meta-analytic pooling or quantitative synthesis. Moreover, of the >100 RCTs, PCSs, and meta-analyses conducted on saturated fat and total mortality/CVD outcomes, all have been undertaken with frequentist statistics. The frequentist paradigm can only report 'failure to find an effect' and requires the expert opinion-based GRADE framework to estimate effect sizes. Evidence-based methods expressly exclude expert opinion. Bayesian methods enable direct probability statements about effect magnitude. For example, when an umbrella review reports no significant effect on total mortality, Bayesian analysis can affirmatively state the probability that the true effect lies within a clinically negligible range. We therefore undertook the first quantitative synthesis within the context of an umbrella review on this topic.

Methods

The reporting of this umbrella review is consistent with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[3]

For RCT, PICO analysis is:

The **Population** of interest is non-pregnant, nonlactating adult women and men in apparently good health, who may have had a prior cardiovascular event.

The **Intervention** was lower levels of saturated fat intake for at least six months.

The **Comparison** was higher levels of saturated fat intake over the same period as the intervention.

The **Outcomes** were hard clinical endpoints: Total mortality, cardiovascular disease incidence or mortality, coronary heart disease incidence or mortality, stroke incidence.

Eligibility criteria and searches

We included systematic reviews and meta-analyses of randomized controlled trials (RCTs) as well as observational studies (cohort, case-control, and cross-sectional designs). To be eligible, reviews were required to report on hard clinical endpoints: all-cause mortality, coronary heart disease (CHD) incidence or mortality, cardiovascular disease (CVD) incidence or mortality, and stroke incidence or

mortality. The focus was on studies of total SFA intake in adult men and adult non-pregnant women published in peer-reviewed journals in the English language. Excluded were reviews that were

a) limited to intermediate risk factors (e.g., HDL/LDL cholesterol, total cholesterol, blood pressure, insulin sensitivity).
b) of dietary patterns (e.g., Mediterranean, DASH, vegan, vegetarian)
c) focused on pregnant women or children
d) published only as preprints or scoping reviews
e) compared high fat vs. low fat versions of a food (e.g., cheese, milk, yogurt), unless the total intake of that food was comparable across groups.

We conducted a systematic PubMed search (October 6, 2025) for English-language human studies from January 1, 2000, to December 31, 2025, combining MeSH terms and title/abstract keywords for saturated fat/dairy exposures, cardiovascular outcomes, and evidence syntheses (systematic reviews and meta-analyses) (**Supplement 1**). The search (i) yielded 1,291 results. To improve precision without losing coverage, we ran a refined search that disabled MeSH auto-explosion for exposure terms (e.g., Fatty Acids, Saturated[mh:noexp], Dairy Products[mh:noexp]); required cardiovascular endpoints as MeSH Major Topics (e.g., Coronary Disease[majr], Mortality[majr]); used adjacency for key phrases in titles/abstracts (e.g., “coronary heart disease”[tiab:~0]); excluded common non-targets (preprints, protocols, scoping reviews); applied a human-studies filter that also captures in-process records (Humans[mh] OR humans/randomized/randomised/prospective[tiab]); and paired publication-type tags with text word variants to robustly retrieve evidence syntheses. The refined query (ii) reduced the retrieval from 1,291 to 376 records. Two persons independently manually screened these 376 records by title/abstract and, when necessary, full text. In total, 26 systematic reviews/meta-analyses met the inclusion criteria, specifically 17 reviews of PCSs and 9 reviews of randomized controlled trials. Records of search strings are in the Appendix. We further checked a 2024 umbrella review on saturated fat and CVD and found no eligible papers missing.[2]

Statistical Analysis

Characteristics of each meta-analysis and systematic review were extracted and organized using Microsoft Excel spreadsheets. We recorded study design (e.g., number of cohorts, total participants), follow-up duration, exposure contrast for SFA (dose–response, highest vs lowest, or replacement), outcomes, key findings, and authors’ conclusions.

We used Bayesian analysis to synthesize findings from existing meta-analyses in separate umbrella reviews of RCTs and PCSs. For CHD incidence and stroke incidence in PCS, we returned to the primary studies within those reviews, extracted study-level estimates, and performed *de novo* meta-analyses. We did a *de novo* meta-analysis of PCS CHD incidence because saturated fat intake yielded the highest Bayesian umbrella relative risk, and for PCS stroke incidence, to further verify a protective effect of saturated fat in Bayesian umbrella analysis.

We chose a Bayesian framework for these umbrella reviews due to its distinct advantages in evidence synthesis, particularly when navigating the complexities of overlapping primary studies and heterogeneous evidence common in nutrition research.[4] Unlike the dichotomous conclusions often drawn from frequentist p-values, a Bayesian analysis provides a more intuitive and clinically useful output: a full posterior probability distribution for every parameter. This enables a transition from estimates of statistical significance to quantify the probability that an effect exceeds various thresholds of clinical relevance or, conversely, the probability that it lies within a region of practical nullity.[5] The random-effects models were specified with weakly informative priors, a standard practice

that regularizes estimates while allowing the synthesized data to drive the conclusions.[6] This approach formally accounts for uncertainty in all parameters, including the between-study heterogeneity expressed as the precision, τ ($=1/\sigma^2$), and provides a robust, transparent, and more complete representation. We ran sensitivity analyses with pessimistic priors and found no changes in the posterior probabilities that would materially alter the conclusions (not presented here).

Relative risks (RR) for all-cause mortality, CHD/CVD mortality, and CHD/CVD incidence were synthesized using a Bayesian random-effects meta-analysis on the log scale. We first extracted the pooled (review-level) adjusted RRs and 95% confidence intervals from the selected reviews. Each respective multivariable-adjusted RR and its 95% CI were transformed into a log effect size and standard error. We fit a random effects model with weakly informative priors: Normal(0,5) for the overall mean (log RR) and half-Normal(0.5) for the between-study SD. Primary outputs were the posterior median RR with 95% credible interval and posterior probabilities that the pooled effect exceeded prespecified thresholds (RR>1.00, 1.05, 1.10) or within a region judged null, without clinical importance (0.95–1.05).

The models were fitted in rstan in R v4.2.3, with four Hamiltonian Monte Carlo chains for 4,000 iterations each, using 1,000 warmup iterations for adaptation and retaining 12,000 post warmup draws for inference. Sample code is presented in **Supplement 2**.

Quality assessment of methods and evidence

Two reviewers assessed the risk of bias of each included systematic review/ meta-analysis using ROBIS. Both reviewers independently rated the study based on its eligibility criteria, identification and selection of studies, data collection and study appraisal, and synthesis and findings by recording responses of “Yes / Probably Yes / Probably No / No / No Information”, then rated domain-level concerns as “Low/ High/ Unclear”, which determined the overall risk of bias. Discrepancies were discussed and resolved between reviewers.

GRADE encoded in Bayesian analyses

The GRADE (Grading of Recommendations Assessment, Development and Evaluation) framework emerged to address fundamental challenges in evidence synthesis, particularly the difficulty of translating frequentist statistical outputs into clear clinical guidance. GRADE provides a structured system for expert-based appraising the certainty of evidence by considering factors like risk of bias, inconsistency, indirectness, and imprecision.[7,8] It serves as an essential structured layer on top of traditional statistical results, such as p-values and confidence intervals, which by themselves do not convey the probability of an effect. A confidence interval, for instance, may span clinically trivial to highly important effect sizes. Yet, the frequentist paradigm offers no probability distribution over this range, necessitating the systematic but post-hoc judgments that GRADE provides.[9]

Bayesian methods offer a path to integrate these judgments directly into the statistical model itself, producing a more unified and intuitive result. It removes the injection of expert opinion into the final assessment of effects. Instead of a multi-step process of calculating a statistic and then separately rating its certainty by expert opinion, a Bayesian analysis yields a single posterior probability distribution. This distribution naturally quantifies uncertainty, allowing for direct probabilistic statements such as "there is an 85% probability that the true effect lies between X and Y." Critically, the inputs to this model—the priors—allow for the explicit inclusion of existing knowledge and constraints. While the choice of priors requires careful justification, a conservative approach can be used to anchor the analysis in reality without dictating its conclusion. By employing weakly informative priors, for example, a model can be constrained to focus on biologically or chemically plausible effect

sizes, preventing unrealistic conclusions while still allowing the data from the studies to drive the final result. This addresses concerns about subjectivity while improving model stability and is the approach we used.

This integrated approach directly models most of the domains that GRADE assesses. Between-study heterogeneity, which GRADE handles with an expert opinion based downgrade for "inconsistency," is modeled directly within a Bayesian hierarchical framework, incorporating that variance into the final posterior distribution.[5] Likewise, concerns about imprecision are not a separate opinion-based judgment call but are reflected directly in the width of the posterior distribution. The transparency of the Bayesian approach comes from making all assumptions—including the priors and model structure—explicit and quantitative, rather than embedding them in separate qualitative expert opinion based rating rules.[10,11]

Results and Discussion

Our search and resulting final count of meta-analyses considered is presented in Figure 1 as a PRISMA flow chart. We found 9 RCTs and 17 PCSs that met our criteria.

Quality Assessments, RCT

We first prepared a Study Matrix showing the underlying studies contained in all nine RCTs and in the 17 PCSs (**Supplement 3**).

The nine RCTs published from 2001 to 2025 included 35 studies in total published from 1963 to 2016. RCTs were conducted in Europe and the United States at various places and times, with some as primary prevention and others as secondary prevention. We note that habitual diets and lifestyles were radically different in these places and times, including in ways that are now accepted as strong risk factors for atherosclerosis and total mortality, such as smoking and alcohol use.

The nine meta-analyses had many studies in common. We drew data from each of them, as available, for total mortality, CHD mortality, CVD mortality, CHD incidence, and CVD incidence. The CCA for these outcomes were 19%, 34%, 21%, 32%, 22%, respectively, all considered in the very high band on the arbitrary scaling of CCA. Out of nine meta-analyses, the most common ones were Rose 1965 (7), MRC 1968 (9), Dayton 1969 (8), Leren 1966/1970 (8), Woodhill 1978 (6), Burr 1989 (6), Frantz 1989 (7), Watts 1992 (7). We also note that *few of these studies were double-blinded*. Participants and or investigators knew the study group participants by virtue of knowing that they were consuming habitual foods or were being provided with a special diet. We did not tabulate the percentage of non-blinded studies.

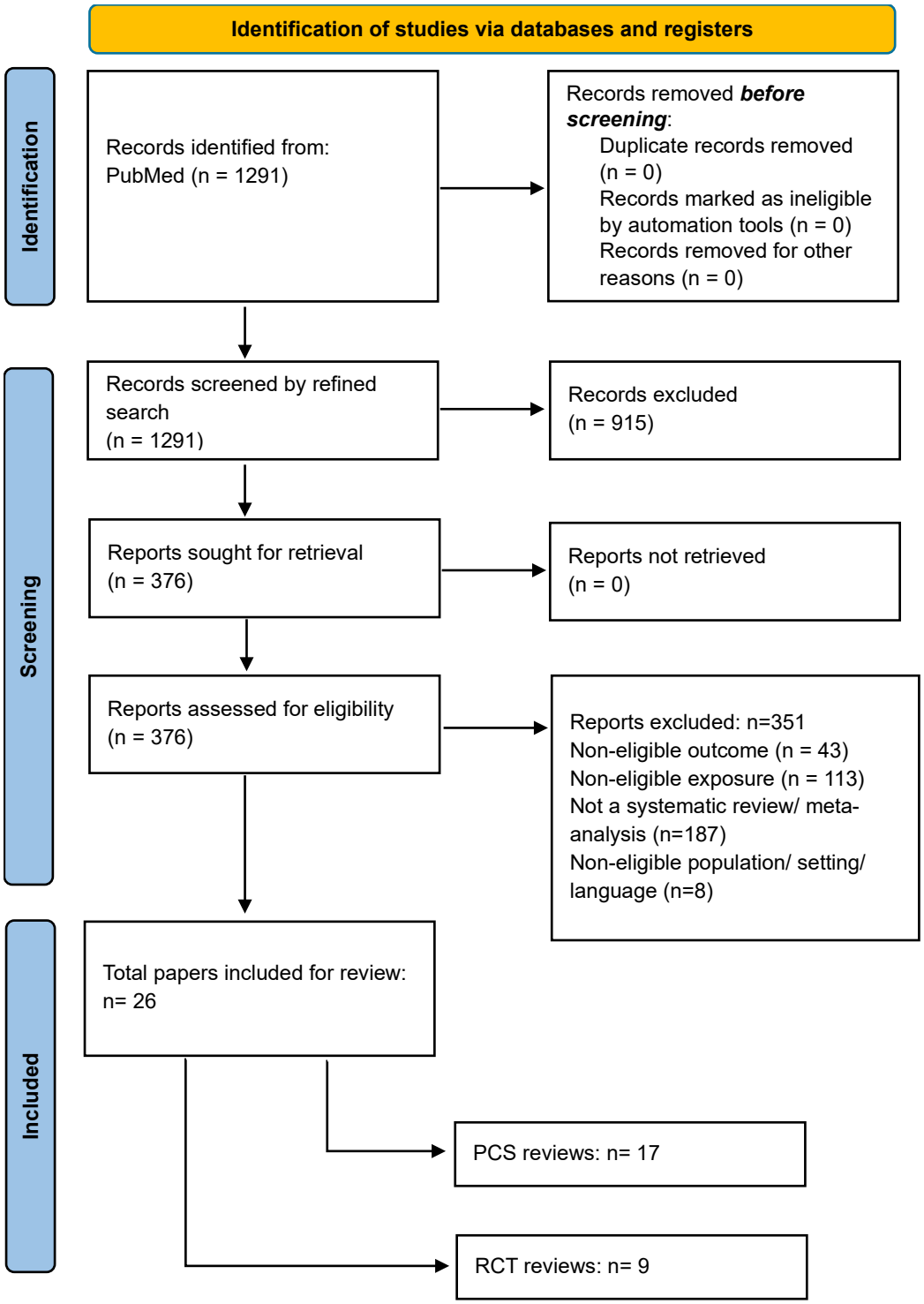


Figure 1. PRISMA 2020 flow diagram of the study selection process. A total of 1,291 records were identified through PubMed. After screening and eligibility assessment, 26 papers were included in the umbrella reviews. One paper had both RCT and PCS meta-analyses, 8 had only RCT meta-analyses, and 16 had only PCS meta-analyses. No duplicate records were identified, automation tools excluded no reports, and no reports were lost to retrieval.

We next looked at the interventions in most of the studies that were common to most meta-analyses to verify that they were, in fact, studies of saturated fat intake, as they have been claimed. Examination of selected studies shows that a key feature of many of the underlying studies widely considered as tests of saturated fat was the presence of partially hydrogenated oils (PHO) in the form of partially hydrogenated fish (including whale) oil (PHFO) in Europe and partially hydrogenated vegetable oil (PHVO) in the US. PHO was present in the comparator group in all of the meta-analyses. The minimum percentage of studies in which PHO was in the comparator group ranged from 29% to 88%. We concluded that these were studies of saturated and PHO-based fats compared to an intervention, not of saturated fats alone.

We incorporated these findings into our Robis analysis (**Supplement 4**). As a formal matter, Robis Domain 1, evaluates whether studies meet review criteria. All reviews were about the effects of saturated fat intake. However, most underlying studies included comparator groups with indeterminate amounts of trans fats from PHO. This confounding makes it impossible to attribute observed effects specifically to saturated fats, rendering the eligibility criteria inappropriate for the stated review question and thus, the objective bias is rated High.

We pivoted to a different question, namely the effects of saturated fat and PHO ("SF+PHO"). We then performed a new Robis evaluation for this intervention and proceeded with the data analysis.

PHO and Trans Fatty Acids

The term "trans" fat has become synonymous with fats that contain partially hydrogenated oils (PHO). The primary source of trans fats in Europe and other parts of the world from at least the 1950s to the 1980s was partially hydrogenated marine oils, specifically whale and fish oils. These were major components of spreads and shortenings for many years. Of particular importance to meta-analyses is the use of these oils in everyday foods in most of the countries where CVD studies were conducted. Trans and unusual isomers were known to be prominent in human tissue, including the aorta, by the early 1980s.[12] We[13] and others[14,15] have reviewed many of these studies previously, including their frank mischaracterization as having control groups free of "trans" fat.[16] One of the core studies conducted in Oslo expressly states, *"Nearly all marine fat used for human consumption, 40-50 g per day per head, is hydrogenated and used in the manufacture of margarine*"[1,17] an amount estimated to deliver almost 10 g/d of trans fatty acids.[15] Trans fatty acids were shown in 1990 to have rapid adverse effects on plasma lipoproteins,[18] which was later directly related to intake of PHO containing *trans* fatty acids and CHD events.[19] Because habitual diets served as controls for many of the vegetable oil interventions, many of these studies cannot ascribe their effects to the level of saturated fat precisely for this reason. And because all nine studies included multiple studies with PHO in the control groups, none of these metanalyses themselves is a pure test of saturated fat; they are all tests of saturated fat plus some PHO ("trans") fat. For completeness, we note that PHO contains many more unnatural fatty acids apart from fatty acids with trans double bonds; for instance, they contain *cis* isomers in unnatural positions[2,20] as well as a range of conjugated polyunsaturates,[21] the nutritional properties of which are unknown. Process contaminants are also likely to play roles in the atherogenicity of oils. Harsh processing of refined bleached deodorized (RBD) coconut oil and palm kernel oil before 1990 or so led to atherogenic properties. Virgin coconut oil made from fresh coconut meat, mildly processed, has identical levels of saturated fat to RBD but does not induce metabolic changes that lead to atherogenic plasma lipids.[22] We did two Robis assessments for RCTs, the first for the planned saturated fat. On the principle that the intervention-comparator groups were not different in only saturated fats, we assessed every paper as having *high bias* because the control groups had uncontrolled amounts of PHO by the ROBIS Domain 1 criteria. We also assessed

them as being tests of SF+PHO. Because intervention oils displaced habitual intake, they simultaneously reduced both saturated fat and PHO. Thus, observed effects cannot be attributed to changes in saturated fat alone—they reflect the combined reduction of SF+PHO. We then did a separate Robis assessment on the basis that all studies are SF+PHO (Supplement 4).

Relative Risks, RCTs

RCT Results are presented in **Supplement 5**. We provide an outline of our interpretation of the results here. We assessed that these meta-analyses had large numbers of studies of mixed SF+PHO; we use this term to avoid confusion. In these statistics, a *lower relative risk (RR) favors the reduction of SF+PHO*; that is, a lower RR means SF+PHO is harmful.

All cause mortality. We conclude with high confidence that SF+PHO effect is neutral for all cause mortality. The Bayesian probability that the relative risk (RR) lies between 0.95 and 1.05 is 95% (1.00 ± 0.05). We judge this interval to be below a clinically important effect.

CHD Mortality. We conclude with high confidence that SF+PHO has a neutral effect based on a 45% chance that mid-RR is less than 1.00. In other words, the distribution of likely RR is approximately symmetric around 1.

CVD Mortality: We conclude with moderate confidence (83% chance) that there is benefit to reduced SF+PHO. The mid-RR reflects a 6.6% benefit, with a 60% chance of ≥5% and 29% of ≥10% benefits, respectively.

CHD Incidence: We conclude with high confidence of a moderate benefit of reduced SF+PHO based on a 97% chance that the RR is < 1. The mid-RR reflects an 11% benefit, with 89% and 55% chances of ≥5% and ≥10% benefits, respectively.

CVD Incidence. We conclude with high confidence of a moderate benefit of reduced SF+PHO on the basis of a 94% chance RR < 1%. mid-RR indicates 16% benefit, and 79% chance of ≥10% benefit.

Integrating these results, we note that all cause and CHD mortality are not related to SF+PHO, while some signal is present for a benefit for reduced SF+PHO on CHD and CVD incidence and CVD mortality. These benefits are clinically small and are undoubtedly related to other factors not captured by SF+PHO. Moreover, these results imply compensatory health benefits in parameters not captured in these measurements, so as to balance the increased relative risk from CVD/CHD.

Quality Assessments, PCS

PCS results are also in Supplement 5. We provide an outline of our interpretation of the results here. We identified 17 meta-analyses published from 2009 to 2024, reviewing a total of 327 PCS published from 1953 to 2023. We did not examine the individual meta-analyses for evidence that they were quantifying PHO along with SF. All meta-analyses, regardless of publication date, included many studies from the 20th century when PHOs were widely present in the food supply. On the principle that changes of saturated fat were correlated with changes in PHO, we will retain the SF+PHO terminology, assigning effects to mixed saturated fat and PHO. In these statistics, a *higher relative risk (RR) favors the reduction of SF+PHO*, that is, SF+PHO is harmful.

The 17 meta-analyses had outcomes for total mortality, CHD mortality, CVD mortality, Stroke mortality, CHD incidence, CVD incidence, and Stroke Incidence. The CCA for these outcomes was 9% (moderate), 10% (high), 14% (high), 7% (moderate), 11% (high), 0.3% (slight), and 10% (moderate), respectively, with CCA bands in parentheses.

Relative Risks, PCSs

PCS RR sign is reversed compared to RCTs: *RR greater than 1 favors intervention* (SF+PHO harmful).

All cause mortality. We conclude with high confidence that SF+PHO effect is neutral for all cause mortality. The Bayesian probability that the relative risk (RR) lies between 0.95 and 1.05 is 79% (1.00 ± 0.05). 5% chance that mid-RR > 1.05 (5% chance that SF+PHO is harmful. We judge this to be below a clinically important effect.

CHD Mortality. We conclude with high confidence that SF+PHO has a neutral effect based on a 52% chance that mid-RR is = 1.00 ± 0.05; 63% chance mid-RR is greater than 1; 13% chance that mid-RR > 1.10 (13% chance that more SFA-PHO is 10% more harmful than less SFA-PHO).

CVD Mortality. We conclude with high confidence that SF+PHO is not harmful based on a 90% chance that the mid-RR lies between 0.95 and 1.05 (1.00 ± 0.05).

Stroke Mortality. We conclude with moderate confidence that SF+PHO is protective against stroke mortality based on 74% chance mid-RR < 1 (beneficial).

CHD Incidence. We conclude with high confidence that SF+PHO is neutral based on 83% chance mid-RR = 1.00 ± 0.05 and mid-RR of 1.027.

CVD Incidence. We conclude with high confidence that SF+PHO is neutral based on 97% chance mid-RR = 1.00 ± 0.05.

Stroke Incidence. We conclude with high confidence that SF+PHO is protective against stroke incidence based on 99.9% chance of RR<1 (protective); 0.12% chance of harm from SF+PHO.

PCS de novo meta-analyses

In two cases, we performed *de novo* meta-analyses starting with the data from the underlying studies. We considered Stroke Incidence and CHD Incidence.

Stroke incidence. From 18 studies, we extracted 55 RRs and related data for analysis. We conclude with high confidence that SF+PHO is mildly protective against stroke incidence with RR <1 of 98.4%. Precision (τ) is lower than for the umbrella review, as expected from the deduplication of redundant studies. This analysis is concordant with the stroke evidence from the umbrella review just above.

CHD Incidence. From 21 studies, we extracted 39 RRs and related data. We conclude with moderate confidence that CHD incidence is mildly greater with SF+PHO, based on mid-RR = 1.065 (6.5% increased risk); 74% chance that SF+PHO RR is <1.1 (10%). Compared to the neutral umbrella review results, this analysis points to mild harm.

GRADE analysis for study quality

The use of GRADE assessments for Bayesian methods is uncommon because Bayesian results provide quantitative assessments of some GRADE parameters. For instance, Bayesian $\tau = (1/\sigma^2)$ (precision = inverse of the variance) is corresponds to the Imprecision and the width of the posterior distribution corresponds to the Inconsistency. In addition, GRADE is not commonly used with Umbrella reviews, though its use is increasing.

We developed a GRADE analysis for the nine RCT meta-analyses only (**Supplement 6**), including a familiar matrix format and a second tab with details. We evaluated the studies as of SF+PHO. We found that all meta-analysis were very low to moderate quality and most had critical limitations.

Our conclusions are based on the quantitative synthesis of data afforded by Bayesian analysis that provides evidence of the absence of an effect, rather than the frequentist absence of evidence, within the constraints of the quality assessments.

Conclusions. Integrating RCT and PCS results.

Total mortality. RCT and PCS are both **neutral** with high confidence.

CHD mortality. RCT and PCS are both **neutral** with high confidence.

CVD mortality. RCT shows with moderate confidence a mild benefit in the reduction of SF+PHO. PCS shows with high confidence SF+PHO is neutral. Integrating these results, we favor RCT and **conclude with low confidence that the reduction of SF+PHO mildly reduces CVD mortality**.

CHD incidence. RCT and the *de novo* meta-analysis concluded with moderate confidence that SF+PHO mildly increases CHD incidence. The umbrella results conclude a neutral effect. Integrating these results, favoring the RCT, and noting the concordance of the *de novo* meta-analysis, we conclude that there is **moderate evidence that SF+PHO mildly increases CHD incidence**.

CVD incidence. RCT evidence provides high confidence that the reduction of SF+PHO moderately reduces CVD incidence, while PCS evidence provides high confidence of a neutral effect. Favoring RCT, we conclude that there is **moderate evidence of mild benefit from reducing SF+PHO**.

Stroke mortality and incidence. Stroke mortality from the one PCS umbrella analysis provides moderate evidence of mild protection. Stroke incidence in the umbrella review and *de novo* meta-analysis both provide **strong evidence of a mild benefit of SF+PHO**.

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Supplement 1

Search Strings

PubMed search string retrieving 1291 results

((("Fatty Acids, Saturated"[MeSH Terms] OR "saturated fat*"[tiab] OR "saturated fatty acid*"[tiab] OR SFA[tiab]) AND ("Cardiovascular Diseases"[MeSH Terms] OR "Coronary Disease"[MeSH Terms] OR "Myocardial Infarction"[MeSH Terms] OR "Stroke"[MeSH Terms] OR "Peripheral Arterial Disease"[MeSH Terms] OR "heart disease*"[tiab] OR "myocardial infarction"[tiab] OR "ischemic heart disease"[tiab] OR "ischaemic heart disease"[tiab] OR "peripheral artery disease"[tiab] OR "peripheral arterial disease"[tiab] OR PAD[tiab] OR CHD[tiab] OR CVD[tiab] OR mortality[tiab] OR "all-cause mortality"[tiab])) AND (Meta-Analysis[pt] OR Systematic Review[pt] OR "systematic review"[tiab] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab])) OR ("pooled analysis"[tiab] AND "dietary fat"[tiab] AND ("coronary heart disease"[tiab] OR "ischemic heart disease"[tiab])) OR ("dietary fat guidelines"[tiab] AND ("randomized"[tiab] OR "randomised"[tiab]) AND ("meta-analysis"[tiab] OR "systematic review"[tiab])) OR ((Dairy Products[MeSH Terms] OR dairy[tiab] OR milk[tiab] OR cheese[tiab] OR yogurt[tiab] OR yoghurt[tiab]) AND ("Cardiovascular Diseases"[MeSH Terms] OR "coronary heart disease"[tiab] OR "ischemic heart disease"[tiab] OR "ischaemic heart disease"[tiab] OR "myocardial infarction"[tiab] OR stroke[tiab] OR CHD[tiab] OR CVD[tiab] OR atherosclerotic[tiab]) AND (Meta-Analysis[pt] OR Systematic Review[pt] OR "systematic review"[tiab] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab])) AND english[lang] AND ("2000/01/01"[dp] : "2025/12/31"[dp])

PubMed Search String retrieving 376 results

(((Fatty Acids, Saturated[mh:noexp] OR "saturated fat*"[tiab] OR "saturated fatty acid*"[tiab] OR SFA[tiab]) AND (diet*[tiab] OR intake[tiab] OR consum*[tiab] OR replac*[tiab] OR substitut*[tiab] OR restrict*[tiab] OR isocaloric[tiab] OR isoenergetic[tiab] OR macronutrient*[tiab] OR circulating[tiab] OR plasma[tiab] OR serum[tiab] OR biomarker*[tiab]) AND (Coronary Disease[majr] OR Myocardial Infarction[majr] OR Stroke[majr] OR Peripheral Arterial Disease[majr] OR Cardiovascular Diseases[majr] OR Mortality[majr] OR "coronary heart disease"[tiab:~0] OR "ischemic heart disease"[tiab:~0] OR "ischaemic heart disease"[tiab:~0] OR "myocardial infarction"[tiab:~0] OR stroke[tiab] OR "peripheral arter* disease"[tiab] OR "all-cause mortality"[tiab] OR "all cause mortality"[tiab] OR "cardiovascular mortality"[tiab] OR "cardiovascular disease"[tiab:~0] OR "atherosclerotic cardiovascular disease"[tiab:~0]) AND (Meta-Analysis[pt] OR Systematic Review[pt] OR "systematic review"[tiab] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab] OR "network meta-analysis"[tiab] OR "network meta-analyses"[tiab] OR "network meta analysis"[tiab:~0] OR "umbrella review"[tiab] OR "pooled analysis"[tiab])) OR ((Dairy Products[mh:noexp] OR dairy[tiab] OR cheese[tiab] OR yogurt[tiab] OR yoghurt[tiab] OR butter[tiab] OR "dairy fat"[tiab]) AND (diet*[tiab] OR intake[tiab] OR consum*[tiab] OR replac*[tiab] OR substitut*[tiab] OR restrict*[tiab] OR isocaloric[tiab] OR isoenergetic[tiab] OR macronutrient*[tiab] OR circulating[tiab] OR plasma[tiab] OR serum[tiab] OR biomarker*[tiab]) AND (Coronary Disease[majr] OR Myocardial Infarction[majr] OR Stroke[majr] OR Peripheral Arterial Disease[majr] OR Cardiovascular Diseases[majr] OR Mortality[majr] OR "coronary heart disease"[tiab:~0] OR "ischemic heart disease"[tiab:~0] OR "ischaemic heart disease"[tiab:~0] OR "myocardial infarction"[tiab:~0] OR stroke[tiab] OR "all-cause mortality"[tiab] OR "all cause mortality"[tiab] OR "cardiovascular mortality"[tiab] OR "cardiovascular disease"[tiab:~0] OR "atherosclerotic cardiovascular disease"[tiab:~0]) AND (Meta-Analysis[pt] OR Systematic Review[pt] OR "systematic review"[tiab] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab] OR "network meta-analysis"[tiab] OR "network meta-analyses"[tiab] OR "network meta analysis"[tiab:~0] OR "umbrella

review"[tiab] OR "pooled analysis"[tiab])) OR ("dietary fat guidelines"[tiab:~0] AND (randomized[tiab] OR randomised[tiab]) AND ("meta-analysis"[tiab] OR "systematic review"[tiab])) OR (macronutrient*[tiab] AND (substitut*[tiab] OR replac*[tiab] OR exchang*[tiab] OR isocaloric[tiab] OR isoenergetic[tiab]) AND ("coronary heart disease"[tiab:~0] OR "ischemic heart disease"[tiab:~0] OR "ischaemic heart disease"[tiab:~0] OR "myocardial infarction"[tiab:~0] OR stroke[tiab] OR "all-cause mortality"[tiab] OR "all cause mortality"[tiab] OR mortality[tiab] OR "cardiovascular mortality"[tiab] OR "cardiovascular disease"[tiab:~0] OR "atherosclerotic cardiovascular disease"[tiab:~0]) AND (Meta-Analysis[pt] OR Systematic Review[pt] OR "systematic review"[tiab] OR "meta-analysis"[tiab] OR "meta-analyses"[tiab] OR "network meta-analysis"[tiab] OR "network meta-analyses"[tiab] OR "network meta analysis"[tiab:~0])) OR ("dietary factors"[tiab] AND "coronary heart disease"[tiab:~0] AND ("systematic review"[tiab] OR Meta-Analysis[pt] OR Systematic Review[pt]))) AND English[lang] AND (Humans[mh] OR humans[tiab] OR randomized[tiab] OR randomised[tiab] OR prospective[tiab]) AND ("2000/01/01"[dp] : "2025/12/31"[dp]) NOT preprint[pt] NOT (protocol[tiab] OR "scoping review"[tiab])

Supplement 2

Sample Code

```
# ==== Packages & Stan options ====
# Code for CVD Mortality

library(dplyr)
library(rstan)
if (!requireNamespace("rstudioapi", quietly = TRUE)) {
  options(mc.cores = 1)
} else {
  options(mc.cores = parallel::detectCores())
}
rstan_options(auto_write = TRUE)

# ==== Stan model: normal–normal RE meta on log scale ====
stan_code_meta <- "
data {
  int<lower=1> K;              // number of estimates
  vector[K] y;                 // log RR/HR per study
  vector<lower=0>[K] se;       // SE of log RR/HR
  real<lower=0> tau_prior_sd;  // half-normal SD for heterogeneity
}
parameters {
  real mu;                     // overall mean (log RR)
  real<lower=0> tau;           // between-study SD
}
model {
  mu  ~ normal(0, 5);                 // weakly informative
  tau ~ normal(0, tau_prior_sd);      // half-normal
  y   ~ normal(mu, sqrt(se .* se + tau^2));   // marginal likelihood
}
generated quantities {
  real RR_mu    = exp(mu);            // pooled RR on natural scale
  real theta_new = normal_rng(mu, tau); // true effect in a new study (log scale)
  real RR_pred = exp(theta_new);        // prediction for a new study's true
effect
}
"

# ==== Helpers: turn (RR, LCL, UCL) into (logRR, SE) ====
ci_to_log_es <- function(rr, lcl, ucl) {
  y  <- log(rr)
  se <- (log(ucl) - log(lcl)) / (2*1.96)
  list(y = y, se = se)
}

make_stan_data <- function(df, rr_col="RR", lcl_col="LCL", ucl_col="UCL",
                           tau_prior_sd = 0.5) {
```

```
  es <- ci_to_log_es(df[[rr_col]], df[[lcl_col]], df[[ucl_col]])
  list(K = length(es$y), y = as.vector(es$y), se = as.vector(es$se),
       tau_prior_sd = tau_prior_sd)
}

summarise_draws <- function(fit, probs = c(0.025, 0.5, 0.975)) {
  dr <- rstan::extract(fit)
  out <- tibble(
    pooled_RR_median   = quantile(exp(dr$mu), 0.5),
    pooled_RR_95_LCI   = quantile(exp(dr$mu), 0.025),
    pooled_RR_95_UCI   = quantile(exp(dr$mu), 0.975),
    Pr_RR_gt_1         = mean(dr$mu > 0),                # Pr(RR>1)
    Pr_RR_gt_1_05      = mean(dr$mu > log(1.05)),        # Pr(RR>1.05)
    Pr_RR_gt_1_10      = mean(dr$mu > log(1.10)),        # Pr(RR>1.10)
    Pr_0_95_lt_RR_lt_1_05 = mean(dr$mu > log(0.95) & dr$mu < log(1.05)),
    tau_median         = median(dr$tau),
    tau_95_LCI         = quantile(dr$tau, 0.025),
    tau_95_UCI         = quantile(dr$tau, 0.975),
    pred_RR_median     = quantile(dr$RR_pred, 0.5),      # prediction (true
effect in a new study)
    pred_RR_95_LCI     = quantile(dr$RR_pred, 0.025),
    pred_RR_95_UCI     = quantile(dr$RR_pred, 0.975)
  )
  out
}

# 1) REVIEW-LEVEL pooling (replace with your review rows)
allcause_reviews <- tibble::tribble(
  ~label,                              ~RR,   ~LCL,  ~UCL,
   "de Souza 2015 (CVD mort)",              0.97,  0.84,  1.12,
  "Kim 2021 (highest vs lowest)",          1.02,  0.92,  1.12,
  "Kim 2021 (per 5%E higher SFA)",         1.03,  1.00,  1.07,
  "Ma 2024 (CVD mort)",                    1.03,  0.98,  1.08,
  "Mazidi 2020 (CVD mort)",                0.96,  0.84,  1.11
)

dat_rev <- make_stan_data(allcause_reviews, rr_col="RR", lcl_col="LCL",
ucl_col="UCL", tau_prior_sd = 0.5)
fit_rev <- stan(model_code = stan_code_meta, data = dat_rev,
                iter = 4000, warmup = 1000, chains = 4, seed = 2025,
                control = list(adapt_delta = 0.98, max_treedepth = 14))
summary_rev <- summarise_draws(fit_rev)
print(summary_rev)
```


1. Leren P. The effect of plasma cholesterol lowering diet in male survivors of myocardial infarction. A controlled clinical trial. *Acta Med Scand Suppl*. 1966;466:1-92 (see p 35).
2. Tyburczy C, Major C, Lock AL, et al. Individual trans octadecenoic acids and partially hydrogenated vegetable oil differentially affect hepatic lipid and lipoprotein metabolism in golden Syrian hamsters. *J Nutr*. Feb 2009;139(2):257-63. doi:10.3945/jn.108.098004

Supplement 3

RCT Study Matrix

		Hooper 2001 (BMJ)	Skeaff & Miller 2009 (Ann Nutr Metab)	Mozaffarian 2010 (PLoS Med)	Schwingshackl & Hoffmann 2014 (BMJOpen)	Harcombe 2015 (Open Heart)	Ramsden 2016 (BMJ)	Hamley 2017 (Nutrition J)	Hooper 2020 (Cochrane)		Included in X meta-analyses	Comments
1963	National Diet-Heart Study (NDHS, multiple centers)	X									1	
1965	Ball 1965				X						1	
1965	Research Committee 1965		X								1	Control diet not controlled (continued normal diet), 600kcal differences between control and diet groups. MI relapse rates n.s.
1965	Rose 1965		X		X	X	X	X	X	X	7	Control group was unblinded and not treated.
1966	Leren 1966									X	1	Same diet as Leren 1970
1968	MRC 1968	X	X	X	X	X	X	X	X	X	9	Control diet not controlled; persons were free living and no mention of food provided
1968	National Diet-Heart Study (NDHS, 1968)							X			1	Mental hospital sub-study; too brief to evaluate hard clinical endpoints. Free living participants prescribed visible fats expressly containing margarines and shortenings).
1969	Dayton 1969	X	X	X		X	X	X	X	X	8	Control diet not controlled. VA hospital fed ad libitum hospital food, and free living daily.
1970	Leren 1970	X	X	X	X	X	SA	X	X		7	Control group had "40-50 g/d hydrogenated marine (fish/whale) oil"
1978	Oxford 1978	X									1	This is a CHO intake and diabetes study
1978	Woodhill 1978	X			X	X	X	X	X		6	Control diet not controlled. Diet group provided advice on reducing SFA intake.
1979	Houtsmuller 1979								X		1	This is a study on LA and diabetic micro- and macroangiopathy
1979	Turpeinen 1979		X	X				X			3	from the abstract: "…control with a normal hospital diet."
1980	Houtsmuller 1980							X			1	This is a study on LA and diabetic micro- and macroangiopathy
1983	Miettinen 1983		X	X							2	Women Finnish mental hospital study. Control diet not controlled "continued with normal diet"

		Hooper 2001 (BMJ)	Skeaff & Miller 2009 (Ann Nutr Metab)	Mozaffarian 2010 (PLoS Med)	Schwingshackl & Hoffmann 2014 (BMJOpen)	Harcombe 2015 (Open Heart)	Ramsden 2016 (BMJ)	Hamley 2017 (Nutrition J)	Hooper 2020 (Cochrane)		Included in X meta-analyses	Comments
1989	**Burr 1989**		X	X	X		SA	X	X	X	6	Advice only. No diet control
1989	**Frantz 1989**	X	X	X		X	X	X		X	7	1.3-2.3 TFA, as per Ramsden 2010
1992	**Watts 1992**	X	X	X	X		SA	X	X	X	7	Randomized to "usual care"
1993	**Sollentuna diet**	X									1	Reduced fat and exercise study, no hard clinical endpoints
1994	**Black 1994**	X							X		2	This is a study on low-fat diets and actinic keratosis
1994	**de Lorgeril 1994**				X						1	The Lyon Diet Heart Study. Control group received no dietary advice; experimental group received PHO-containing margarine.
1994	**Stanford weight 1994**	X									1	This is a calorie restriction/ exercise study
1995	**Toronto Polyp Prevention 1995**	X									1	Not a sat fat study, nor does it have hard clinical endpoints
1997	**MSFAT**	X									1	low fat food lead to lower total fat intake, no significant differences on cardiovascular risk factors
1998	**Turku weight (mixed; vegetarian)**	X									1	This is just a calorie deficit trial looking at lipid related cardiovascular risk factors
2001	**Moy 2001**								X		1	Only Counseling as intervention, looking only at risk factors and behavioral outcomes
2003	**Sondergaard 2003**				X						1	Mediterranean dietary advice only, no hard clinical endpoints
2004	**Ley 2004**								X		1	Control diet ad libitum; Intervention group counseled on reduced-fat, otherwise ad libitum diet.
2006	**Howard 2006**		X		X				X		3	Control is usual diet, intervention is "intensive behavior-modification counseling", no food provided.
2006	**Michalsen 2006**				X						1	No diet provided, control group ad libitum; intervention group Mediterranean diet education. No hard clinical endpoints measured.
2006	**WINS 2006**								X		1	Also only counseling vs. no conuseling. No food provided, diets were self selected. No hard clinical endpoints.

		Hooper 2001 (BMJ)	Skeaff & Miller 2009 (Ann Nutr Metab)	Mozaffarian 2010 (PLoS Med)	Schwingshackl & Hoffmann 2014 (BMJOpen)	Harcombe 2015 (Open Heart)	Ramsden 2016 (BMJ)	Hamley 2017 (Nutrition J)	Hooper 2020 (Cochrane)		Included in X meta-analyses	Comments
2013	Ramsden 2013									X	1	Control diet not controlled. Intervention group provided safflower oil and safflower oil margarine to replace butter and common margarines.
2016	Vijayakumar 2016									X	1	Participants were given commercial coconut or sunflower oil. No difference were found after two years in cardiovascualr risk factors
2016	Ramsden 2016						X				1	MCE, "free surplus USDA food commodities including common margarines and shortenings were key components of the control diet"
	number with trans fatty acids in control (estimated)	4	6	7	5	5	4	8	6	7		
	number of studies	14	11	8	11	6	6	11	13	9		
	% studies with trans or uncontrolled control	29%	55%	88%	45%	83%	67%	73%	46%	78%		

SA - Sensitivity analysis

Supplement 3 (continued)

PCS Study Matrix

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
1953	London Bus and Bank Study1953							X		
1958	Baltimore Longitudinal Study of Aging (BLSA) 1958				X					
1964	Glostrup Population Studies Multi–centre 1964		X		X					
1965	Hegsted et al. – 1965	X								
1966	Borchgrevink et al. – 1966 – Alpha–linolenic	X								
1967	Serum – 1967				X					
1968	Medical Research Council (MRC) Trial				X					
1968	Natvig et al. – 1968 – Alpha–linolenic	X								
1969	Dayton – 1969				X					
1970	Borchgrevink/OSLO				X					
1970	Leren – 1970				X					
1970	Whitehall Study				X					
1972	Finnish Clinic Health Examination Survey (FCHES)				X					
1972	Men – 1972				X					
1972	Finnish Mobile Clinic Health Study (FMC)		X							
1973	Los Angeles Veterans Study (LA Veteran)				X					
1973	Sydney Diet Heart Study (SDHS)				X					
1976	Bang et al. – 1976	X								
1977	Men – 1977				X					
1979	Finnish Mental Hospital Study (FMHS)				X					
1980	Before – 1980				X					
1980	Puerto Rico Heart Health Program							X		
1981	Shekelle – 1981			X		X				
1983	Lipid Research Clinics (LRC)				X	X			X	

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
1983	Miettinen et al. – 1983 – Finnish Mental Hospital	X								
1983	Seven Countries Study							X		
1984	Kingdom – 1984				X					
1984	McGee – 1984			X			X			
1984	Oxford Vegetarian Study				X					
1985	Both – 1985				X					
1985	Fresh – 1985				X					
1985	Health and Lifestyle Survey (HLS)				X				X	
1985	Ireland–Boston Diet Heart Study (IBDH)				X					
1985	Kushi – 1985			X		X			X	
1985	McGee – 1985 – USA Honolulu Heart Program					X		X		
1985	Kushi 1985 (Ireland–Boston Diet Heart Study)									
1986	Israeli Ischemic Heart Disease Study (IIHD)		X		X	X				
1986	Keys et al. – 1986	X								
1986	Quebec Cardiovascular Study (QCS)				X					
1988	Adventist Health Study (AHS)		X							
1989	Atherosclerosis Risk in Communities (ARIC)		X		X					
1989	Burr et al. – 1989	X								
1989	Witteman - 1989									
1990	After – 1990				X					
1991	Both – 1991				X					
1991	Leaf et al. – 1991 – Leaf trial	X								
1991	Posner 1991	X		X	X	X		X		
1991	Posner – 1991					X				
1992	STARS				X					
1993	Cardiovascular Health Study (CHS)				X					
1993	Denmark – 1993				X					

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
1993	Fehily – 1993			X						
1993	Goldbourt – 1993			X		X	X			
1993	Rohan – 1993									
1993	Cardiovascular Health Study (CHS), trans fat — Wang 2014					X				
1993	EPIC — Spanish centers									
1994	Black et al. – 1994	X								
1994	Dwyer – 1994									
1994	Western Electric Study							X		
1995	Ascherio – 1995									
1995	Grundt et al.				X					
1995	HARP				X					
1995	Kromhout et al. – 1995 – ATBC	X	X		X	X			X	
1995	Physicians' Health Study (PHS)				X					
1995	Scottish Heart Health Study (SHHEC)				X					
1996	Ascherio 1996	X		X		X			X	
1996	Ascherio – 1996					X			X	
1996	Esrey – 1996					X			X	
1996	Ascherio 1996 (HPFS)									
1997	Clarke et al. – 1997	X								
1997	Daviglus et al. – 1997 – HPFS	X	X		X	X			X	
1997	Gillman – 1997					X	X	X		X
1997	Hu – 1997 – NHS		X		X	X				
1997	Mann – 1997			X						
1997	Pietinen P - 1997	X		X		X			X	
1997	Pietinen – 1997 – Finland					X			X	
1997	Seino F - 1997					X				
1997	Seino – 1997					X	X			X
1997	Singh et al. – 1997	X								

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
1997	UK health-conscious cohort — Mann 1997					X				
1998	Honolulu Heart Study (HHS) 1998				X			X		
1998	Leng 1998				X					
1998	Multiple Risk Factor Intervention Trial (MRFIT) 1998				X					
1998	Singapore Chinese Health Study (SCHS) 1998									
1999	GISSI–Prevenzione Investigators – 1999				X					
1999	Holmes – 1999									
1999	Hu – 1999 – Nurses' Health Study		X			X				
1999	Lyon Diet Heart Study 1999				X					
1999	NHANES – 1999									
1999	Payette – 1999									
1999	von Schacky 1999				X					
2000	Liu – 2000									
2000	Ludwigshafen 2000				X					
2000	Palli – 2000									
2001	Iso – 2001					X	X			X
2001	Nilsen et al.				X					
2001	Oomen 2001 (Zutphen trans fat)	X				X				
2001	Oomen – 2001				X	X				
2001	Oomen 2001 (Zutphen trans fat)									
2001	Yuan 2001									
2002	Bemelmans et al. – 2002 – Alpha–linolenic	X								
2002	Boniface 2002			X		X			X	
2002	Boniface – 2002					X			X	
2002	Bucher et al. – 2002	X								
2002	Danish National Birth Cohort (DNBC)				X					
2002	He – 2002 – JAMA									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2002	Hu – 2002 – JAMA									
2003	Aric – 2003		X		X					
2003	Erkkila et al. – 2003 – EUROASPIRE	X			X					
2003	From – 2003				X					
2003	He 2003			X		X			X	
2003	He – 2003					X	X		X	X
2003	Hu – 2003									
2003	Iso – 2003						X			X
2003	Jelis – 2003				X					
2003	Oct – 2003				X					
2003	Oh – 2005					X				
2003	Trichopoulou – 2003 – EPIC Greece									
2003	Zhang – 2003									
2003	Mozaffarian 2003 cohort									
2004	Borugian – 2004									
2004	Jakobsen 2004	X		X						
2004	Jakobsen – 2004									
2004	Sauvaget 2004			X						
2004	Sauvaget – 2004						X			X
2004	Tanasescu2004									
2004	Tanasescu – 2004		X			X				
2005	Albert – 2005 – Circulation									
2005	Kelemen – 2005									
2005	Kelemen – 2005 – IWHS (USA)		X							
2005	Leaf et al. – 2005 – Leaf trial	X								
2005	Leosdottir – 2005				X	X				
2005	Mozaffarian et al. – 2005	X								
2005	Mozaffarian – 2005									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2005	Nakamura et al. – 2005 – EUROASPIRE	X								
2005	Raitt et al. – 2005 – Raitt trial	X			X					
2005	Solfrizzi – 2005									
2005	Trichopoulou – 2005									
2005	Tucker et al. – 2005 – Iowa Women	X		X						
2005	Tucker – 2005 – USA Baltimore Longitudinal Study of Aging					X				
2005	WHS — Women's Health Study		X							
2006	Howard et al. – 2006 – Women's Health Initiative	X								
2006	Jarvinen – 2006 – Br J Nutr									
2006	SOFA				X					
2006	Trichopoulou – 2006									
2006	Xu J, 2006	X		X	X	X			X	
2006	Xu – 2006					X			X	
2006	Wiberg 2006					X				
2006	Iso 2006									
2006	Järvinen 2006									
2007	Lagiou – 2007									
2007	Leosdottir – 2007					X				
2007	Smit – 2007									
2007	Trichopoulou – 2007									
2007	Yokoyama et al. – 2007 – Yokoyama	X								
2007	Yokoyama – 2007 – JELIS – Lancet – PMID:17398308				X					
2008	GISSI–HF				X					
2008	Streppel – 2008 – Zutphen Study – Eur Heart J									
2008	Virtanen – 2008 – Am J Clin Nutr									
2009	Alpha Omega				X					
2009	Boden–Albala 2009				X					

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2009	Boden–Albala – 2009									
2009	Dijkstra – 2009 – Rotterdam Study – Eur J Heart Fail									
2009	Halbesma – 2009									
2009	Jakobsen – 2009 – Am J Clin Nutr									
2009	Levitan – 2009 – Eur Heart J									
2009	Montonen – 2009 – J Nutr									
2009	SU.FOL.OM3				X					
2009	Virtanen – 2009 – Circulation									
2010	Bates – 2010									
2010	de Goede – 2010 – J Nutr									
2010	Fung – 2010									
2010	Heine–Broring – 2010 – Rotterdam Study – Am J Clin Nutr									
2010	Jakobsen – 2010 – Am J Clin Nutr									
2010	National Health Screening Service (NHSS)				X	X				
2010	Preis – 2010									
2010	Yamagishi – 2010 – JACC – Am J Clin Nutr – PMID:20685950						X			X
2010	EPIC-NL (Dutch EPIC)									
2011	Akbaraly – 2011 – Whitehall II – Am J Clin Nutr									
2011	Atkinson 2011				X	X				
2011	Atkinson – 2011						X			
2011	de Goede – 2011 – PLoS One									
2011	Houston 2011									
2011	Houston – 2011									
2011	Vedtofte – 2011 – Am J Clin Nutr									
2011	Chinese cohorts (Zhang/Zhuang)									
2011	Belin – 2011 – Women's Health Initiative – Circ Heart Fail									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2012	Chiuve 2012									
2012	Chiuve – 2012									
2012	de Oliveira Otto MC 2012									
2012	de Oliveira Otto – 2012									
2012	Dilis – 2012 – EPIC Greece				X					
2012	Kokura Study (KOKURA)				X					
2012	Laake – 2012 – NCS (Norway)									
2012	Larsson 2012									
2012	Larsson – 2012						X			X
2012	Misirli – 2012					X	X			
2012	Nagata 2012				X				X	
2012	Nagata – 2012 – Takayama study Japan								X	
2012	Nilsson – 2012		X		X					
2012	OPERA				X					
2012	ORIGIN				X					
2012	Risk and Prevention Study (RPS)				X					
2012	Strom – 2012									
2012	Wallstrom 2012			X	X	X				
2012	Wallstrom – 2012					X	X			X
2012	Yaemsiri 2012									
2012	Yaemsiri – 2012						X			X
2012	de Goede 2010–2012									
2013	Argos – 2013									
2013	Argos – 2013 – HEALS (Bangladesh)									
2013	Chien – 2013 – Japan (Chin–Shan)									
2013	Kiage – 2013 – REGARDS – Am J Clin Nutr					X				
2013	October – 2013				X					

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2013	Simila – 2013									
2013	Yamagishi K 2013				X	X				
2013	Yamagishi – 2013					X	X			X
2013	Yu – 2013 – ATBC		X			X			X	
2013	de Oliveira Otto 2012/2013									
2014	Haring – 2014									
2014	Kiage 2014					X				
2014	Levine – 2014									
2014	Levine – 2014 – NHANES III (USA)									
2014	Miyagawa – 2014									
2014	OPACH				X					
2014	Rebello – 2014									
2014	Santos – 2014									
2014	Virtanen 2014				X	X				
2014	Virtanen – 2014					X				
2014	Wakai – 2014				X	X				
2014	Wakai – 2014									
2014	Vedtofte 2011/2014									
2014	Farvid 2014 (linoleic pooled)									
2014	Amiano 2014									
2014	Miyagawa 2014									
2015	Campmans–Kuijpers – 2015									
2015	Chiuve – 2015									
2015	Guasch–Ferre 2015									
2015	Guasch–Ferre – 2015									
2015	Li – 2015									
2015	Nagata – 2015									
2015	Puaschitz 2015									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2015	Puaschitz – 2015									
2015	Li 2015									
2016	Campmans–Kuijpers – 2016									
2016	Chen – 2016									
2016	Courand – 2016									
2016	Hernandez–Alonso – 2016									
2016	Hernandez–Alonso, 2016									
2016	Owen – 2016									
2016	Praagman 2016	X		X	X					
2016	Praagman – 2016									
2016	Song – 2016									
2016	Song – 2016 – NHS & HPFS (USA)		X			X			X	
2016	Wang 2016	X								
2016	Wang – 2016									
2016	Xu – 2016									
2016	Zong – 2016		X			X			X	
2016	Sala-Vila 2016									
2017	Dehghan – 2017									
2017	Dinesen 2017									
2017	Holmes – 2017									
2017	Rhee – 2017									
2017	Sluijs2017									
2017	Sluijs – 2017									
2017	Wang – 2017					X			X	
2017	Zaslavsky – 2017									
2017	Nagata Japanese cohort									
2017	Rhee 2017									
2017	Dehghan 2017 (PURE)									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2018	AlEssa – 2018									
2018	Arthur – 2018									
2018	Dominguez – 2018 – SUN (Spain)									
2018	Ricci – 2018									
2018	Ricci – 2018 – NHANES (USA)									
2018	Seidelmann – 2018									
2018	Song – 2018 – Mendonca et al.,2020									
2018	Tharrey – 2018									
2018	Zhuang – 2018									
2019	Budhathoki – 2019									
2019	Chan – 2019									
2019	Jiao – 2019		X			X			X	
2019	Kurihara – 2019									
2019	Mazidi – 2019									
2019	Okada – 2019									
2019	Praagman – 2019				X					
2019	Virtanen – 2019									
2019	Zhuang – 2019									
2019	Zhuang, 2019a									
2019	Praagman 2016–2019									
2020	Chen – 2020									
2020	Chen – 2020									
2020	Ho – 2020									
2020	Ho – 2020									
2020	Huang – 2020									
2020	Huang – 2020									
2020	Langsetmo – 2020									
2020	Lelli – 2020									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2020	Lin – 2020									
2020	Mao – 2020 – CHNS (China)									
2020	Mao – 2020									
2020	Mazidi – 2020									
2020	Mendonca – 2020		X			X				
2020	Mirmiran – 2020									
2020	Miyazawa – 2020									
2020	Shan – 2020									
2020	Shan – 2020 – NHANES (USA)									
2020	Trevisan – 2020									
2020	Wu – 2020 – CHNS (China)									
2021	Akter – 2021									
2021	Fontana – 2021 – EPIC–Italy (Italy)									
2021	Kwon – 2021 – KoGES (Korea)									
2021	Kwon – 2021									
2021	Laguna – 2021									
2021	Sadeghi – 2021									
2021	Sun – 2021									
2021	Sun – 2021 – WHI (USA)									
2021	Yao – 2021									
2021	Steur 2021									
2021	Voortman 2021									
2021	Glenn 2021									
2022	Das – 2022 – CHAMP (Australia)									
2022	Merono – 2022 – InCHIANTI (Italy)									
2022	Zeng – 2022 – NHANES (USA)									
2022	Zhou – 2022									
2023	Bajracharya – 2023									

Year	Study	Skeaff 2009 (Ann Nutr Metab)	Jakobsen 2009 AJCN	Siri-Tarino 2010 (AJCN)	Chowdhury 2014 (Ann Intern Med)	De Souza 2015 (BMJ)	Cheng 2016 (Neurol Sci)	Harcombe 2017a BJSM (Pre-1983)	Harcombe 2017b BJSM (Current)	Muto 2018 (J Atheroscler Thromb)
2023	Zhao – 2023 – NIH–AARP (USA)									
	Number of studies	31	17	17	78	53	15	8	22	11

Supplement 3 (continued)

PCS Study Matrix Part 2

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
1953	London Bus and Bank Study1953								
1958	Baltimore Longitudinal Study of Aging (BLSA) 1958								X
1964	Glostrup Population Studies Multi–centre 1964								X
1965	Hegsted et al. – 1965								
1966	Borchgrevink et al. – 1966 – Alpha–linolenic								
1967	Serum – 1967								
1968	Medical Research Council (MRC) Trial								
1968	Natvig et al. – 1968 – Alpha–linolenic								
1969	Dayton – 1969								
1970	Borchgrevink/OSLO								
1970	Leren – 1970								
1970	Whitehall Study								
1972	Finnish Clinic Health Examination Survey (FCHES)								
1972	Men – 1972								
1972	Finnish Mobile Clinic Health Study (FMC)								X
1973	Los Angeles Veterans Study (LA Veteran)								
1973	Sydney Diet Heart Study (SDHS)								
1976	Bang et al. – 1976								
1977	Men – 1977								
1979	Finnish Mental Hospital Study (FMHS)								
1980	Before – 1980								
1980	Puerto Rico Heart Health Program								
1981	Shekelle – 1981			X					
1983	Lipid Research Clinics (LRC)								X
1983	Miettinen et al. – 1983 – Finnish Mental Hospital								
1983	Seven Countries Study								X
1984	Kingdom – 1984								
1984	McGee – 1984							X	X

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
1984	Oxford Vegetarian Study								
1985	Both – 1985								
1985	Fresh – 1985								
1985	Health and Lifestyle Survey (HLS)								X
1985	Ireland–Boston Diet Heart Study (IBDH)								
1985	Kushi – 1985			X					
1985	McGee – 1985 – USA Honolulu Heart Program			X					X
1985	Kushi 1985 (Ireland–Boston Diet Heart Study)								X
1986	Israeli Ischemic Heart Disease Study (IIHD)								X
1986	Keys et al. – 1986								
1986	Quebec Cardiovascular Study (QCS)								
1988	Adventist Health Study (AHS)								X
1989	Atherosclerosis Risk in Communities (ARIC)								X
1989	Burr et al. – 1989								
1989	Witteman - 1989	X							
1990	After – 1990								
1991	Both – 1991								
1991	Leaf et al. – 1991 – Leaf trial								
1991	Posner 1991	X							X
1991	Posner – 1991							X	
1992	STARS								
1993	Cardiovascular Health Study (CHS)								X
1993	Denmark – 1993								
1993	Fehily – 1993							X	X
1993	Goldbourt – 1993			X				X	X
1993	Rohan – 1993							X	
1993	Cardiovascular Health Study (CHS), trans fat — Wang 2014								X
1993	EPIC — Spanish centers								X
1994	Black et al. – 1994								
1994	Dwyer – 1994							X	
1994	Western Electric Study								X
1995	Ascherio – 1995							X	

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
1995	Grundt et al.								
1995	HARP								
1995	Kromhout et al. – 1995 – ATBC								X
1995	Physicians' Health Study (PHS)								
1995	Scottish Heart Health Study (SHHEC)								
1996	Ascherio 1996	X							
1996	Ascherio – 1996			X				X	
1996	Esrey – 1996			X		X		X	X
1996	Ascherio 1996 (HPFS)								X
1997	Clarke et al. – 1997								
1997	Daviglus et al. – 1997 – HPFS								X
1997	Gillman – 1997		X					X	X
1997	Hu – 1997 – NHS							X	X
1997	Mann – 1997			X				X	
1997	Pietinen P - 1997	X							
1997	Pietinen – 1997 – Finland			X		X		X	
1997	Seino F - 1997	X							
1997	Seino – 1997		X					X	
1997	Singh et al. – 1997								
1997	UK health-conscious cohort — Mann 1997								X
1998	Honolulu Heart Study (HHS) 1998								
1998	Leng 1998								
1998	Multiple Risk Factor Intervention Trial (MRFIT) 1998								X
1998	Singapore Chinese Health Study (SCHS) 1998								X
1999	GISSI–Prevenzione Investigators – 1999								
1999	Holmes – 1999							X	
1999	Hu – 1999 – Nurses' Health Study							X	X
1999	Lyon Diet Heart Study 1999								
1999	NHANES – 1999							X	
1999	Payette – 1999							X	
1999	von Schacky 1999								
2000	Liu – 2000							X	
2000	Ludwigshafen 2000								X

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2000	Palli – 2000							X	
2001	Iso – 2001		X					X	
2001	Nilsen et al.								
2001	Oomen 2001 (Zutphen trans fat)	X							X
2001	Oomen – 2001				X				X
2001	Oomen 2001 (Zutphen trans fat)								X
2001	Yuan 2001								X
2002	Bemelmans et al. – 2002 – Alpha–linolenic								
2002	Boniface 2002	X							X
2002	Boniface – 2002			X				X	X
2002	Bucher et al. – 2002								
2002	Danish National Birth Cohort (DNBC)								
2002	He – 2002 – JAMA				X				
2002	Hu – 2002 – JAMA				X				
2003	Aric – 2003								X
2003	Erkkila et al. – 2003 – EUROASPIRE								
2003	From – 2003								
2003	He 2003	X							
2003	He – 2003		X					X	
2003	Hu – 2003							X	
2003	Iso – 2003		X					X	
2003	Jelis – 2003								
2003	Oct – 2003								
2003	Oh – 2005							X	
2003	Trichopoulou – 2003 – EPIC Greece			X					X
2003	Zhang – 2003							X	
2003	Mozaffarian 2003 cohort								X
2004	Borugian – 2004							X	
2004	Jakobsen 2004	X							
2004	Jakobsen – 2004							X	
2004	Sauvaget 2004	X							
2004	Sauvaget – 2004		X	X				X	
2004	Tanasescu2004	X							
2004	Tanasescu – 2004							X	X

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2005	Albert – 2005 – Circulation				X				
2005	Kelemen – 2005							X	
2005	Kelemen – 2005 – IWHS (USA)						X		
2005	Leaf et al. – 2005 – Leaf trial								
2005	Leosdottir – 2005			X		X		X	X
2005	Mozaffarian et al. – 2005								
2005	Mozaffarian – 2005				X				
2005	Nakamura et al. – 2005 – EUROASPIRE								
2005	Raitt et al. – 2005 – Raitt trial								
2005	Solfrizzi – 2005			X		X		X	
2005	Trichopoulou – 2005			X					X
2005	Tucker et al. – 2005 – Iowa Women								
2005	Tucker – 2005 – USA Baltimore Longitudinal Study of Aging			X		X		X	X
2005	WHS — Women's Health Study								X
2006	Howard et al. – 2006 – Women's Health Initiative								
2006	Jarvinen – 2006 – Br J Nutr				X				
2006	SOFA								
2006	Trichopoulou – 2006			X					X
2006	Xu J, 2006	X							X
2006	Xu – 2006			X		X		X	X
2006	Wiberg 2006								
2006	Iso 2006								X
2006	Järvinen 2006								X
2007	Lagiou – 2007							X	
2007	Leosdottir – 2007							X	
2007	Smit – 2007							X	
2007	Trichopoulou – 2007							X	
2007	Yokoyama et al. – 2007 – Yokoyama								
2007	Yokoyama – 2007 – JELIS – Lancet – PMID:17398308								
2008	GISSI–HF								
2008	Streppel – 2008 – Zutphen Study – Eur Heart J				X				X
2008	Virtanen – 2008 – Am J Clin Nutr				X				X
2009	Alpha Omega								

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2009	Boden–Albala 2009	X							
2009	Boden–Albala – 2009							X	
2009	Dijkstra – 2009 – Rotterdam Study – Eur J Heart Fail				X				X
2009	Halbesma – 2009							X	
2009	Jakobsen – 2009 – Am J Clin Nutr				X				
2009	Levitan – 2009 – Eur Heart J				X				
2009	Montonen – 2009 – J Nutr				X				
2009	SU.FOL.OM3								
2009	Virtanen – 2009 – Circulation				X				X
2010	Bates – 2010							X	
2010	de Goede – 2010 – J Nutr				X				
2010	Fung – 2010							X	
2010	Heine–Broring – 2010 – Rotterdam Study – Am J Clin Nutr				X				X
2010	Jakobsen – 2010 – Am J Clin Nutr				X				
2010	National Health Screening Service (NHSS)								
2010	Preis – 2010							X	
2010	Yamagishi – 2010 – JACC – Am J Clin Nutr – PMID:20685950		X					X	X
2010	EPIC-NL (Dutch EPIC)								X
2011	Akbaraly – 2011 – Whitehall II – Am J Clin Nutr					X			
2011	Atkinson 2011	X							X
2011	Atkinson – 2011		X					X	
2011	de Goede – 2011 – PLoS One				X				
2011	Houston 2011	X							
2011	Houston – 2011							X	
2011	Vedtofte – 2011 – Am J Clin Nutr				X				
2011	Chinese cohorts (Zhang/Zhuang)								X
2011	Belin – 2011 – Women's Health Initiative – Circ Heart Fail				X				
2012	Chiuve 2012	X							
2012	Chiuve – 2012							X	
2012	de Oliveira Otto MC 2012	X							
2012	de Oliveira Otto – 2012				X				
2012	Dilis – 2012 – EPIC Greece			X				X	X

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2012	Kokura Study (KOKURA)								
2012	Laake – 2012 – NCS (Norway)						X		
2012	Larsson 2012	X							
2012	Larsson – 2012		X					X	
2012	Misirli – 2012			X					X
2012	Nagata 2012	X					X		
2012	Nagata – 2012 – Takayama study Japan			X		X		X	
2012	Nilsson – 2012							X	X
2012	OPERA								
2012	ORIGIN								
2012	Risk and Prevention Study (RPS)								
2012	Strom – 2012				X				
2012	Wallstrom 2012	X							X
2012	Wallstrom – 2012		X					X	X
2012	Yaemsiri 2012	X							
2012	Yaemsiri – 2012		X					X	
2012	de Goede 2010–2012								X
2013	Argos – 2013							X	
2013	Argos – 2013 – HEALS (Bangladesh)						X		
2013	Chien – 2013 – Japan (Chin–Shan)			X					
2013	Kiage – 2013 – REGARDS – Am J Clin Nutr					X			
2013	October – 2013								
2013	Simila – 2013							X	
2013	Yamagishi K 2013	X					X		X
2013	Yamagishi – 2013		X					X	X
2013	Yu – 2013 – ATBC							X	X
2013	de Oliveira Otto 2012/2013								X
2014	Haring – 2014							X	
2014	Kiage 2014	X							
2014	Levine – 2014							X	
2014	Levine – 2014 – NHANES III (USA)			X			X		
2014	Miyagawa – 2014							X	
2014	OPACH								
2014	Rebello – 2014							X	

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2014	Santos – 2014							X	
2014	Virtanen 2014	X					X		X
2014	Virtanen – 2014			X		X		X	X
2014	Wakai – 2014						X		
2014	Wakai – 2014			X		X		X	
2014	Vedtofte 2011/2014								X
2014	Farvid 2014 (linoleic pooled)								X
2014	Amiano 2014								X
2014	Miyagawa 2014								X
2015	Campmans–Kuijpers – 2015							X	
2015	Chiuve – 2015							X	
2015	Guasch–Ferre 2015	X					X		
2015	Guasch–Ferre – 2015			X		X		X	
2015	Li – 2015							X	
2015	Nagata – 2015							X	
2015	Puaschitz 2015	X							
2015	Puaschitz – 2015							X	
2015	Li 2015								X
2016	Campmans–Kuijpers – 2016								X
2016	Chen – 2016							X	
2016	Courand – 2016							X	
2016	Hernandez–Alonso – 2016							X	
2016	Hernandez–Alonso, 2016						X		
2016	Owen – 2016							X	
2016	Praagman 2016	X					X		
2016	Praagman – 2016							X	X
2016	Song – 2016							X	
2016	Song – 2016 – NHS & HPFS (USA)						X		X
2016	Wang 2016	X					X		
2016	Wang – 2016					X		X	
2016	Xu – 2016							X	
2016	Zong – 2016							X	X
2016	Sala-Vila 2016								X
2017	Dehghan – 2017		X	X		X		X	
2017	Dinesen 2017	X							

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2017	Holmes – 2017							X	
2017	Rhee – 2017							X	
2017	Sluijs2017	X							X
2017	Sluijs – 2017		X						X
2017	Wang – 2017			X					
2017	Zaslavsky – 2017							X	
2017	Nagata Japanese cohort								X
2017	Rhee 2017								X
2017	Dehghan 2017 (PURE)								X
2018	AlEssa – 2018							X	
2018	Arthur – 2018							X	
2018	Dominguez – 2018 – SUN (Spain)						X		X
2018	Ricci – 2018					X		X	
2018	Ricci – 2018 – NHANES (USA)						X		
2018	Seidelmann – 2018							X	
2018	Song – 2018 – Mendonca et al.,2020							X	
2018	Tharrey – 2018							X	
2018	Zhuang – 2018					X			
2019	Budhathoki – 2019							X	
2019	Chan – 2019							X	
2019	Jiao – 2019							X	X
2019	Kurihara – 2019							X	
2019	Mazidi – 2019			X					
2019	Okada – 2019							X	
2019	Praagman – 2019								X
2019	Virtanen – 2019							X	X
2019	Zhuang – 2019			X		X		X	
2019	Zhuang, 2019a						X		
2019	Praagman 2016–2019								X
2020	Chen – 2020							X	
2020	Chen – 2020						X		X
2020	Ho – 2020							X	
2020	Ho – 2020						X		
2020	Huang – 2020							X	
2020	Huang – 2020						X		

Year	Study	Zhu 2019 (Lipids Health Dis)	Kang 2020 (NMCD)	Mazidi 2020 (Clin Nutr)	Schwab 2021 (FNR) **EJCN not FNR	Kim 2021 (Clin Nutr)	Wallerer 2024 (eClinicalMedicine)	Ma 2024 (Nutrients)	Jayedi 2024 CRFSN (Coronary events)
2020	Langsetmo – 2020							X	
2020	Lelli – 2020							X	
2020	Lin – 2020							X	
2020	Mao – 2020 – CHNS (China)						X		
2020	Mao – 2020							X	
2020	Mazidi – 2020							X	
2020	Mendonca – 2020							X	X
2020	Mirmiran – 2020							X	
2020	Miyazawa – 2020							X	
2020	Shan – 2020							X	
2020	Shan – 2020 – NHANES (USA)						X		
2020	Trevisan – 2020							X	
2020	Wu – 2020 – CHNS (China)						X		
2021	Akter – 2021							X	
2021	Fontana – 2021 – EPIC–Italy (Italy)						X		X
2021	Kwon – 2021 – KoGES (Korea)						X		
2021	Kwon – 2021							X	
2021	Laguna – 2021						X		
2021	Sadeghi – 2021							X	
2021	Sun – 2021							X	X
2021	Sun – 2021 – WHI (USA)						X		X
2021	Yao – 2021							X	
2021	Steur 2021								X
2021	Voortman 2021								X
2021	Glenn 2021								X
2022	Das – 2022 – CHAMP (Australia)						X		
2022	Merono – 2022 – InCHIANTI (Italy)						X		
2022	Zeng – 2022 – NHANES (USA)						X		
2022	Zhou – 2022						X		
2023	Bajracharya – 2023						X		X
2023	Zhao – 2023 – NIH–AARP (USA)						X		
	Number of studies	30	14	27	21	14	14	87	86

Supplement 4

Risk of Bias for RCTs

Risk of Bias for **Saturated Fat**

All are rated High because of confounding with PHO.

Review	D1	D2	D3	D4	Overall ROB
Hooper 2001 (BMJ)	High	Low	Low	Low	High
Skeaff & Miller 2009 (Ann Nutr Metab)	High	High	High	Low	High
Mozaffarian 2010 (PLoS Med)	High	Low	High	Low	High
Schwingshackl & Hoffmann 2014 (BMJ Open)	High	Low	Low	Low	High
Harcombe 2015 (Open Heart)	High	Low	High	Low	High
Ramsden 2016 (BMJ)	High	High	Unclear	Low	High
Hamley 2017 (Nutrition Journal)	High	High	High	High	High
Hooper 2020 (Cochrane)	High	Low	Low	Low	High
Yamada 2025 (JMA Journal)	High	Low	Low	Low	High

Risk of Bias RCT for Saturated Fat+PHO

Review	D1	D2	D3	D4	Overall ROB	One line rationale
Hooper 2001 (BMJ)	Low	Low	Low	Low	Low	Preregistered Cochrane review with a comprehensive search (multi-databases + trial registries + grey literature + expert contact), no language limits; duplicate screening and extraction; Cochrane risk-of-bias (and GRADE) applied; random-effects with meta-regression and funnel-plot checks; prespecified hard outcomes
Skeaff & Miller 2009 (Ann Nutr Metab)	Low	High	High	Low	High	Broad question mixing cohorts and RCTs; English-only despite multi-database search; no report of duplicate screening/extraction; some data taken from prior reviews; no domain-based trial-level RoB; random-effects with heterogeneity/sensitivity checks, but D2–D3 methods weaknesses dominate
Mozaffarian 2010 (PLoS Med)	Low	Low	High	Low	High	QUOROM/PRISMA-aligned with an extensive search (multi-DB + grey + expert contact), independent duplicate screening/extraction, and RE meta-analysis with heterogeneity, meta-regression, and funnel checks; however, trial appraisal used Jadad scores (not a domain-based Cochrane RoB)
Schwingshackl & Hoffmann 2014 (BMJ Open)	Low	Low	Low	Low	Low	Secondary-prevention RCTs; broad search (MEDLINE/EMBASE/Cochrane) with no language/date limits + reference checks; independent duplicate screening and extraction; Cochrane RoB applied; random-effects MA with uni/multivariable meta-regression, sensitivity and funnel-plot checks
Harcombe 2015 (Open Heart)	Low	Low	High	Low	High	No preregistered protocol and a narrow search (MEDLINE + Cochrane only); no duplicate data extraction; relies on PEDro “quality score” instead of a domain-based risk-of-bias tool; heterogeneity and publication-bias checks were done, but weaknesses in data collection/appraisal
Ramsden 2016 (BMJ)	Low	High	Unclear	Low	High	PRISMA-style with a meta-analysis, but the main paper omits core methods (databases/strings, whether screening/extraction were duplicated, which RoB tool) and limits to English—details only in a web appendix—so D2 (identification/selection) concerns and unclear RoB appraisal
Hamley 2017 (Nutrition Journal)	Low	High	High	High	High	No preregistered protocol and a sparse, older search; duplicate data extraction not reported; relies on quality scores (not a domain-based RoB tool); pools heterogeneous contrasts (reduce fat, replace SFA→PUFA, extreme quantiles) with limited small-study bias checks—weaknesses in appraisal/synthesis
Hooper 2020 (Cochrane)	Low	Low	Low	Low	Low	Preregistered Cochrane review with a broad search of databases and registries (CENTRAL, MEDLINE, Embase; WHO ICTRP, ClinicalTrials.gov), independent duplicate screening in Covidence and duplicate extraction, Cochrane risk-of-bias with author contact, random-effects meta-analysis with meta-regression, subgroup and funnel-plot checks, and GRADE certainty ratings
Yamada 2025 (JMA Journal)	Low	Low	Low	Low	Low	PROSPERO-registered review with a broad search (CENTRAL, PubMed, Ichu-shi) and no language limits; independent dual screening in Rayyan with two-reviewer extraction; trial bias assessed using RoB 2; random-effects meta-analysis with I^2 and funnel-plot checks; outcomes (mortality/CVD) pre-specified

PCS Risk of Bias

Review	D1	D2	D3	D4	Overall ROBIS
Cheng et al. (2016)	Low	Low	Low	Low	Low
Chowdhury et al. (2014)	Low	Low	Low	Low	Low
de Souza et al. (2015)	Low	High	Low	Low	High
Harcombe et al. (2017a)	Low	Low	Low	Low	Low
Harcombe et al. (2017b)	Low	Low	Low	Low	Low
Jakobsen et al. (2009)	Low	High	High	Low	High
Jayedi et al. (2024)	Low	Low	Low	Low	Low
Kang et al. (2020)	Low	High	Low	Low	High
Kim et al. (2021)	Low	High	Low	Low	High
Ma et al. (2024)	Low	Low	Low	Low	Low
Mazidi et al. (2020)	Low	High	Low	Low	High
Muto & Ezaki (2018)	Low	High	High	Low	High
Schwab et al. (2014)	Low	High	Low	Low	High
Siri-Tarino et al. (2010)	Low	Low	High	Low	High
Skeaff & Miller (2009)	Low	High	High	Low	High
Wallerer et al. (2024)	Low	Low	Low	Low	Low
Zhu et al. (2019)	Low	Low	High	Low	High

Review. Readable rationale (no grade sentence)

Cheng et al. (2016). Multi-database (PubMed/Embase/Web of Knowledge), no language restrictions, handsearch and author contact. Dual independent extraction with third reviewer, The Newcastle–Ottawa Scale used. Appropriate FE/random-effects synthesis with subgroup analysess, sensitivity analyses and Egger.

Chowdhury et al. (2014). Predefined protocol, Multi-database (MEDLINE, Science Citation Index, CENTRAL), no language restrictions, Handsearch and author contact. Dual independent data extraction with third adjudication, The Newcastle–Ottawa Scale for cohorts and the Cochrane risk-of-bias tool for RCTs. Appropriate random-effects/FE synthesis with heterogeneity, meta-regression and Egger.

de Souza et al. (2015). WHO-guided protocol, A comprehensive multi-database search (MEDLINE/Embase/CENTRAL/CINAHL/EBMR), no language restrictions, Handsearch. Appendix strategies, Dual independent extraction and duplicate RoB (the Newcastle–Ottawa Scale) and GRADE certainty ratings. Appropriate random-effects synthesis with heterogeneity, meta-regression, sensitivity analyses and small-study bias checks. BUT single-screener at title/abstract stage → D2 High → Overall High.

Harcombe et al. (2017a). Prespecified historical scope, Multi-database (MEDLINE/EMBASE/Cochrane Library) search with manual reference-list checks. Dual independent inclusion decisions, Structured RoB appraisal, Narrative synthesis aligned with heterogeneous/limited pre-1983 data.

Harcombe et al. (2017b). Prospective-cohort scope, MEDLINE/Embase/Cochrane Library search, PRISmeta-analysis flow, Dual independent eligibility. Cochrane-style RoB appraisal, Random-effects meta-analysis with heterogeneity, sensitivity analyses and Egger.

Jakobsen et al. (2009). Individual participant data pooled analysis with predefined cohort eligibility. Identification via literature searches and expert inquiry and participation by agreement, No trial-level RoB tool. Robust substitution modelling with confounder adjustment and random-effects pooling → Overall High.

Jayedi et al. (2024). PROSPERO protocolled, 5-database search w/ no language restrictions, Independent duplicate screening and extraction, ROBINS-I and RoB 2. Random-effects and dose–response, heterogeneity and small-study bias checks, GRADE certainty ratings.

Kang et al. (2020). Prospective cohorts, PubMed/Embase/CENTRAL/Web of Science but restricted to English. Independent independent duplicate screening and dual extraction, The Newcastle–Ottawa Scale RoB. Random-effects and linear/nonlinear dose–response and Egger/Begg → D2 English/grey limits.

Kim et al. (2021). Prospective cohort-only, PubMed and Web of Science, restricted to English, Handsearch. Dual data extraction and dual the Newcastle–Ottawa Scale RoB, Random-effects dose–response with heterogeneity/Egger, D2 limits → Overall High.

Ma et al. (2024). PROSPERO, PubMed/Embase/CENTRAL, No language restrictions, Independent duplicate screening and extraction, The Newcastle–Ottawa Scale used. Random-effects dose-response and Egger/Begg.

Mazidi et al. (2020). Prospective cohort and meta-analysis. A comprehensive multi-database search search stated 'without language restriction' but full texts then excluded non-English. Independent duplicate screening and extraction, The Newcastle–Ottawa Scale RoB. Random-effects model and I^2 statistics, Egger/Begg → D2 contradictions/restricted to English.

Muto & Ezaki (2018). Prespecified cohort-only criteria with subtype focus, Single-database PubMed and reference lists. Independent independent duplicate screening but no reported independent duplicate data extraction or primary-study RoB tool. Random-/fixed-effects, I^2 statistics, Egger/funnel-plot assessment, Extensive subgroup analysesing → D2/D3 concerns.

Schwab et al. (2014). A priori, detailed eligibility, independent independent duplicate screening, dual quality appraisal, but search is lmited to Pubmed, Swemed, with no registries.

Siri-Tarino et al. (2010). Prospective cohort meta-analysis, MEDLINE and EMBASE and hand-search, Independent duplicate data extraction, Reproducible terms. No language restrictions reported, Non-standard cohort RoB (quality score, not the Newcastle–Ottawa Scale/ROBINS-I). Random-effects with heterogeneity, meta-regression, influence and funnel-plot assessment → D3 High → Overall High.

Skeaff & Miller (2009). Multi-database search (Cochrane, MEDLINE, EMBASE, SCOPUS, WoS, PubMed) but restricted to English. Independent duplicate screening/extraction not reported, No validated primary-study RoB tool. Random-effects meta-analysis with heterogeneity and funnel-plot assessment tests, Cautious interpretation → Overall High.

Wallerer et al. (2024). PROSPERO-registered, MEDLINE/Embase/Scopus and no language restrictions and backward citation, Independent duplicate screening and extraction. ROBINS-E used, Random-effects Nmeta-analysis with heterogeneity/incoherence/small-study bias checks. GRADE certainty ratings-driven interpretation.

Zhu et al. (2019). Cohort-focused with clear eligibility, MEDLINE/Embase/Cochrane search, Independent duplicate screening and extraction. Random-effects and meta-reg, Egger and leave-one-out, Dose-response via splines. But no formal primary-study RoB tool reported → D3 High → Overall High

Supplement 5

RCT based Umbrella Analyses Part 1

	Bayesian Relative Risk (RR)	Lower 95% CrI	Upper 95% CrI	Bayesian Probability of RR<0.90	Bayesian Probability of RR<0.95	Bayesian Probability of RR<1	Bayesian Probability of RR>1	Bayesian Probability of RR>1.05	Bayesian Probability of RR>1.10	Bayesian Probability of 0.95<RR<1.05
All cause Mortality	0.983	0.908	1.070	1.70%	16.48%	69.23%	30.77%	4.95%	0.93%	78.57%
CHD Mortality	1.021	0.867	1.189	4.97%	15.09%	37.32%	62.68%	32.58%	12.95%	52.33%
CVD Mortality	1.021	0.959	1.068	0.43%	1.87%	17.63%	82.37%	8.18%	0.59%	89.96%
Stroke Mortality	0.888	0.486	1.741	53.09%	64.72%	74.09%	25.91%	19.64%	15.63%	15.64%
CHD Incidence	1.027	0.974	1.083	0.01%	0.43%	13.03%	86.97%	16.28%	1.15%	83.28%
CVD Incidence	0.979	0.950	1.012	0.03%	2.45%	93.43%	6.58%	0.44%	0.08%	97.11%
Stroke Incidence	0.905	0.857	0.943	40.13%	98.67%	99.88%	0.12%	0.01%	0.00%	1.33%

De novo based Meta analyses

	Bayesian Relative Risk (RR)	Lower 95% CrI	Upper 95% CrI	Bayesian Probability of RR<0.90	Bayesian Probability of RR<0.95	Bayesian Probability of RR<1	Bayesian Probability of RR>1	Bayesian Probability of RR>1.05	Bayesian Probability of RR>1.10	Bayesian Probability of 0.95<RR<1.05
Stroke Incidence	0.939	0.886	0.994	8.20%	66.59%	98.40%	1.60%	0.02%	0.00%	33.39%
CHD Incidence	1.065	0.973	1.188	0.03%	0.73%	8.61%	91.39%	61.38%	25.73%	37.88%

Higher RR favors intervention (SF-PHO harmful)

RCT

Higher RR favors intervention (SF-PHO harmful)

- All cause mortality: Strong Confidence for neutral effect. 79% chance mid-RR = 1.00 ± 0.05; 5% chance that mid-RR > 1.05 (5% chance SFA/PHO harmful)
- CHD Mortality: High confidence for neutral effect. . 52% chance mid-RR = 1.00 ± 0.05; 13% chance that mid-RR > 1.10 (13% chance SFA/PHO harmful)
- CVD Mortality: Strong confidence (90% chance) for neutral effect. 90% chance mid-RR = 1.00 ± 0.05
- Stroke Mortality: Moderate confidence for SFA/PHO protective (26% chance mid-RR <1)
- CHD Incidence: Moderate confidence of neutral effect of SFA/PHO. 83% chance mid-RR = 1.00 ± 0.05
- CVD Incidence: Strong Confidence for neutral effect. 97% chance mid-RR = 1.00 ± 0.05; 0.4% chance that mid-RR > 1.05 (5% chance SFA/PHO harmful)
- Stroke Incidence: Strong Confidence for protective effect: 99.9% chance of RR<1 (protective). Mid-RR = 0.91.
- PCS de novo meta-analysis
- *Stroke incidence. High confidence that SF-PHO is mildly protective against stroke incidence with RR <1 of 98.4%. Precision (τ) is lower than for the umbrella review, as expected from the deduplication of redundant studies.*
- *CHD Incidence. Moderate confidence that CHD incidence is mildly greater with SF-PHO based on mid-RR = 1.065 (6.5% increased risk); 74% chance that SF-PHO RR is <1.1 (10%). Compared to the neutral umbrella review results, this analysis points to mild harm.*

RCT based Umbrella Analyses Part 2

	Heterogeneity τ	Heterogeneity τ Lower 95% CrI	Heterogeneity τ Upper 95% CrI	Predicted Relative Risk (RR)	Predicted RR Lower 95% CrI	Predicted RR Upper 95% CrI	%CCA	CCA Band	# of reviews	# of RRs extracted from reviews	Reviews included
All cause Mortality	0.084	0.042	0.198	0.982	0.786	1.221	9%	Moderate	5	7	de Souza 2015, Kim 2021, Ma 2024, Mazidi 2020, Wallerer 2024
CHD Mortality	0.144	0.036	0.358	1.021	0.681	1.503	10%	High	5	7	de Souza 2015, Harcombe 2017, Mazidi 2020, Skeaff 2009, Jakobsen 2009
CVD Mortality	0.022	0.001	0.130	1.022	0.908	1.128	14%	High	4	5	de Souza 2015, Kim 2021, Ma 2024, Mazidi 2020
Stroke Mortality	0.280	0.030	0.950	0.885	0.315	2.698	7%	Moderate	2	2	Cheng 2016, Mazidi 2020
CHD Incidence	0.048	0.002	0.142	1.028	0.887	1.190	11%	High	6	9	Chowdhury 2014, de Souza 2015, Jayedi 2024, Siri-Tarino 2010, Skeaff 2009
CVD Incidence	0.013	0.001	0.074	0.979	0.920	1.043	0%	Slight	4	7	Ma 2024, Siri-Tarino 2010, Zhu 2019, Schwab 2014
Stroke Incidence	0.031	0.002	0.118	0.906	0.798	1.006	10%	High	6	10	Cheng 2016, Kang 2020, De Souza 2015, Muto 2018, Siri Tarnio 2010, Ma 2024

De novo based Meta analyses

	Heterogeneity τ	Heterogeneity τ Lower 95% CrI	Heterogeneity τ Upper 95% CrI	Predicted Relative Risk (RR)	Predicted RR Lower 95% CrI	Predcited RR Upper 95% CrI	-	-	# of studies	# of RRs extracted from reviews	Studies included
Stroke Incidence	0.143	0.087	0.215	0.936	0.694	1.267	-	-	18	55	McGee 1984, Goldbourt 1993, Gillman 1997, Seino 1997, Iso 2001, He 2003, Iso 2003, Sauvaget 2004, Wiberg 2006, Leosdottir 2007, Atkinson 2011, Misirli 2012, Wallstrom 2012 (Men), Wallstrom 2012 (Women), Yaemsiri 2012, Larsson 2012, Yamagishi 2010, Yamagishi 2013, Sluijs 2017
CHD Incidence	0.221	0.124	0.358	1.064	0.660	1.730	-	-	21	39	Ascherio 1996, ATBC Pietinen 1997, Boniface 2002 (Men), Boniface 2002 (Women), Dehghan 2017, Esrey 1996, Framingham Heart Study, Goldbourt 1993, Guasch-Ferre 2015, HPFS Ascherio 1996, Kushi 14985, Leosdottir 2005, Mann 1997, Nagata 2012, Pietinen 1997, Sauvaget 2004, Shekelle 1981, Strongheart Study, Tucker 2005, Virtanen 2014, Wakai 2014, Xu 2006

PCS based Umbrella Analyses Part 1

RCT-based Umbrella analyses

	Bayesian Relative Risk (RR)	Lower 95% CrI	Upper 95% CrI	Bayesian Probability of RR<0.90	Bayesian Probability of RR<0.95	Bayesian Probability of RR<1	Bayesian Probability of RR>1	Bayesian Probability of RR>1.05	Bayesian Probability of RR>1.10	Bayesian Probability of 0.95<RR<1.05
All cause Mortality	0.983	0.908	1.070	1.70%	16.48%	69.23%	30.77%	4.95%	0.93%	78.57%
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CVD Mortality	1.021	0.959	1.068	0.43%	1.87%	17.63%	82.37%	8.18%	0.59%	89.96%
Stroke Mortality	0.888	0.486	1.741	53.09%	64.72%	74.09%	25.91%	19.64%	15.63%	15.64%
CHD Incidence	1.027	0.974	1.083	0.01%	0.43%	13.03%	86.97%	16.28%	1.15%	83.28%
CVD Incidence	0.979	0.950	1.012	0.03%	2.45%	93.43%	6.58%	0.44%	0.08%	97.11%
Stroke Incidence	0.905	0.857	0.943	40.13%	98.67%	99.88%	0.12%	0.01%	0.00%	1.33%

De novo based Meta analyses

	Bayesian Relative Risk (RR)	Lower 95% CrI	Upper 95% CrI	Bayesian Probability of RR<0.90	Bayesian Probability of RR<0.95	Bayesian Probability of RR<1	Bayesian Probability of RR>1	Bayesian Probability of RR>1.05	Bayesian Probability of RR>1.10	Bayesian Probability of 0.95<RR<1.05
Stroke Incidence	0.939	0.886	0.994	8.20%	66.59%	98.40%	1.60%	0.02%	0.00%	33.39%
CHD Incidence	1.065	0.973	1.188	0.03%	0.73%	8.61%	91.39%	61.38%	25.73%	37.88%

PCS based Umbrella Analyses Part 2

	Heterogeneity τ	Heterogeneity τ Lower 95% CrI	Heterogeneity τ Upper 95% CrI	Predicted Relative Risk (RR)	Predicted RR Lower 95% CrI	Predicted RR Upper 95% CrI	%CCA	CCA Band	# of reviews	# of RRs extracted from reviews	Reviews included
All cause Mortality	0.084	0.042	0.198	0.982	0.786	1.221	9%	Moderate	5	7	de Souza 2015, Kim 2021, Ma 2024, Mazidi 2020, Wallerer 2024
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Stroke Mortality	0.280	0.030	0.950	0.885	0.315	2.698	7%	Moderate	2	2	Cheng 2016, Mazidi 2020
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De novo based Meta analyses

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CHD Incidence	0.221	0.124	0.358	1.064	0.660	1.730	-	-	21	39	Ascherio 1996, ATBC Pietinen 1997, Boniface 2002 (Men), Boniface 2002 (Women), Dehghan 2017, Esrey 1996, Framingham Heart Study, Goldbourt 1993, Guasch-Ferre 2015, HPFS Ascherio 1996, Kushi 14985, Leosdottir 2005, Mann 1997, Nagata 2012, Pietinen 1997, Sauvaget 2004, Shekelle 1981, Strongheart Study, Tucker 2005, Virtanen 2014, Wakai 2014, Xu 2006

PCS

Higher RR favors intervention (SF-PHO harmful)

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- Stroke Mortality: Moderate confidence for SFA/PHO protective (26% chance mid-RR <1)
- CHD Incidence: Moderate confidence of neutral effect of SFA/PHO. 83% chance mid-RR = 1.00 ± 0.05
- CVD Incidence: Strong Confidence for neutral effect. 97% chance mid-RR = 1.00 ± 0.05; 0.4% chance that mid-RR > 1.05 (5% chance SFA/PHO harmful)
- Stroke Incidence: Strong Confidence for protective effect: 99.9% chance of RR<1 (protective). Mid-RR = 0.91.
- PCS *de novo* meta-analysis
- *Stroke incidence. High confidence that SF-PHO is mildly protective against stroke incidence with RR <1 of 98.4%. Precision (τ) is lower than for the umbrella review, as expected from the deduplication of redundant studies.*
- *CHD Incidence. Moderate confidence that CHD incidence is mildly greater with SF-PHO based on mid-RR = 1.065 (6.5% increased risk); 74% chance that SF-PHO RR is <1.1 (10%). Compared to the neutral umbrella review results, this analysis points to mild harm.*

Supplement 6

GRADE for RCT

GRADE Evidence Assessment: Comparison Across Meta-Analyses

SF+PHO rated

Question: Should reduced saturated fatty acid intake be used for prevention of cardiovascular disease and mortality?

Study & Outcome	№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Intervention			Certainty	Importance
HOOPER 2001 - BMJ Systematic Review												
Total mortality	11 trials	randomised trials	serious (-1)	not serious	not serious (0)	not serious	not serious	~30,902 p-y	-	RR 0.98 (0.86-1.12)	MODERATE	CRITICAL
CV mortality	11 trials	randomised trials	serious (-1)	serious (-1)	not serious (0)	not serious	not serious	~30,902 p-y	-	RR 0.91 (0.77-1.07)	LOW	CRITICAL
Combined CV events	14 trials	randomised trials	serious (-1)	serious (-1)	not serious (0)	not serious	not serious	~30,902 p-y	-	RR 0.84 (0.72-0.99)	LOW	IMPORTANT
SKEAFF & MILLER 2009 - Dietary Fat RCTs												
CHD mortality	5 RCTs	randomised trials	serious (-1)	not serious	very serious (-2)	serious (-1)	not serious	2,181	-	RR 0.52 (0.30-0.87)	VERY LOW	CRITICAL
Combined CHD events	8 RCTs	randomised trials	serious (-1)	not serious	very serious (-2)	not serious	not serious	-	-	RR 0.68 (0.52-0.90)	VERY LOW	IMPORTANT
MOZAFFARIAN 2010 - PUFA for SFA RCTs												
Combined CHD events	8 RCTs	randomised trials	serious (-1)	not serious	very serious (-2)	not serious	not serious	13,614	-	RR 0.81 (0.70-0.95)	VERY LOW	CRITICAL
SCHWINGSHACKL & HOFFMANN 2014 - Secondary Prevention												
All-cause mortality	12 RCTs	randomised trials	serious (-1)	serious (-1)	very serious (-2)	not serious	not serious	7,150	-	RR 0.92 (p=0.60, I^2=59%)	VERY LOW	CRITICAL
CV mortality	12 RCTs	randomised trials	serious (-1)	serious (-1)	very serious (-2)	not serious	not serious	7,150	-	RR 0.96 (p=0.84, I^2=69%)	VERY LOW	CRITICAL
HARCOMBE 2015 - RCTs Available to 1977-1983 Guidelines												
All-cause mortality	6 RCTs	randomised trials	very serious (-2)	serious (-1)	serious (-1)	serious (-1)	serious (-1)	2,467 men	-	RR 0.996 (0.865-1.147)	VERY LOW	CRITICAL

CHD mortality	6 RCTs	randomised trials	very serious (-2)	serious (-1)	serious (-1)	serious (-1)	serious (-1)	2,467 men	-	RR 0.989 (0.784-1.247)	VERY LOW	CRITICAL
RAMSDEN 2016 - Minnesota Coronary Experiment & Meta-Analysis												
CHD mortality (Meta-analysis)	5 RCTs	randomised trials	very serious (-2)	not serious	serious (-1)	serious (-1)	serious (-1)	-	-	RR 1.13 (0.83-1.54)	VERY LOW	CRITICAL
HAMLEY 2017 - Adequately Controlled Trials Only												
Major CHD events	Multiple RCTs	randomised trials	very serious (-2)	serious (-1)	not serious (0)	not serious	not serious	-	-	RR 1.06 (0.86-1.31)	MODERATE	CRITICAL
HOOPER 2020 - Cochrane Review												
All-cause mortality	11 RCTs (12 comp)	randomised trials	serious (-1)	not serious	not serious (0)	not serious	not serious	55,858	-	RR 0.96 (0.90-1.03)	MODERATE	CRITICAL
CV mortality	10 RCTs (11 comp)	randomised trials	serious (-1)	not serious	serious (-1)	serious (-1)	not serious	53,421	-	RR 0.95 (0.80-1.12)	LOW	CRITICAL
Combined CV events	12 RCTs (15 comp)	randomised trials	serious (-1)	serious (-1)	serious (-1)	not serious	not serious	53,758	-	RR 0.83(0.70-0.98)	MODERATE	IMPORTANT
YAMADA 2025 - Meta-Analysis												
All-cause mortality	9 RCTs	randomised trials	serious (-1)	not serious	not serious (0)	not serious	serious (-1)	-	-	RR 0.988 (0.943-1.037)	MODERATE	CRITICAL
CVD mortality	9 RCTs	randomised trials	serious (-1)	serious (-1)	not serious (0)	serious (-1)	serious (-1)	-	-	RR 1.026 (0.911-1.151)	VERY LOW	CRITICAL
CHD incidence	9 RCTs	randomised trials	serious (-1)	serious (-1)	not serious (0)	not serious	serious (-1)	-	-	RR 0.895 (0.801-1.001)	MODERATE	CRITICAL

Key findings:

• All 9 meta-analyses rated as VERY LOW or LOW certainty evidence

• Most studies show null or modest effects on mortality outcomes

• Hamley 2017 shows that adequately controlled trials have null effects (RR 1.06)

GRADE certainty ratings: ⊕⊕⊕⊕ HIGH | ⊕⊕⊕○ MODERATE | ⊕⊕○○ LOW | ⊕○○○ VERY LOW

RCT Evidence Table

Citation	Last search date	Databases	No. RCTs (total studies)	Eligibility criteria	Population	Interven-tion	Compara-tor	Out-comes reported	Pooled effect(s) & Model	Heterogen-eity. (I^2)	Certainty (GRADE?)	RoB method	Funding/ COI & Notes
Hooper 2001 (BMJ)	May 1999	Cochrane Library; MEDLINE; Embase; CAB Abstracts; CVRCT registry; SIGLE, plus bibliography screening and expert contact	11	Adequately randomised RCTs in adults, ≥6-month intervention or follow-up; aim to reduce/modify dietary fat or cholesterol; must report mortality/CVD morbidity. Excluded omega-3–only interventions, multifactorial trials, and non-truly-randomised designs.	Randomized controlled trials in adult participants receiving dietary fat reduction/modification (≥6 months). Excluded children, pregnant, acutely ill; included both primary and secondary prevention populations (many post-MI; mostly male).	Low SFA	High SFA	All cause Mortality	RR = 0.98 (95% CI 0.86-1.12)	p (het) = 0.30	ns	Custom trial-quality assessment (pre-RoB2): evaluated randomization method, blinding of physicians/participants, and differences in care; inclusion/validity/data extraction done in duplicate. No named RoB tool reported.	ns
								CVD mortality	RR = 0.91 (95% CI = 0.77–1.07)	ns	ns		ns
								CVD incidence	RR = 0.84 (95% CI = 0.72–0.99)	p (het) = 0.16	ns		*
Skeaff & Miller 2009 (Ann Nutr Metab)	No report	Cochrane Library, Medline, Embase, SCOPUS, Web of Science and PubMed. Searches were limited to English-language publications; reference lists (including systematic reviews) were also searched.	7	Included prospective cohorts and RCTs focused on CHD outcomes (death/events; RCTs also total mortality). N-3 RCTs had to increase fish/fish-oil/purified n-3 LCPUFA intake. Excluded cohorts without RR estimates, studies outside predefined diet-fat categories, MRFIT, CVD-only endpoints, and certain n-3 trials (plus one with methodological concerns).	about 280,000 participants and ~6,600 CHD deaths over ~3.7 million person-years, largely North America/Europe; 19/28 cohorts were men-only, with Nurses' Health Study covering most women; recruitment ages 40–65 years.	High PUFA	High SFA	CHD mortality	RR = 0.84 (95% CI = 0.62–1.12)	I^2 = 0.0%, p (het) = 0.874	No GRADE/SoF framework	No dedicated trial- or cohort-level RoB tool	ns
								CHD incidence	RR = 0.83 (95% CI = 0.69–1.00)	I^2 = 40.3%, p (het) = 0.137	ns		ns
								All cause mortality	RR = 0.88 (95% CI = 0.76–1.02	I^2 = 4.7%, p (het) = 0.400	ns		ns

Citation	Last search date	Databases	No. RCTs (total studies)	Eligibility criteria	Population	Interven-tion	Compara-tor	Out-comes reported	Pooled effect(s) & Model	Heterogen-eity. (I^2)	Certainty (GRADE?)	RoB method	Funding/ COI & Notes
Mozaffarian 2010 (PLoS Med)	June 2009	MEDLINE, Embase, AGRIS, AMED, HMIC, PsycINFO, Cochrane Library, Web of Knowledge, CABI, CINAHL, plus conference abstracts (Zetoc), Faculty of 1000, grey literature (SIGLE), related articles/hand-searching, and direct author/expert contact for unpublished trials or missing data.	8	Included RCTs in adults that increased total or n-6 PUFA (vs control) for ≥1 year, with no major concomitant interventions, and that reported hard CHD events (MI, CHD death, sudden death). Allowed both feeding and dietary-advice designs; primary vs secondary prevention was not restricted. Excluded non-randomized/observational studies, n-3–focused interventions, studies with only intermediate/"soft" endpoints, and commentaries/reviews/duplicates.	Adults randomized to increase total or n-6 PUFA intake in place of SFA for ≥1 year, with an appropriate control group, and reporting hard CHD events (MI and/or CHD/cardiac death). Trials included both primary and secondary prevention, using either feeding or dietary-advice designs.	High PUFA	High SFA	CHD incidence, 5% repl with "PUFA"	RR = 0.90 (95% CI = 0.83–0.97)	I^2=37%, p (het)=0.13	No GRADE/SoF framework	Jadad scale (randomization, blinding, withdrawals/dropouts; 0–5 points). Trials had modest and relatively homogeneous quality (scores 2 or 3); all had blinded endpoint assessment.	*
Schwingshackl & Hoffmann 2014 (BMJ Open)	February 2014	MEDLINE, EMBASE, Cochrane Trial Register (Cochrane Library); reference lists of retrieved articles/reviews were also checked.	12	Secondary-prevention RCTs, ≥12-month follow-up, comparing reduced (<30% TEC) and/or modified fat diets vs control; must report hard outcomes (all-cause/cardiovascular mortality, combined cardiovascular events, MI) with event counts; established CHD/CAD only. Excluded non-randomised, multifactorial programs, trials not distinguishing SFA differences between arms, and studies not strictly in established CHD/CAD.	Adults with established CHD/CAD only: survivors of myocardial infarction, stable/unstable angina pectoris, acute coronary insufficiency, or CAD verified by coronary angiography; randomized controlled trials with ≥12 months follow-up comparing reduced (<30% TE) and/or modified fat diets vs control.	Low SFA	High SFA	CVD mortality CVD incidence	RR = 0.96 (95% CI = 0.66–1.31) RR = 0.85 (95% CI = 0.65–1.34)	I^2 = 0% I^2 = 57%	Moderate Moderate	Cochrane Collaboration Risk-of-Bias tool	ns ns

Citation	Last search date	Databases	No. RCTs (total studies)	Eligibility criteria	Population	Interven-tion	Compara-tor	Out-comes reported	Pooled effect(s) & Model	Heterogen-eity. (I^2)	Certainty (GRADE?)	RoB method	Funding/ COI & Notes
								MI incidence	RR = 0.76 (95% CI = 0.54–1.09)	I^2 = 19%	Moderate		ns
								All cause mortality	RR = 0.92 (95% CI = 0.68–1.25)	I^2 = 0%	Moderate		ns
Harcombe 2015 (Open Heart)	No report	MEDLINE and the Cochrane Library (AMED, CAB Abstracts, CINAHL, EMBASE, HMIC, SIGLE	6	Systematic review restricted to RCTs in adults lasting ≥1 year, explicitly targeting reduction/modification of dietary fat or cholesterol, and reporting all-cause mortality, CHD mortality, and cholesterol. Excluded observational, non-randomized, or multifactorial designs; specific non-randomized historical trials (e.g., Anti-Coronary Club, Finnish Mental Hospital) were excluded.	Randomised dietary fat intervention studies in human adults (≥1-year duration) reporting all-cause and CHD mortality; five trials were secondary-prevention only; LA Veterans mixed primary/secondary; all participants were men.	Low SFA	High SFA	CHD Mortality	RR = 0.99 (95% CI 0.78-1.25)	I^2 = 30.6%	No GRADE/SoF framework	PEDro scale	ns
								All-cause mortality	RR = 1.00 (95% CI 0.87-1.15)	I^2 = 15.7%	ns		ns
Ramsden 2016 (BMJ)	25 Sept 2015	PubMed, EMBASE, CINAHL, plus hand-searching of prior reviews/trials, grey literature, and direct contact with investigators/families to obtain unpublished endpoints.	6	MCE trial: Adults (≥20) in a nursing home or state mental hospitals were randomized to a high–linoleic acid diet vs control; the paper analyzes recovered data. Systematic review/meta-analysis: Included English-language RCTs replacing SFA with linoleic acid–rich oils (individual randomization, no major co-interventions, hard mortality endpoints). Excluded trials with large n-3 EPA/DHA, advice-only without oil provision, and studies with only intermediate endpoints.	Randomized controlled trials (since ~1950) that individually randomized participants and replaced saturated fat with linoleic-acid–rich vegetable oil (e.g., corn/soy/safflower) vs usual care, without large n-3 EPA/DHA co-interventions or other major concomitant interventions, and reported CHD or all-cause mortality.	High PUFA	High SFA	CHD mortality	HR = 1.13 (95% CI = 0.83–1.54)	I^2 = 45.1%	No GRADE/SoF framework	Domain-based risk-of-bias assessment, two independent raters.	ns
								All cause mortality	HR = 1.07 (95% CI = 0.90–1.27	I^2 = 38.8%			ns

Citation	Last search date	Databases	No. RCTs (total studies)	Eligibility criteria	Population	Interven-tion	Compara-tor	Out-comes reported	Pooled effect(s) & Model	Heterogen-eity. (I^2)	Certainty (GRADE?)	RoB method	Funding/ COI & Notes
Hamley 2017 (Nutrition Journal)	No report	PubMed, EMBASE, CINAHL; plus hand-searching, grey literature, and contacting study investigators/families.	5	This meta-analysis included randomized trials that replaced SFA with mostly n-6 PUFA and reported CHD events, CHD mortality, or total mortality. Inclusion required a control group and evidence of a ≥20% simultaneous decrease in SFA and increase in n-6 PUFA in the intervention (or dietary advice strongly indicating this). Trials failing that shift were excluded; remaining trials were further classified as adequately vs inadequately controlled based on additional dietary/non-dietary differences, with FMHS also excluded in a separate analysis due to inadequate randomization.	Adult RCTs that replaced SFA with mostly n-6 PUFA vs usual diet; trials included free-living and institutionalized participants, with and without prior CHD; outcomes were CHD events/mortality and total mortality.	Low SFA	High SFA	CHD incidence	RR = 1.02 (95% CI = 0.84–1.23)	I^2 = 72%	No GRADE/SoF framework	Authors assessed domains including random sequence generation, allocation concealment, blinding of participants/personnel and outcome assessment, selective reporting, differences in between-group care, and study-specific biases; additionally, trials were classified as "adequately controlled" vs "inadequately controlled" based on potential confounding differences between groups.	ns
								CHD mortality	RR = 1.13 (95% CI = 0.91–1.40)	I^2 = 65%	ns		ns

Citation	Last search date	Databases	No. RCTs (total studies)	Eligibility criteria	Population	Interven-tion	Compara-tor	Out-comes reported	Pooled effect(s) & Model	Heterogen-eity. (I^2)	Certainty (GRADE?)	RoB method	Funding/ COI & Notes
								All cause mortality	RR = 1.07 (95%CI = 0.90–1.26)	I^2 = 26%	ns		ns
Hooper 2020 (Cochrane)	Oct 2019	CENTRAL, MEDLINE, Embase; plus ClinicalTrials.gov and WHO ICTRP	15	Included RCTs in adults (not acutely ill/pregnant/lactating) with an intention to reduce SFA *or* documented statistically significant SFA reduction, comparing against higher SFA/usual diet, with no multifactorial co-interventions, ≥24-month duration, and mortality or CVD morbidity reported. Designs could be individual or cluster RCTs (≥6 clusters). Excluded non-randomised/uncertain randomisation, multifactorial programmes (unless separable via factorial design), weight-loss-only arms, Atkins-type or fat-substitute interventions, enteral/parenteral and formula weight-reducing diets, and trials with no primary outcome events.	Adults (≥18 y), with or without CVD; trials ≥24 months; excluded acutely ill and pregnant/breastfeeding women.	Low SFA	High SFA	All cause mortality	RR = 0.96 (95% CI = 0.90–1.03)	I^2 = 2%	Moderate	Cochrane Risk of Bias tool (Higgins 2011)	ns
								CVD incidence	RR = 0.83 (95% CI = 0.70–0.98)	I^2 = 36%	Moderate		*
								CVD mortality	RR = 0.95 (95% CI = 0.80–1.12)	I^2 = 2%	Moderate		ns
								CHD incidence	RR = 0.83 (95% CI = 0.68–1.01)	I^2 = 62%	Very low		ns
								CHD mortality	RR = 0.97 (95% CI = 0.82–1.16)	I^2 = 9%	Low		ns

Citation	Last search date	Databases	No. RCTs (total studies)	Eligibility criteria	Population	Interven-tion	Compara-tor	Out-comes reported	Pooled effect(s) & Model	Heterogen-eity. (I^2)	Certainty (GRADE?)	RoB method	Funding/ COI & Notes
Yamada 2025 (JMA Journal)	April 2023	Cochrane CENTRAL, PubMed, and Ichu-shi; reference lists also checked.	9	Included randomized controlled trials in adults that restricted saturated fat (SFA) and reported cardiovascular disease outcomes; no language limits. Excluded arbitrarily evaluated surrogate/imaging outcomes (ECG or coronary angiography changes).	Adults in randomized controlled trials of saturated-fat reduction vs usual diet; outcomes: CVD mortality, all-cause mortality, myocardial infarction, and coronary artery events	Low SFA	High SFA	CVD mortality	RR = 0.94 (95% CI = 0.75–1.19)	ns	No GRADE/SoF framework	Cochrane RoB 2	ns
								CHD incidence	RR = 0.85 (95% CI = 0.65–1.11)	ns	ns		ns
								MI incidence	RR = 0.85 (95% CI = 0.71–1.02)	ns	ns		ns
								All cause mortality	RR = 1.01 (95% CI = 0.89–1.14)	ns	ns		ns

PCS Evidence Table

Study	Description	Model	Parameter	Data
Cheng 2016	Prospective cohort studies only; must report RR with 95% CI for stroke vs. SFA intake and use multivariable adjustment (e.g., alcohol, smoking, BP). If duplicate cohorts existed, the most recent/longest follow-up report was used. No language limits. Excluded non-prospective designs, reviews, non-human studies, and abstracts/reports without RR+95% CI for SFA–stroke associations.	h-l	Stroke incidence	RR = 0.89 (95% CI = 0.82–0.96)
			ischemic stroke	RR = 0.90 (95% CI = 0.82–0.99)
			Hemorrhagic stroke	RR = 0.76 (95% CI = 0.62–0.92)
			Stroke mortality	RR = 0.75 (95% CI = 0.59–0.94)
Chowdhury 214	Included prospective cohorts (≥1-year follow-up) and randomized trials in general or stable-CVD adult populations that assessed dietary intake, biomarkers, or supplement/dietary interventions of fatty acids with coronary outcomes (MI, CHD, angina, coronary death, angiographic stenosis; generally excluding sudden cardiac death from definitions when possible). No language restrictions. Studies not meeting these design/outcome requirements were not included.	dose response	CHD incidence	RR = 1.03 (95% CI 0.98–1.07)
de Souza 2015	Included: human observational studies (prospective cohorts, case-control, nested designs) that reported an association between saturated or trans fat intake (dietary self-report or biomarker) and all-cause mortality, CHD/CVD outcomes, ischemic stroke, or type 2 diabetes. No language limits.	dose response	Most adjusted estimates	
			All cause morality	RR = 0.99 (95% CI = 0.91–1.09)
			CHD mortality	RR = 1.15 (95% CI = 0.97–1.36)

Study	Description	Model	Parameter	Data
			CVD mortality	RR = 0.97 (95% CI = 0.84–1.12)
			CHD incidence	RR = 1.06 (95% CI = 0.95–1.17)
			Ischemic stroke incidence	RR = 1.02 (95% CI = 0.90–1.15)
Harcombe 2017a	Included prospective cohort studies of adult humans that reported CHD mortality and provided dietary fat intake plus serum cholesterol data. Excluded RCTs, cross-sectional, and case–control designs.	dose response	All cause and CHD mortality	1 direct asso with SFA, others ns
Harcombe 2017b	Systematic review limited to prospective cohort studies in adults that reported CHD mortality, with dietary fat intake and serum cholesterol data available; excluded RCTs, cross-sectional, and case–control designs.	dose response	CHD mortality	RR = 1.1 (95% CI = 0.94–1.30)
Jakobsen 2009	Included cohort studies meeting Pooling Project standards (≥150 CHD events; usual diet data; validated/repeatable diet assessment). Within included cohorts, participants were excluded if <35 years, had prior CVD/diabetes/cancer (non-melanoma skin cancer excepted), or had extreme energy intakes (±3 SD from study-specific mean).	replacement	CHD Incidence	PUFA, HR = 0.87 (95% CI = 0.77–0.97) MUFA, HR = 1.19 (95% CI = 1.00–1.42) Carbohydrate, HR = 1.07 (95% CI = 1.01–1.14)
			CHD Mortality	PUFA, HR = 0.74 (95% CI = 0.61–0.89) MUFA, HR = 1.01 (95% CI = 0.73–1.41) Carbohydrate, HR = 0.96 (95% CI = 0.82–1.13)

Study	Description	Model	Parameter	Data
Jayedi 2024	Prospective observational designs (cohort, case-cohort, nested case-control) in adults ≥18 assessing dietary fats or biomarkers (total and subtypes) across ≥2 exposure categories with coronary events outcomes and adjusted effect sizes were eligible; RCTs in adults testing fatty-acid interventions with any control were also eligible. No language/date/publication limits. Excluded retrospective studies and those in children, adolescents, pregnant/breastfeeding women, critically ill, or institutionalized elders; duplicates handled by preferring dose-response-suitable or most recent reports.	dose response	CHD incidence	RR = 1.03 (95% CI = 0.99–1.08)
Kang 2020	Prospective cohort studies in adults (≥18 y) that assessed usual dietary SFA via reliable diet questionnaires, defined stroke with standard clinical criteria, and reported RRs/HRs with 95% CIs were eligible. Studies needed ≥3 exposure categories (or a continuous dose metric) to support dose–response analysis. Excluded were cohorts with prior stroke at baseline and studies with insufficient/irretrievable outcome data; among duplicate cohorts, the most comprehensive/longest follow-up report was used. The search was English-only and did not include unpublished reports.	h-l, dose response	Stroke incidence	High v. low, RR = 0.87 (95% CI = 0.78–0.96) Dose-response, RR=0.94 (95% CI 0.89–0.98)
Kim 2021	Included prospective cohort studies that assessed dietary fat/fatty acids and reported all-cause, CVD, or cancer mortality, with RR (or calculable) and 95% CI. Excluded studies focused only on omega-3 PUFA, and cohorts with pre-existing disease at baseline; among duplicate cohorts, the larger or longer follow-up	dose response	All cause mortality	Highest v. lowest, RR = 1.03 (95% CI = 0.94–1.13) 5% energy increment, RR= 1.02 (95% CI = 1.00–1.05)

Study	Description	Model	Parameter	Data
	report was used. The search targeted English-language full-length articles up to February 2020.			
			CVD mortality	Highest v. lowest, RR = 1.02 (95% CI = 0.92–1.12) 5% energy increment, RR = 1.03 (95% CI = 1.00–1.07)
Ma 2024	Prospective cohort studies (no language/time limits) that reported HR or RR with 95% CI, included ≥1 exposure of interest (dietary macronutrients: protein, fat, carbohydrate) and ≥1 outcome of interest (all-cause, CVD, cancer mortality or CVD events) were eligible. Excluded were duplicates; non-cohort/non-human designs (case reports, letters, reviews, meta-analyses, ecological studies); and studies with insufficient data or conducted in children.	dose rsponse	All cause mortality CVD mortality CVD incidence Stroke incidence	RR = 1.05 (95% CI = 0.98–1.13) RR = 1.03 (95% CI = 0.98–1.08) RR = 0.96 (95% CI = 0.92–1.02) RR= 0.92 (95% CI = 0.82–1.02)
Mazidi 2020	Prospective cohort studies of dietary fat intake and mortality were eligible if they reported multivariable-adjusted effect estimates; non-cohort designs, non-English papers, animal/younger (<20) or diseased baseline populations, and studies lacking usable RRs/HRs/ORs were excluded.	dose response	All cause mortality CVD mortality CHD mortality	HR= 1.04 (95% CI = 0.98–1.11) HR = 0.96 (95% CI = 0.84–1.11) HR = 1.10 (1.01–1.20)

Study	Description	Model	Parameter	Data
			Stroke mortality	HR = 1.03 (95% CI = 0.85–1.26)
			CVD incidence and mortality, and total mortality	RR = 1.06 (95% CI = 0.96–1.15)
Muto 2018	Included prospective cohort studies linking dietary saturated fat to incident or fatal intracerebral hemorrhage or ischemic stroke, requiring CT/MRI/autopsy confirmation (or death-certificate–based stroke from the 1980s onward). Excluded cohorts that did not separate stroke subtypes or lacked imaging-based diagnosis; specific well-known cohorts (Honolulu Heart Program, Caerphilly, EPIC, Framingham, HPFS, Ni-Hon-San) were excluded for those reasons.	dose response	Intracerebral hemorrhage	HR = 0.69 (95% CI = 0.48–1.00)
			Ischemic stroke	HR = 0.89, (95% CI = 0.82–0.96)
Schwab 2021	Included original human studies (plus SRs for certain questions) from 2000–2012, limited to RCTs, prospective cohorts, and nested case–control designs in adults 18–70 who were disease-free at baseline (overweight, dyslipidemia, or glucose intolerance allowed; BMI ≤30 kg/m²). Studies had to examine the amount and/or quality of dietary fat, use standard diet assessments or biomarkers, and (for RCTs) meet minimum duration and dropout thresholds; cohorts needed ≥4 years follow-up (≥5 years for cancer). Cross-sectional, animal, and most retrospective designs were excluded; scope specifically excluded TFA/CLA/dietary cholesterol and postprandial lipemia studies. Papers could also be excluded for wrong topic, wrong exposure (whole foods), inadequate design, non-	dose response, replacement	CVD incidence	-

Study	Description	Model	Parameter	Data
	Caucasian-only populations, too few subjects/too short duration, or missing nutrient data.			
Siri-Tarino 2010	Eligible studies were prospective cohorts in generally healthy adults that specifically analyzed saturated fat intake and reported hard CVD outcomes (CHD and/or stroke), not risk factors. An example exclusion was a study with inconsistent effect estimate reporting that couldn't be resolved with authors.	replacement	CHD incidence Stroke incidence CVD incidence	RR = 1.07 (95% CI = 0.96–1.19) RR = 0.81 (95% CI = 0.62–1.05) RR = 1.00 (95% CI = 0.89–1.11)

Study	Description	Model	Parameter	Data
Skeaff 2009	English-language cohort studies and RCTs on dietary fat and CHD were eligible. Cohorts needed CHD death/events (including non-fatal CHD); RCTs focused on total mortality (and, for n-3 trials, also restenosis/revascularization, non-fatal MI, angina). They accepted dietary assessment or biomarkers for exposure (but MUFA required dietary assessment). Excluded were cohorts without RR estimates, multifactorial trials (e.g., MRFIT), studies not fitting the intervention categories (e.g., olive oil arm), CVD-only (not CHD) endpoints, ALA-supplement trials, and trials with methodological concerns.	h-l, dose response	CHD incidence CHD mortality	High v. low: RR = 0.93 (95% CI = 0.83–1.05) Dose-response: RR =1.03 (95% CI = 0.87–1.22) High v. low: RR = 1.14 (95% CI = 0.82–1.60) Dose response RR = 1.11 (95% CI = 0.75–1.65)
Wallerer 2024	Eligible studies were prospective observational designs in adults (≥18 y) from generally healthy populations, reporting isocaloric substitution analyses (macronutrients or their subtypes) using established methods (leave-one-out/partition) with the outcome all-cause mortality; duplicate cohort reports were resolved by favoring the larger case count or longer follow-up, and conference abstracts with sufficient methods/results were eligible. Excluded were studies only in children/adolescents/pregnant women and publications without all-cause mortality data.	replacement	All cause mortality	Replacement of SFA with PUFA, HR = 0.86 (95% CI = 0.81–0.91) Replacement of SFA with MUFA, HR = 0.91 (95% CI = 0.86–0.97) Replacement of trans-fat with SFA, HR =0.85 (95% CI = 0.75–0.97) Replacement of carbohydrate with SFA, HR = 1.06 (95% CI, = 1.00–1.13) Replacement of protein with SFA, HR = 1.01 (95% CI = 0.91–1.13)

Study	Description	Model	Parameter	Data
Zhu 1019	Included cohort or nested case-control studies that examined dietary total fat or fat subclasses (SFA, TFA, MUFA, PUFA) vs CVD outcomes, and reported RR/HR with 95% CI; duplicates were resolved by choosing the most recent or largest report. For dose-response, studies needed ≥3 exposure categories (or equivalent data) plus cases/person-years. Excluded at screening were papers without relevant associations, duplicates, case-control designs (non-nested), and studies "only investigating fat from breakfast."	h-l, rose response	CVD incidence	High v. low, HR = 0.97 (95% CI = 0.93–1.02) Per 5% energy increment, HR = 0.99 (95% CI = 0.95–1.04) Per 5 g/day increment, HR = 0.98 (95% CI = 0.95–1.00)

Appendix 4.8. Effects of Thermally Stressed Added Fats on Cardiometabolic Health

A NARRATIVE REVIEW ON THE EFFECTS OF THERMALLY STRESSED ADDED FATS ON CARDIOMETABOLIC HEALTH

A narrative review

Ameer Y. Taha, PhD
Department of Food Science and Technology
University of California Davis

Abstract

Background: In the US, seed oils account for approximately 70% of added fats to foods, thus being the main source of lipids used in normal cooking (e.g. at home), restaurants (e.g. frying), and industrial making of ultra-processed foods, where oils can be subjected to thermal stress. Several observational studies have shown an association between the intake of thermally stressed foods (e.g. fried foods) and risk of cardiometabolic disease. It is not known whether these findings are corroborated by intervention studies.

Objectives: The overall objective of this narrative review is to understand the effects of oxidized lipids originating from thermally stressed oils on cardiometabolic health. Specifically, two critical questions will be addressed: 1) what are the effects of thermal processing on the composition of added fats and oils, and 2) what are the biochemical (2a) and clinical effects of consuming thermally processed oils (2b)?

Methods: For question (1), a narrative review was conducted based on available literature to provide a summary of the current understanding and knowledge gaps in the field of lipid oxidation in relation to the thermal processing of fats and oils. Question 2a was also based on a literature review on the metabolism of oxidized lipids in vivo. Question 2b involved a systematic PubMed search for controlled intervention human studies that investigated the effects of oxidized lipids or frying on cardiometabolic health outcomes.

Results: The evidence suggests that during thermal treatment (e.g. pan frying), the degree of fatty acid unsaturation in oils rather than antioxidant levels, is a key determinant of the formation of lipid oxidation products (Q1), which are bioavailable upon ingestion (Q2a). The systematic search (Q2b) yielded a total of 6 intervention studies, of which 5 involved acute administration of a test meal containing various types of thermally treated oils mixed with foods, and one was a chronic 4 week study. Three of the 5 acute intervention studies found evidence of increased oxidized lipids within chylomicrons in serum/plasma of participants who consumed a meal containing thermally stressed oils compared to those who did not. Enrichment of oxidized lipids in chylomicrons was exacerbated if participants had diabetes. One study reported increased serum levels of lipid mediators involved in inflammation after a breakfast meal containing different types of fried oils. Another acute intervention study showed that participants receiving a test meal prepared by frying pasta and zucchini in olive oil, decreased the post-prandial insulin response and C-reactive protein increment in obese, but not in lean participants, when compared to weight-matched participants who received a meal prepared by adding olive oil to boiled pasta and grilled zucchini. The only long-term intervention study which fed participants fried versus non-fried meats (4 times a week) for 4 weeks, found impaired glucose response, increased serum markers of systemic inflammation and altered gut microbiota in participants who consumed the fried meats compared to those who did not.

Conclusions: The majority of intervention studies point to evidence of cardiometabolic impairments in humans consuming thermally stressed oils added to foods. These effects were exacerbated by pre-existing metabolic conditions including diabetes and obesity. Future intervention studies are needed to capture the long-term (>4 weeks) effects of oxidized lipids from thermally stressed oils, particularly in relation to oil type, processing methods, duration of human exposure and underlying cardiometabolic status.

Introduction

Food processing is a broad term which describes the physical, chemical and/or thermal treatment of foods to improve safety, quality, and shelf-life. A number of studies have reported a link between the consumption of extensively processed foods (i.e. ultra-processed) and increased risk of weight gain, cardiometabolic impairments and overall mortality [1,2]. The potential adverse health effects of processed foods is of particular concern in countries like the US, where over 50% of daily calories may come from ultra-processed foods [3].

Added fats are a major component of processed foods. In the US, seed oils account for approximately 70% of added fats to foods (USDA), of which many undergo processing involving heat treatment. Seed oils are also used extensively in restaurant settings (e.g. frying), in-home cooking and processing applications (e.g. to make ultra-processed foods). The most common seed oils used in the US are soybean, canola and corn oil.

Processing often involves the application of heat, which can oxidize lipids/fats added to foods, and increase human dietary exposure to oxidized lipids (e.g. those generated during frying or the making of ultra-processed foods). Thermally-induced lipid oxidation results in the degradation of essential vitamins (e.g. vitamin E) and the generation of lipid oxidation products which may have adverse health effects. Likely, there is a range of thermal treatment which results in normal exposures to oxidized lipids, and a level beyond which thermal treatment of oils may cause harm, particularly in the context of cardiometabolic disorders [4-10]. For instance, in a study involving 3 prospective cohorts, cooking meats at higher temperatures or with a greater frequency of open flames, was associated with increased risk of type 2 diabetes [7]. While the findings are associative, they point to a potential link between the degree of thermally treating meats, and the risk of type 2 diabetes.

The overall objective of this narrative review is to understand the effects of oxidized lipids originating from thermally stressed oils on cardiometabolic health. Specifically, two questions will be addressed: 1) what are the effects of thermal processing on the composition of added fats and oils, and 2) what are the (a) biochemical and (b) clinical effects of consuming thermally processed oils? These questions are important in view of several observational studies which showed an association between higher consumption of fried foods and increased risk of all-cause mortality, type 2 diabetes, cardiovascular disease, and some aspects of the metabolic syndrome (e.g. weight gain and hypertension) [4-10]. Thus, it is important to understand the chemistry of what happens exactly when oils undergo thermal treatment, and what this imparts on the body upon ingestion.

The current narrative review will focus on the effects of oxidized lipids originating from thermally stressed oils on cardiometabolic outcomes; it will not address the effects of consuming different dietary fats from different seed oils or other sources on health outcomes, as this is outside of the current scope. The review will focus on seed oils, because they account for approximately 70% of added fats in the US, thus being the

main source of lipids used in cooking, restaurants and food processing applications (e.g. in ultra-processed foods). Also, seed oils contain unsaturated fatty acids, which are more prone to thermal oxidation than saturated fatty acids [11].

As will be discussed below, the evidence indicates that thermally-generated lipid oxidation products are bioavailable when ingested through food, and that they may impair cardiometabolic health.

Methods

For Question 1, which aimed to understand the effects of processing and cooking on the composition of added fats and oils, a narrative review of available literature was conducted to provide a summary of the current understanding and knowledge gaps in the field of lipid oxidation in relation to the thermal processing of fats and oils. Question 2, which aimed to explore the biochemical and clinical effects of consuming processed and thermally stressed fats and oils, was divided into two subparts. Question 2a used a narrative review of available literature to identify pre-clinical and clinical studies on the metabolism of oxidized lipids in vivo (i.e. absorption and distribution). Question 2b involved a systematic PubMed search to identify controlled intervention human studies that investigated the effects of oxidized lipids or frying on cardiometabolic health outcomes.

For question 2b, databases/links used to perform the search included PubMed (UC Davis Library link to access all full text articles) with MeSH search to check for key terms, Web of Science (complementary citation tracking tool for searching the title of an article to find others that have cited it) and Google scholar (complementary citation tracking tool).

Table 1 outlines the search terms used to derive controlled intervention studies that investigated the effects of oxidized lipids or frying on cardiometabolic outcomes. The targeted search was performed to address the pre-specified question of "What are the clinical effects of consuming processed and thermally stressed fats and oils?". Once the search was generated, randomized controlled trials were selected to exclude animal/pre-clinical studies, since the focus of the current narrative is on human intervention studies. Articles were screened to determine whether the studies were based on observational or interventional studies. Only interventional studies were reviewed. Additionally, review articles were screened to find other lead references that did not come up in the main search. Observational studies or meta-analyses mostly based on observational studies were not considered, because unlike interventional studies, the evidence from observational studies establishes associations rather than causation.

Results

Question 1: what effects do processing and cooking have on the composition of added fats and oils?

This section will provide a brief overview on the i) mechanisms of lipid oxidation, ii) how refined oils are processed, iii) estimated daily exposure to oxidized lipids in human diets due to processing and iv) factors that determine oxidized lipid levels in oils. The section will conclude with v) a summary of the evidence, and identifying vi) knowledge gaps that need to be resolved.

i) Mechanisms of Lipid Oxidation:

Seed oils are composed of a group of lipids called triacylglycerols (TAGs). During thermal treatment, TAGs break down into free fatty acids, which can oxidize [12]. Broadly speaking, there are four main types of oxidation products that are formed when TAGs break down during thermal processing – 1) primary oxidation products, 2) secondary volatiles, 3) fragmentation products and 4) addition products.

Primary oxidation products, also known as 'oxylipins', form when unsaturated fatty acids become oxidized (i.e. they gain at least one oxygen molecule). Hydroperoxides, the most studied primary oxidation product, is a type of oxylipin. Others include mono, di and try-hydroxy or epoxy fatty acids [13], including 9- and 13-hydroxyoctadecadienoic acid, 9,10-dihydroxyoctadecenoic acid and 9(10)- epoxyoctadecanoic acid, among others [14]. Primary oxidation products can occur in both cis and trans conformations [15]. In plants and other living organisms, primary oxidation products are typically formed through enzymatic reactions involving lipoxygenase, cyclooxygenase and cytochrome P450 enzymes (reviewed in [16]). This yields specific enantiomers with S conformation [17]. During the thermal treatment of oils, oxylipins can be formed non-enzymatically, resulting in racemic mixtures with both R- and S- conformations.

Secondary oxidation products (i.e. secondary volatiles) form when primary oxidation products break down to yield smaller and relatively volatile molecules which typically have sensory attributes. In plants and animals, secondary volatiles are formed when lyase enzymes act on oxylipins [18], but during thermal treatment, they can be formed non-enzymatically via beta-scission [19]. Examples of secondary volatiles are short-chain carboxylic acids, cyclic fatty acids, ketone esters and aldehydes (e.g. acrolein, hexenal, hexanal, etc.) (reviewed in [11,20]). Secondary volatiles are responsible for odor and flavor in foods. For instance, secondary volatiles such as hexanal are associated with rancid type flavors or odors, whereas hexenals are responsible for earthy green flavors/odors. There are thousands of secondary volatile compounds that can be generated from oxylipin breakdown. Many secondary compounds volatilize or remain in the fat/oil medium within food, depending on their hydrophobicity. More hydrophobic compounds tend to adsorb to lipids in foods, thus contributing to taste/flavor, whereas less hydrophobic compounds tend to volatilize, thus contributing to odor/aromas.

Fragmentation products are typically seen with prolonged thermal treatment, when TAGs in oil break down into mono- and di-acylglycerols [21,22]. Mono-and di-acylglycerols are responsible for the 'foaming' phenomenon seen in fryers. These compounds tend to go to the surface and form a layer there, due to their lower density compared to TAGs. Mono- and di-acylglycerols can break down further into free fatty acids, which can further oxidize into primary and secondary oxidation products.

With more prolonged or excess thermal treatment, TAGs containing oxidized and non-oxidized fatty acids begin to polymerize, leading to oil thickening [21]. At this point, thermal transfer within the oil is diminished, which means that more heat energy is needed to cook foods properly. Oils are usually discarded or diluted with a fresh oil batch once thickening occurs.

Other 'late' oxidation products formed with prolonged thermal treatment of foods interacting with heated oils (e.g. during frying) or lipid-containing foods (e.g. roasting) include advanced lipid-protein and lipid-sugar products (i.e. advanced glycation products; reviewed in [20]). Trans fatty acids can also form during thermal treatment of fats through cis-trans isomerization (reviewed in [11]). However, the extent of trans fatty acids formed during thermal treatment is very low (<~1% of total fatty acids) [23,24] compared to traditional hydrogenation methods, where trans fatty acid levels can reach ~50% of total fatty acids [25]. Thus, trans fatty acids at the levels formed during thermal treatment of oils/foods are not likely to cause significant harm on cardiometabolic risk factors compared to trans fatty acid intake at the levels found in partially hydrogenated oils [26].

ii) Oil Processing:

This section will briefly review the physical and chemical methods used to extract and refine oils prior to commercialization. It is intended to point out that the majority of refined oils sold to consumers are heavily processed through various physical/chemical/thermal treatment methods designed to remove pre-existing lipid oxidation products and increase shelf-life. These oils are then used for frying and other thermal processing applications, which can generate the lipid oxidation products outlined in section (i).

Oils are extracted from seeds mechanically (i.e. pressing) and/or chemically, using hexane as a solvent. The extracted seed oils are often 'refined' to increase their shelf-life. This means that they undergo a series of chemical and thermal steps to remove potential oxidants (e.g. metals), free fatty acids and primary and secondary oxidation products originating from the seeds (as mentioned above, these are naturally formed through enzymatic processes in plants).

The 'refining' process is achieved through 4 steps – degumming, alkalinization, bleaching and deodorization ("DABD") (reviewed in [27,28]). Most commercially available oils go through this process with a few exceptions, such as virgin olive oil. Thus, the difference between "olive oil" and "virgin olive oil" is that the former is refined, whereas

the latter is not. Below, a brief overview will be provided on the refining process, given that it affects basal oxidized lipid levels of commercially available oils.

Degumming involves heating oil with added water, often containing phosphoric acid to remove free metals and phospholipids originating from cell membranes within the seeds. Phospholipids appear as a cloudy emulsion in oils so degumming removes them and results in a clearer oil. The extracted phospholipids can be resold as commercial lecithin, which is used as an emulsifier in various food applications.

The second step of the DABD process is alkalinization, which involves the use of a base to remove naturally occurring free fatty acids in the extracted oil as well as phosphoric acid added during the degumming step. This process may also remove free oxylipins present in the oils.

Bleaching involves heating in the absence of air (80 -100ºC) with bleaching earth (0.2-2%) to remove metal-containing pigments such as chlorophyll. This step is needed because metal in those pigments can act as a pro-oxidant, especially if it dissociates from chlorophyll.

In the final deodorization step, the oil is heated to 250-275ºC under vacuum with steam as a sparge. This promotes 1) the degradation of residual oxylipins into secondary volatiles and 2) the vaporization of secondary volatiles present in the oil. As mentioned above, many secondary volatiles are responsible for the rancid smell of oils, so their vaporization ensures the removal of any odorous compounds naturally generated through lyase enzymes, when the seed is pressed for oil.

The DABD refining process is required to produce a shelf-stable oil. It is designed to minimize the presence of oxylipins, secondary volatiles and pro-oxidants in oils. This is why oxylipin concentrations are lower in refined oils compared to non-refined (i.e. virgin) oils [29]. The DABD process decreases antioxidant levels in oils, which is why antioxidants (e.g. vitamin E) are sometimes added afterwards, particularly if the oils will be used in restaurant or industry settings.

Lipid oxidation products in DABD-processed oils, or foods to which these oils are added, can still be formed during storage due to light exposure or physical-chemical interactions with air present in the oil/food matrix [30,31]. This could determine basal levels of primary, secondary and other oxidation products in oils before they are subjected to thermal treatment, which causes oxidation products to further increase.

iii) Exposure to Oxidized Lipids in Humans From Seed Oils

In general, primary and secondary oxidation products are generated during the early steps of lipid oxidation. Fragmentation and addition products are late oxidation products formed when oils are used repeatedly to fry foods, or are heated for prolonged periods (e.g. days) at high temperatures. Thus, they are generated during the end stages of lipid oxidation, known as the ‘termination’ step. In most processing applications involving thermal treatment, primary and secondary oxidation products are the main species that form and accumulate in foods. Fragmentation and addition products are relatively less

abundant because their formation would be notable in terms of oil texture (thickening), color (foaming) and the formation of rancid-type odors, which would prompt users to change or dilute the oil. Thus, one can infer that primary and secondary oxidation products are the main oil oxidation species that humans consume on a daily basis.

The advent of analytical techniques involving mass-spectrometry and NMR has enabled the quantitation of oxylipins and secondary volatiles in oils and foods subjected to different processing conditions. A study by Richardson et al. showed the presence of low levels of oxylipins in non-thermally treated refined oils (soybean, corn and canola) and in extra-virgin olive oil [14]. Using USDA oil consumption data, the authors estimated daily intake from these oils to be ~1.1 mg per person per day, and that soybean oil contributed the majority (>80%) of oxylipins in the US diet, given that it is the most consumed oil in the US.

It should be pointed out that this 1.1 mg per person per day intake dose represents a lower exposure estimate value, as oxylipin concentrations can increase by at least 2-10-fold when oils or foods containing added fats are thermally treated [14]. A study by Koch et al. which measured oxylipins in processed foods reported concentrations in the range of 0.023 mg/g (hamburger patty) to 1.2 mg/g (falafel) [29]. When corrected for portion size (~68 g for hamburger patty and 70 g for falafel), estimated intake of oxylipins ranged between 1.6 to 81 mg per portion. Thus, depending on portion size and the extent of processing, estimated daily intake of oxylipins in the diet amounts to hundreds of milligrams per day. **The estimated range is 1 to 500 mg per person per day**, where 1 mg per day represents intakes of foods containing added oils not subjected to processing (a highly unlikely scenario), and 500 mg per day represents a scenario where individuals are consuming several portions of heavily processed foods per day. It should be noted that these are only estimates and that future studies are needed to accurately quantify oxylipins in a variety of processed foods habitually consumed in the US, to obtain estimated daily intakes across the population.

Because oxylipins in the diet are made non-enzymatically, exposure through oils/foods is likely to yield racemic mixtures of the same oxylipin (R and S). Endogenously, the body synthesizes S-series oxylipins enzymatically. Questions remain on the impact of chronic exposure to R oxylipin stereoisomers, and whether their biological effects differ from oxylipins in the S conformation.

Data on secondary compound levels in heated oils are sparse, mainly because most studies have used non-quantitative methods such as the TBARS assay to measure aldehydes and other secondary volatiles in oil/food samples. A recent study which quantified secondary volatiles in heated oils estimated their concentrations to be 404 umol/kg of oil [32]. Assuming a representative average molecular weight of 86 g/mol, exposure from secondary volatiles in fried oils amounts to ~35 mg per kg of oil. Assuming a daily intake of 30 to 60 g of oil per day, **estimated daily levels of secondary lipid oxidation products from oils are in the order of a few mg per**

person per day (~1 to 2 mg per person per day). This exposure will depend on whether these compounds volatilize or remain in the food matrix.

iv) Factors That Determine Oxidized Lipid Levels in Foods

It is true that the extent and type of processing determines oxylipin and volatile product formation and exposure through foods. However, the evidence to date indicates that the most important determinant of oxylipin levels in oils is polyunsaturated fatty acid (PUFA) content. In whole foods, both PUFA content and water levels have been shown to modify the extent of primary and secondary product formation.

In oils, studies have consistently shown that the greater the PUFA composition, the greater the extent of lipid oxidation during thermal processing. For instance, oils high in the PUFA, linoleic acid (e.g. soybean, corn oils), generate more primary and secondary oxidation products derived from linoleic acid compared to oils low in linoleic acid (e.g. high monounsaturated fatty acid algae oils, olive oil) [32,33]. Interestingly, one study showed that the vitamin E content in oils was not strongly related to the extent of oxylipin formation following 30 minutes of pan frying [33]. The main determinant of lipid oxidation was oil PUFA composition [33].

Similar to oils, foods with a greater PUFA content oxidize faster during thermal treatment compared to those with less PUFA content. The presence of water in foods, however, is known to accelerate the degradation of oxylipins into secondary compounds. For instance, one study showed that oxylipins decrease in milk subjected to various forms of thermal treatment (e.g. holder pasteurization) [34]. Another showed that residual water in French fries can also degrade oxylipins acquired from the oil they are fried in [35]. Likely degradation products not measured in these studies include secondary volatiles.

v) Summary

The main determinant of lipid oxidation in oils/foods is PUFA composition – more PUFAs mean more lipid oxidation. Thus, to decrease the oxidizability of oils or foods, it would be reasonable to use oils with less PUFA content, such as olive and high monounsaturated fatty acid algae oils. Oils with 10% or less PUFA fatty acid composition would be ideal for minimizing lipid oxidation during thermal treatment, based on studies showing that olive and algae oils with 10% or less PUFA content, are less prone to oxidation compared to oils high in PUFAs such as soybean and corn oil [14,33].

vi) Knowledge Gaps That Need to be Resolved Include:

1) Detailed profiling of oxylipins and secondary volatiles in processed/ultra-processed foods to better estimate intake of oxidized lipids.
2) Better quantification of R and S oxylipin mixtures and secondary volatile derivatives from these mixtures. This is because the R and S oxylipin forms and their derivatives may exert different biological effects from what is formed in vivo (typically S form).

3) Oxylipins and secondary volatiles in other low PUFA oils including beef tallow, butter and coconut oil need to be further characterized before and after thermal treatment or industrial processing.
4) Because low PUFA fats derived from animal sources are likely to contain low levels of cholesterol (e.g. beef tallow and butter), a better understanding of the extent of oxidized cholesterol formation during processing in relation to bioavailability, bioactivity, and effects on cardiometabolic health is needed [36].

Question 2: What are the biochemical and clinical effects of consuming processed and thermally stressed fats and oils?

As mentioned above, when oils oxidize, they form two classes of bioactive compounds of concern to humans; these are primary oxidation products (i.e. oxylipins), and secondary oxidation products which include reactive aldehydes. This section will address the i) biochemical effects of these compounds on the body in terms of absorption and in vivo bioactivity, and ii) discuss the health effects of consuming processed oils, relying on interventional clinical trials. Additional subsections on iii) summary and conclusions and iv) knowledge gaps that need to be resolved will be provided in the end.

With regard to biochemical effects (i), both pre-clinical and human studies will be discussed to highlight knowledge gaps that need to be addressed in humans on the metabolism of oxidized lipids. The health effects section (ii) will focus on interventional human studies retrieved through the systemic search outlined in the Methods. While numerous studies have reported on the adverse effects of dietary exposure to oxidized lipids in animals (rodents mainly), clinically-relevant outcomes in humans remain less understood, which is why the focus of this section will be on human interventional studies.

i) Question 2a: Biochemical Effects of Lipid Oxidation Products – In Vivo Metabolism

Both rodent and human studies have shown that ingested oxylipins are absorbed. One study in rats showed that heavy-isotope labeled (deuterated) 13-hydroxyoctadecadienoic acid, an abundant oxylipin found in high linoleic acid oils and foods, is rapidly absorbed and incorporated into plasma lipoproteins and tissues (heart, adipose and liver) [37], suggesting that oxylipins are absorbed and reside in tissues. Other studies in rodents have shown that upon ingestion, hydroperoxy fatty acids (i.e. hydroperoxides) are either reduced to hydroxy fatty acids or degraded into aldehydes in the gut [38-40]. The resulting hydroxy fatty acids and aldehydes are then absorbed intact.

Human studies also support pre-clinical data showing that oxylipins are absorbed, but direct evidence of tissue incorporation in humans is lacking. Specifically, the evidence in humans demonstrates that various types of oxylipins including hydroxy, dihydroxy, epoxy and diepoxy fatty acids can be absorbed intact.

In one human study, TAGs containing labeled mono- and di-epoxy fatty acids peaked within 2 to 4 hours in plasma following ingestion [20]. The authors estimated (based on the plasma area under the curve) that 17% and 8% of the monoepoxy and diepoxy fatty acid doses provided were absorbed [41]. Similarly, TAGs containing labeled hydroxy and dihydroxy fatty acids were shown to be absorbed at an efficiency of 21% and 4.5%, respectively [42]. In both studies, oxylipins appeared in plasma chylomicrons within an hour after ingestion, peaked at 2 to 6 hours, and were barely detectable after 24 hours. It is not clear from these studies where the remaining oxylipin tracer went; presumably a portion might have been excreted through feces and/or transformed into other oxylipin and secondary degradation products (e.g. aldehydes).

The relatively rapid disappearance of labeled oxylipins from plasma in humans (within 24 hours) is consistent with rodent studies showing that they rapidly clear from plasma because they incorporate into tissues (adipose, liver and heart) [41]. Once in tissues, they reside there for a much longer duration compared to their PUFA precursors. For instance, in rats, the typical half-life of 13-hydroxyoctadecadienoic acid in heart and liver ranges between 3.1 to 3.6 days [37], whereas the half-life of PUFAs in these tissues is between 3 to 5 hours [43]. This suggests that ingested oxylipins reside longer in tissues and are more difficult to clear, compared to their precursor fatty acids. Further studies are needed to confirm these findings in humans.

ii) Question 2b: Clinical Effects of Consuming Processed and Thermally Stressed Fats

Most of the evidence on the effects of oxidized oils on health stems from animal studies [44,45]. In these pre-clinical studies, the intake of oxidized fatty acids through the diet has been shown to promote hypertension and the formation of atherosclerotic lesions (reviewed in [36]). Ingested oxylipins were also found to promote liver inflammation in mice [44].

A comprehensive literature search was performed (see Methods) to retrieve intervention studies that explored the effects of oxidized lipid intake on cardiometabolic risks in humans. This was coupled to known articles by the author on the topic, based on subject matter expertise. Of the 1120+ articles and reviews retrieved and screened, 6 relevant intervention studies were identified and summarized in **Table 2**.

Of the 6 studies identified and discussed below, 3 studies (Studies 1 to 3 in the following paragraphs) showed that dietary intake of thermally stressed oils increased primary and secondary oxidation products in circulating chylomicrons [46-48]. This is concerning because oxidized chylomicrons are processed by the liver into oxidized low-density lipoproteins (oxLDL), which have been associated with increased risk of atherosclerosis, the metabolic syndrome, type 2 diabetes and stroke (both ischemic and hemorrhagic) [49-52].

Study 1: In a study by Strapans et al. [46] participants received a meal containing thermally stressed corn oil with bread, and conjugated dienes were quantified after 4 hours in serum chylomicrons; conjugated dienes are surrogate markers of oxidized fatty

acids (i.e. oxylipins / primary oxidation products). Thiobarbituric Acid Reactive Substances (TBARS) were also measured in whole serum as markers of secondary lipid oxidation products, mainly aldehydes. The study showed that subjects fed corn oil (1 g/kg) containing low (6.5-10 nmol conjugated dienes/mg oil), medium (30-50 nmol conjugated dienes/mg oil) or high (80-120 nmol conjugated dienes/mg oil) levels of conjugated dienes, increased serum conjugated dienes in chylomicrons from 9.7 nmol/µmol TAG (control) to 21.9 nmol/µmol TAG (medium oxidized) and to 46 nmol/µmol TAG (highly oxidized) [46]. TBARS levels representing aldehydes in serum were not detected in subjects fed the low or medium oxidized corn oil diets, but were detected in highly oxidized oil group at a level of 0.14 nmol/µmol TAG. Compared to the control diet (low oxidized corn oil), serum linoleic acid percent composition decreased in the subjects fed the high-oxidized corn oil from 56 to 41%, suggesting displacement of this fatty acid with primary oxidized fatty acids incorporated into serum from the diet [46].

The study also showed, in a subset of participants, that conjugated dienes in chylomicrons peaked after 6 hours post-prandially, and decreased by ~ 8 hours. Additionally, the lag time to copper oxide induced oxidation of serum from subjects fed the highly oxidized oil versus controls, was shortened from 4.3 to 3.2 hours, indicating that oxidized chylomicrons are potentially prone to further lipid oxidation compared to relatively less oxidized chylomicrons.

Study 2: The relationship between dietary and circulating oxylipins may depend on disease status. In humans, the consumption of a singly dietary meal containing low (40-99 µmol/mmol TAG of conjugated dienes) or high oxidized fatty acids (40 to 200 µmol/mmol TAG of conjugated dienes) derived from corn oil, increased oxylipins within 2.5 hours (measured with the conjugated diene method) in serum chylomicrons of diabetic subjects with poor glycemic control, compared to diabetics with good glycemic control or control subjects with normal glycemia [47]. This suggests differences in the absorption or metabolic handling of dietary oxidized lipids in diabetics with poor glycemic control compared to diabetic or healthy individuals with normal glycemic control.

Study 3: Another small human study (n=5) showed that aldehydes increased in serum chylomicrons collected 4 hours after individuals were fed soybean oil thermally treated for 7 hours at 220 °C (peroxide value 4.8 mEq/kg) compared to non-heated soybean oil (1.6 mEq/kg) [48]. No differences in serum TAGs were observed [48]. This suggests that secondary oxidation products in oils are also bioavailable.

Study 4: In a cross-over intervention study, 26 subjects (17 post-menopausal women and 9 men) received a breakfast muffin made with 4 different types of oils pre-heated at 180 °C for 5 min, 10 times a day for 2 days with 30 min cooling intervals [53]. The oils used were 1) refined sunflower oil as control, 2) refined high oleic-sunflower oil with 400 mg/L dimethylsiloxane as an antioxidant, 3) refined high oleic-sunflower oil with 400 mg/L of added polyphenols and 3) non-refined olive oil (i.e. extra-virgin) containing 400 mg/L of natural polyphenols. Oxylipins involved in promoting or dampening inflammation

were measured in serum at baseline (pre-meal) and 2 and 4 hours after administering the breakfast meals. As mentioned earlier, oxylipins can be generated non-enzymatically (e.g. during oil frying), or through various enzymes in the body, where they participate in signaling and immune regulation. In the body, oxylipins are often referred to bioactive lipid mediators, because they facilitate multiple biological processes in vivo (reviewed in [16]).

Compared to baseline, the authors reported significant increases in pro-inflammatory oxylipins (hydroxyoctadecadienoic acids; HODEs) in the group that consumed muffins containing refined sunflower oil (authors did not specify whether this was at 2 or 4 hours post-prandially). Increments in the other groups receiving refined oleic-sunflower oils with added antioxidants and non-refined olive oil containing natural antioxidants, were intermediate relative to baseline [53]. Prostaglandin D2 (PGD2), an oxylipin which has both pro- and anti-inflammatory roles in vivo, also increased significantly in the group that received the sunflower oil muffins, with intermediate changes relative to baseline in the other groups. PGE3, an anti-inflammatory oxylipin, significantly decreased in all 4 groups relative to baseline [53].

A limitation of this study is that it did include a control group which received non-thermally stressed oils. This would have informed whether the observed post-prandial responses were due to oil versus fried oil consumption. Additionally, the study did not include a group that received fried sunflower oil with added antioxidants to enable differentiation between the effects of added antioxidants versus the effects of varying PUFA oil content. Despite these limitations, the findings show differences in post-prandial oxylipin responses following different types of fried oils. In this regard, increases in pro-inflammatory oxylipins (relative to baseline) were most pronounced in refined sunflower oil compared to refined oils with added anti-oxidants or non-refined olive oil containing natural anti-oxidants. Notably, this could be due to the higher PUFA composition of sunflower oil (58%) compared to high-oleic sunflower and olive oils which contain 11-18% PUFAs, since increased PUFA intake has been shown to increase PUFA-derived oxylipins in rodents and humans through enzymatic and non-enzymatic oxidation [54,55].

Study 5: A study by Frenette et al. showed that a test meal made by frying food in 25 g of extra-virgin olive oil decreased plasma insulin and C-peptide post-prandial response in 12 obese, insulin resistant women, compared to a similar calorie-matched test meal cooked by boiling, and containing 25 g of non-thermally treated extra-virgin olive oil [56]. There was a non-significant tread towards a reduction in plasma TAGs relative to baseline, after consuming the fried meal compared to the non-fried control meal in obese subjects. Lean subjects given the same test meals showed no post-prandial changes in plasma insulin, C-peptide or TAGs. Additionally, neither the obese nor lean subjects showed significant differences in plasma post-prandial glucose response after either meals (made with or without fried extra-virgin olive oil). The ‘fried meal’ consisted of penne pasta, courgettes (zucchini) and apple of which the pasta (presumably boiled first) was stir fried for 15 seconds in olive oil that was pre-fried for 3 minutes prior; the

zucchini was also deep-fried in olive oil for an unspecified amount of time. This led the authors to propose that frying the pasta for a short amount of time promoted the formation of amylose lipid complexes that slowed the rate of carbohydrate absorption, resulting in a lower post-prandial insulin and C-peptide response. It is not known whether longer exposure periods of the same test meals fried with the same type of oil or other oils high in PUFAs (e.g. soybean, canola, corn) would lead to similar findings.

Study 6: Most studies investigated the effects of acute intake of fried foods or thermally stressed oils, leaving significant knowledge gaps on the role of more chronic intakes on cardiometabolic endpoints. A recent 4-week intervention study addressed this knowledge gap by testing the effects of chronic fried meat intake at a frequency of 4 times a week, on multiple cardiometabolic markers [57]. The study randomized 58 individuals to a control diet containing meats that were boiled, steamed or dressed with sauce at 100°C, and 59 individuals to an isocaloric experimental diet which contained fried meats cooked at 150°C for <3 min. The types of oil used to fry the meats provided in the intervention arm were not specified, which is a limitation. After the intervention, participants in the group consuming fried meats had a higher body mass index, impaired glucose metabolism, increased serum and fecal markers of inflammation, and reduced richness of gut microbiota. Notably, after the intervention, serum advanced glycation products (markers of fried food consumption) were higher in the group which consumed fried meats compared to the control group, suggesting that participants adhered to their diets during the study.

iii) Summary

There is convincing evidence that dietary oxidized fats (oxylipins and aldehydes) are absorbed and thus bioavailable. Interventional studies in humans show that the acute consumption of thermally stressed oils with food increased circulating lipid oxidation products in chylomicrons and markers of inflammation. These effects were exacerbated by underlying metabolic impairments (e.g. diabetes with impaired glucose regulation). The one study which showed improvements in post-prandial insulin response after acutely administering fried oil with food, demonstrated this effect in lean but not obese individuals, suggesting that healthy adults may metabolically process fried oils differently compared to individuals with metabolic impairments. In contrast, chronic consumption (4 weeks) of fried meat increased body mass index, impaired glucose metabolism, promoted inflammation and disrupted gut microbiota. Thus, it is concluded that consistent with observational studies, interventional studies in humans increase surrogate markers of cardiometabolic disease [4-10].

However, several questions remain unanswered. For instance, it is not known whether effects of thermal processing of fats on cardiometabolic risk factors depend on the type of oil used. Different oils have varying degrees of oxidative potential, with low PUFA oils such as olive oil being more resilient to thermal stress compared to high PUFA oils such as corn, soybean and sunflower. This means that frying with olive oil for instance is likely to result in lower exposure to lipid oxidation products compared to corn, soybean

or sunflower oils. Additionally, the response to various processed oils is likely to depend on age, health status and other individual characteristics. Furthermore, it is not clear how the thermal treatment methods used in some of these studies translate to actual human exposures; in some cases oils were heated for a few days (**Table 2**). Thus, it remains unclear whether the same outcomes would be observed with more realistic exposure to oxidized fats.

The amount and type of oxidation products formed during thermal processing will also depend on the processing methods used (including duration, temperature, container used, etc.) and the interacting food matrix (e.g. different carbohydrates/proteins types in foods) and food additives used (e.g. emulsifiers). Lipid oxidation products formed during these scenarios may also modify health outcomes.

iv) Knowledge Gaps That need to be Resolved:

1) Comparing the effects of different oils subjected to thermal processing on cardiometabolic outcomes. Specifically, whether oils/fats with low oxidative potential (e.g. olive, avocado, lard, etc.) have different effects on cardiometabolic surrogate endpoints compared to oils with high oxidative potential (e.g. high PUFA oils such as soybean, corn, etc). Chronic studies are needed in this regard.
2) How different oil processing methods, including duration of thermal treatment, container used, etc., and interacting food matrix and additives modify exposure to oxidized lipids, and related cardiometabolic risk factors.
3) Health effects of exposure to the R/S racemic mixtures typically formed when oils/foods are processed remain unknown.
4) Knowledge on how much oxidized lipids in the diet could be tolerated in humans remains unknown. Thus, more data is needed on exposure to oxidized lipids from thermally processed oils, and how these lipids are handled with age and by individuals with underlying metabolic impairments compared to those without.

Discussion

The evidence suggests that the PUFA content of added fats determines the extent of their oxidation during thermal processing. Oils high in PUFAs are more susceptible to heat-induced oxidation compared to low PUFA oils. Therefore, to reduce dietary exposure to oxidized lipids, there is strong evidence to suggest that low PUFA oils such as olive oil or high-monounsaturated fatty acid algae oils could be used for cooking and processing, instead of high PUFA oils such as soybean, corn, safflower and sunflower.

There are other low-PUFA oil alternatives such as beef tallow, butter, coconut oil, avocado oil and others, but these have not been studied in terms of lipid oxidation species that are produced when they are processed. Future studies should investigate the oxidative stability of these oils during thermal and other types of processing. Additionally, studies should differentiate between the oxidative stability of animal versus plant low-PUFA fats, because animal sources are likely to contribute oxidized cholesterol when processed. Plant sources are likely to contribute oxysterols. Dietary

exposure to oxidized cholesterol and oxidized sterols, as well as related health impacts need further investigation.

Lastly, with oils, studies are needed to understand their interaction with food matrices (carbohydrates, proteins, and other micronutrients such as iron) and food additives (e.g. emulsifiers) during processing, as well as the impact of exposure from the R and S forms on metabolic health. At present, it is not known how exposure to the R/S racemic mixture differs from the forms made naturally in the body via enzymes (primarily S).

There is strong evidence in humans showing that oxylipins and secondary compounds (aldehydes) are bioavailable. In agreement with observational evidence, interventional studies suggest that acute and chronic exposure to thermally processed oils or foods impairs surrogate cardiometabolic endpoints. Future chronic intervention studies are needed to compare the effects of different thermally stressed oils/fats provided at clinically relevant exposure levels on cardiometabolic endpoints to better guide the specific oil types that could be used for various processing applications including in-home cooking, restaurants or industry.

Conclusion

Dietary consumption of oxidized lipids from oils ranges between 1 to 500 mg per person per day, depending on processing methods. Added fats high in polyunsaturated fatty acids (PUFAs) are more susceptible to thermally induced lipid oxidation compared to oils low in PUFAs. Oxidized fats are bioavailable in humans (high certainty) and contribute to circulating oxidized lipid levels (high certainty). Thus, to reduce exposure to oxidized fats in the diet, it is recommended that low PUFA oils such as olive oil or high monounsaturated algae oils be used for cooking and processing. Other low PUFA oils including butter, beef tallow, coconut oil and avocado oil are of interest, but studies are needed to better understand their oxidative stability and contribution to oxidized lipid formation when processed in foods. Observational evidence has shown a link between the consumption of fried foods and cardiometabolic disease (low certainty), and this is supported by a few intervention studies which showed that exposure to thermally stressed oils or fried foods impairs cardiometabolic surrogate markers, particularly in individuals with pre-existing metabolic impairments (medium certainty based on the limited number of studies). Future intervention studies are needed to capture the long-term (>4 weeks) effects of oxidized lipids from thermally stressed oils on cardiometabolic and other health outcomes, particularly in relation to oil type (low vs high-PUFA oils), processing methods, dose and duration of human exposure and underlying cardiometabolic status.

Table 1: Search terms used to derive intervention studies that investigated the effects of oxidized lipids on cardiometabolic outcomes.

Exposure from seed or vegetable oils	Main outcomes
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in seed oils	Mortality
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in vegetable oils	Cardiometabolic
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in plant oils	Cardiovascular disease
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in soybean oil	Dyslipidemia
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in Corn oil	Pre-diabetes
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in Safflower oil	Diabetes
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in Sunflower oil	Insulin Resistance
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in Canola oil	Biomarkers (LDL, Cholesterol, Triglycerides, Glucose, insulin)
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in Olive oil	Pathway biomarkers (Inflammation, oxidative stress)
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes Beef tallow	Obesity
Oxidized fatty acids / oxidized lipids or fats /oxylipins / aldehydes in Butter	Hypertension
French fries / fried / frying	body weight
lipid hydroperoxides / linoleate hydroperoxide / lipid peroxides	BMI

Table 2: Intervention studies on the effects of thermally stressed oils or fried food consumption on cardiometabolic surrogate endpoints in humans

Study	Sample size and cohort Inclusion/exclusion criteria	Sex (age)	Randomized?	Fasted?	Intervention	Formulations and doses provided	Measurements taken	Outcomes
Strapans 1994 [46]	Cross over design with 3 test oils with a 14 day washout period in between. n=6 for test oils (Exp 1) and n=4 for time-course of oxidized lipid clearance (Exp 2).	Healthy males (24-45 yrs)	Not stated	Yes (12 hours)	Control oil: corn oil containing 0.14 mg/g oil of V-E , 6.5-10 nmol conjugated dienes / mg oil, 0 nmol TBARS/mg oil, and 62.45% LA Medium oxidized oil[1]: Added 0.14 mg/g oil of V-E and contained 30-50 nmol conjugated dienes/mg oil, 0.041 nmol TBARS/mg oil[1] and 59.86% LA Highly oxidized oil[1]: Added 0.14 mg/g oil of V-E and contained 80-120 nmol conjugated dienes/mg oil, 0.103 nmol TBARS/mg oil and 57.1% LA	1 g/kg of corn oil with 100 g of carbohydrate in the form of 3 to 4 slices of white bread	Exp 1 : Collected blood at baseline and after 4 hours. Measured serum conjugated dienes in triglycerides in chylomicron fraction and LA (n=6 per test oil). Exp 2: For time-course of oxidized lipid clearance, control and highly oxidized oil were administered and blood collected at baseline and every 2 hours for 8 hours for conjugated diene measurements. Also measured lag time to copper oxide induced oxidation.	Exp 1 (n=6; cross-over design): Serum LA ↓ from 56% (control) to 54% (medium oxidized) and 41% (highly oxidized). Conjugated dienes in chylomicrons ↑ from 9.7 nmol/µmol TAG (control) to 21.9 nmol/µmol TAG (medium oxidized) and 46 nmol/µmol TAG (highly oxidized) TBARS in chylomicrons only detected in highly oxidized oil group at 0.14 nmol/µmol TAG Exp 2 (n=4): Conjugated dienes increased to ~110 nmol/µmol TAG in the highly oxidized group by 6 hours, and started to decrease by 8 hours (to ~50 nmol/µmol TAG). It was ~20 nmol/µmol TAG in the control group.

Study	Sample size and cohort Inclusion/exclusion criteria	Sex (age)	Randomized?	Fasted?	Intervention	Formulations and doses provided	Measurements taken	Outcomes
								Lag time to copper oxide induced oxidation of serum from subjects fed the highly oxidized oil versus controls, was shortened from 4.3 to 3.2 hours (suggests oxidized chylomicrons further susceptible to lipid oxidation independent of LA content)
Strapans et al., 1999 [47]	31 diabetics including 22 HBA1>10% and 9 with HBA1>7.7%, and 24 controls matched for sex and serum triglycerides and cholesterol	Males (52-64 yrs)	Not stated	Yes (12 hours)	11 controls were given a 'low oxidized' lipid-based diet 13 controls were given a 'high oxidized' lipid-based diet 4 diabetics with good glycemic control were given the 'low oxidized' lipidbased diet 5 diabetics with good glycemic control were given the 'high oxidized' lipid-based diet 8 diabetics with poor glycemic control were given the 'low oxidized' lipid-based diet 14 diabetics with poor glycemic control were given the 'high oxidized' lipid-based diet	Corn oil was oxidized for 1 to 3 days to generate 1) low oxidized lipid diet containing 40-99 umol of conjugated dienes / mmol TAG, and 2) high oxidized lipid diet containing 100-200 umol of conjugated diene/mmol TAG Participants received 1 ml /kg body weight of low or high oxidized lipids from tocopherol depleted corn oil, mixed with 100 g of potatoes.		Dienes in chylomicrons increased in diabetics with poor glycemic control receiving a low or high oxidized corn oil diet Increases were higher in those who received the high oxidized corn oil diet relative to low. Changes persisted after correcting for TAGs.

Study	Sample size and cohort Inclusion/exclusion criteria	Sex (age)	Randomized?	Fasted?	Intervention	Formulations and doses provided	Measurements taken	Outcomes
Naruszewicz et al., 1987 [48]	Cross—over design on n=5	Males (3 normolipidemic and 2 hypotriglyceridemic; 25-38 yrs)	Not stated	Yes (unspecified period)	Control oil: 100 g fresh soybean oil (1.6 mEq/kg peroxide value and 7.8 mEq/kg carbonyl value) Oxidized oil: 100 g heated soybean oil for 7 h at 220°C (4.8 mEq/kg peroxide value and 35.6 mEq/kg carbonyl value)		Collected blood at baseline and 4 hours later	Exp 1 (or A) : TBARS in plasma increased by 1.4 to 7 fold relative to baseline across the 5 subjects after consuming heated sobean oil; this increase was not seen after consuming non-heated oil (expressed as µmol MDA/l)* Exp 2 (or B): Repeat of Exp 1 in three normolipidemic subjects, showed similar increase 4.2 to 7 fold versus baseline after consumption of heated soybean oil. Fold change appeared higher in the normolipidemic subjects (2.6 to 7 fold) compared to hyperlipidemic subjects (1.4 to 2.2 fold). No stats done due to sample size.
Ferreiro-Vera et al., 2013 [53]	Cross-over design on 26 obese (non-diabetic) individuals with BMI of 30-04 kg/m^2. Excluded individuals with kidney, pancreas,	17 post-menopausal women (48-70 yrs) and 9 men (39-70 yrs)	Yes	Not stated	4 groups given muffin breakfast containing four different oils heated at 180 °C for 5 min, 10 times a day for 2 days with 30 min cooling intervals. Muffins given every 2 weeks for 8 weeks (cross-over design):	0.45 mL oil/kg	Serum oxylipins at baseline and and 4 hours post-prandially.	9- and 13-HODE significantly increased from baseline in individuals receiving fried sunflower oil (Group 1), but not other fried oils contaning added or

Study	Sample size and cohort Inclusion/exclusion criteria	Sex (age)	Randomized?	Fasted?	Intervention	Formulations and doses provided	Measurements taken	Outcomes
	lung, liver or thyroid disease.				Group 1 (control): Sunflower oil Group 2: Refined high oleic-sunflower oil with 400 mg/L dimethylsiloxane Group 3: Refined high oleic-sunflower oil with 400 mg/L added polyphenols. Group 4: Extra-virgin olive oil containing 400 mg/L polyphenols.			natural antioxidants(Groups 2, 3 and 4). PGD2 significantly increased from baseline after fried sunflower oil intake (Group 1), and tended to increase in the other groups as well (Groups 2, 3 and 4). PGE3 decreased from baseline in all groups, but the reduction was greatest (and significant) after fried olive oil intake (Group 4).
Farnetti et al., 2011 [56]	12 obese insulin-resistant non-diabetic women (BMI 32.8) + 5 healthy lean women (BMI 22.2) *Study was not a cross-over design. Meals were given 1 week apart.	Females: Obese 41 yrs); lean (43 yrs)	Yes	Yes (unspecified period)	Control: 60g of boiled pasta, 150 g of courgettes (zucchini) gilled for 4 min, 150 g of apple and 35 g of extra-virgin olive oil Experimental: 60g of pasta stir fried in 10 g of extra-virgin olive oil for 3 min, 150 g of courgettes (zucchini) deep-fried in extra-virgin olive oil for unspecified amount of time (15 g of oil was retained in the courgettes post frying), and 150 g of apple.	Meals administered 1 week apart, per subject.	Plasma post-prandial glucose, insulin and C-peptide response (baseline and every 30 min over a 3 hour period). TAGs measured at baseline and after 3 hours post- prandially.	In obese women, feeding a meal prepared with fried extra-virgin oil reduced post-prandial insulin and c-peptide response. A non-statistical trend towards lower post-prandial TAGs was observed in obese women. No significant changes were observed in post-prandial responses in lean women.

Study	Sample size and cohort Inclusion/exclusion criteria	Sex (age)	Randomized?	Fasted?	Intervention	Formulations and doses provided	Measurements taken	Outcomes
Gao et al, 2021 [57]	N=65 per arm (130 total); final sample size was 58 controls, 59 intervention. Healthy overweight adults with BMI>24 kg/m^2 Excluded: individuals taking pro/pre/antibiotics within 3 months of enrollment, with diabetes, dyslipidemia or GI disease, received surgery within 3 months, exercised frequently or took protein supplements, had smoking or drinking habits.	Mixed (18-35 yrs). Included 53.45-55.93 % females	Yes	Yes (12 hours)	4 week dietary intervention consisting of: Control: meat cooked with boiling, steaming or dressing with sauce at 100°C (unspecified amount of time) Experimental: Frying meat at 150°C for <3 min (oil used not specified)	Meats were provided 4x a week	Specified primary outcomes: Serum glucose, oral glucose tolerance test, fecal microbiota Specified secondary outcomes: LPB, sCD14, adiponectin, FGF21, cytokines Other outcomes: BMI, protein digestibility advanced glycation products, amongst others listed in the article	After intervention, participants in the group consuming fried meats had higher BMI and advanced glycation products in serum. Calculated Protein digestibility was lower in individuals who consumed fried meats versus controls. Insulinogenic index was higher in the group consuming fried meats than controls whereas muscle insulin response index was higher. Serum inflammatory biomarkers (LPS, LBP/sCD14, TNF-a, IL-1β, and IL-10) were higher after fried meat consumption, whereas FGF21 were lower. Gut microbiota richness was lower in the fried meat group compared to controls. Compared to the control group, fecal concentrations of

Study	Sample size and cohort Inclusion/exclusion criteria	Sex (age)	Randomized?	Fasted?	Intervention	Formulations and doses provided	Measurements taken	Outcomes
								carnitine and methylglutaric acid were higher, whereas valeric acid, butyric acid, and indolepropionic acid were lower in the fried meat group

[1]Achieved by storing in air for unspecified amounts of time.

*It is not clear if the TBARS data, which measure malonaldehydes, were measured in whole plasma or in chylomicrons. The article says TBARS was measured in both. Table 1 shows TBARS units in µmol MDA/L, suggesting these are whole plasma measurements, not measurements in chylomicrons.

Abbreviations in table: BMI, body mass index; FGF21, fibroblast growth factor 21 (FGF21); IL, Interleukin; LA, linoleic acid; LPS, lipopolysaccharide; LPB, LPS binding protein; MDA, Malonaldehyde; sCD14, soluble LPS receptor CD14; TBARS, Thiobarbituric Acid Reactive Substances; TAGs, triacylglycerols; TNF-a, Tumor Necrosis Factor-Alpha.

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Appendix 4.9. High-Quality, Nutrient-Dense Protein Foods

PRIORITIZE HIGH QUALITY, NUTRIENT DENSE PROTEIN FOODS AS PART OF A HEALTHY DIETARY PATTERN

Rapid Systematic Review (for Weight Management)
&
Narrative Review (for Nutrient Adequacy)

Donald Layman, PhD, FASN
Professor Emeritus
Department of Food Science and Human Nutrition
University of Illinois at Urbana-Champaign

Heather Leidy, PhD, FASN
Associate Professor
Department of Nutritional Sciences
Department of Pediatrics – Dell Medical School
University of Texas at Austin

Abstract

Objectives: The purpose of this report was to evaluate the experimental evidence supporting daily protein intakes between 1.2-1.6 g protein/kg body weight for improved weight management (WM) in adults and nutrient adequacy (NA) across most life stages.

Methods: Given the time constraints, a rapid systematic review was completed for WM, utilizing the Cochrane Rapid Review Methods Guidance, whereas a narrative review was completed for NA. The population of interest was adults (ages 19+ years) for WM and children through older adults (ages 2+ y) for NA. For both reviews, the intervention/exposure was higher-protein diets, containing ≥1.2 g pro/kg body weight; ≥ 20% of daily energy as protein; and/or protein foods. The comparator included protein intakes between 0.8-1.1 g protein/kg of body weight; between 10-18% of daily energy as protein; and/or the removal/elimination of a protein food from the diet. Studies were excluded if the exposure/intervention had <130 g carbohydrates; were not matched in energy to the comparator (control); were isolated amino acid or protein supplement studies; had dietary fat intake >35% of daily energy; or had protein intakes similar to the comparator (i.e., intakes <5% energy (as protein) differential). Energy restriction and energy balance trials were all included. For WM, studies of ≥12 weeks in duration were included. Comparisons between animal and plant source protein foods were also included when possible. Primary outcomes for WM were body weight, fat mass, and lean/fat-free mass and the prevalence of nutrient (in)adequacy and specific quantities of nutrients for NA. The respective literature searches were performed; the evidence was synthesized and evaluated; and recommendations developed when appropriate.

Results: The strength of evidence that protein intakes between 1.2 – 1.6 g protein/kg body weight improve WM in adults was moderate to strong and was strong for NA across most life stages.

Conclusions: The evidence supports a recommended healthy range of dietary protein as 1.2-1.6 g protein/kg body weight for health promotion and disease prevention which can be accomplished by prioritizing high quality, nutrient and protein dense unprocessed or minimally processed animal and plant protein foods, including red meat.

RELEVANCE & GOALS

The purpose of this report is to highlight the importance of prioritizing high quality, nutrient dense protein foods when creating dietary guidelines for lifelong health. Protein is arguably the most essential of all nutrients.

As a result, the Dietary Reference Intakes created by the Institute of Medicine include a minimum requirement of 0.8 g protein/kg body weight per day and a safe and acceptable range for dietary protein of 10% to 35% of daily calories. However, for the past 20 years, the Dietary Guidelines for Americans (DGAs) have failed to incorporate the entire range of protein and instead have modelled and recommended dietary patterns at the lower end with little to no experimental evidence to support this approach. Since 2010, the DGAs have further eroded the quality of the diet with recommendations to shift towards eating a plant-based dietary pattern through the reduction and/or replacement of high quality animal source protein foods with plant sources based on goals to reduce saturated fat and increase fiber intake while ignoring nutrient density for essential amino acids or micronutrients. To further compound this, the "protein ounce equivalents" tool was developed in 2005 to encourage the exchange of animal source protein foods with plant sources. Although protein ounce equivalents continue to be utilized within the guidelines, it has been repeatedly documented as not equivalent in total protein, essential amino acids, or energy and not based on scientific evidence.

Collectively, the fallacies with the previous recommendations have the potential to reduce protein density and quality within the diet, encourage carbohydrate amounts that far exceed requirements, and increase the difficulty in establishing appropriate calorie levels. Unfortunately, the DGAs have failed to improve the health of Americans with many adults living with obesity and other chronic diseases, including type 2 diabetes and cardiovascular disease. The DGAs have the opportunity to recognize that healthy dietary patterns come in many forms to provide Americans with the flexibility to consume healthy foods they enjoy, including high quality protein foods from a combination of animal and plant sources. Further, consumer-facing dietary guidelines are needed and should prioritize dietary protein to provide practical recommendations for personalized nutrition and health.

Importantly, one of the most significant roles of dietary protein is providing the essential amino acids for health, growth, and maintenance. For adults, achieving and maintaining a healthy weight, including optimal body composition, is of critical importance for health promotion and disease prevention. The extensive published research on weight management is used in this report to provide experimental evidence for prioritizing dietary protein for adult health. The current report prioritizes weight management evidence exclusively from randomized controlled trials to inform food-based recommendations within the dietary guidelines. This report also summarizes the existing evidence to examine the contribution of high quality, animal and plant source protein foods on nutrient adequacy.

Introduction

Protein foods provide the essential amino acids (EAAs) required to support all life-sustaining structures and functions of the body. Because of their diverse and unique roles, amino acids are perhaps the most essential of all nutrients requiring consistent daily supply from high quality protein foods and in proportion to lean body mass.

One of the most obvious roles of amino acids is in development and maintenance of muscles and bones for functional mobility and a body composition consistent with cardiometabolic health. Achieving and maintaining a healthy weight, including optimal body composition, is of critical importance for health promotion and disease prevention. Yet, over 74% of U.S. adults and 35% of young people are living with overweight/obesity [1, 2].

A substantial body of evidence from randomized controlled trials (RCTs) exists supporting the unique role of high quality, nutrient dense dietary protein as part of a healthy dietary pattern consistent with weight management to achieve and/or maintain a healthy body weight and body composition. However, previous dietary guideline committees have failed to incorporate this evidence into dietary recommendations. Thus, the purpose of this report is to critically and systematically evaluate the weight management evidence exclusively from RCTs to inform food-based recommendations within the Dietary Guidelines for Americans (DGAs).

Although protein foods are primarily recognized for specific protein and EAAs contents, they are also the most nutrient dense foods. They contain many of the at-risk vitamins and minerals of concern for underconsumption either for the entire population or certain life stages, including those nutrients not readily found in any of the other food groups (e.g., heme iron, vit B12, vit D). Thus, protein foods are essential, calorie-efficient, and enjoyed by many Americans as part of our cultural eating habits. This report will also summarize the existing evidence to examine the contribution of high quality, animal and plant source protein foods on nutrient adequacy.

The DGAs recommend a variety of animal source protein foods (ASPFs) and plant source protein foods (PSPFs) to provide enough total protein to satisfy the minimum requirements set at the Recommended Dietary Allowance (RDA) of 0.8 g/kg body weight for adults and to ensure the dietary patterns meet most nutrient needs [3, 4]. However, over the past 20 years, an extensive body of research has underscored the unique and diverse metabolic roles of protein, and now there is compelling evidence that consuming *additional* foods that provide protein at quantities *above the RDA* may be a key dietary strategy to combat obesity in the U.S *(while staying within calorie limits by reducing nutrient-poor carbohydrate foods)*.

Instead of incorporating this approach, the past iterations of the DGAs have eroded daily protein quantity by shifting protein recommendations to PSPFs, including beans, peas, and lentils, while reducing and/or de-emphasizing intakes of ASPFs, including meats, poultry, and eggs. The shift towards PSPFs was intended to reduce adiposity and risks of chronic diseases but was primarily informed by epidemiological evidence on

dietary patterns, even in some cases when experimental evidence from randomized controlled trials (RCTs) was available to more specifically inform this recommendation. Another key aspect that DGA committees have inadequately considered are the nutrient consequences when shifting from ASPFs to PSPFs. ASPFs not only provide EAAs, they also provide a substantial amount of highly bioavailable essential micronutrients that are under-consumed. Encouraging Americans to move away from these foods may further compromise the nutrient inadequacies already impacting many in the U.S., especially our young people.

Compounding this is the recent evidence highlighting the fallacies of using the unsubstantiated concept of protein ounce equivalents within food pattern (substitution) modeling, leading to recommended reductions in daily protein intakes and protein quality since ASPFs and PSPFs are not equivalent in terms of total protein or EAA density. Given that 1) there is no Tolerable Upper Intake Level (UL) for dietary protein established by the Dietary Reference Intakes (DRIs) and 2) consuming high quality ASPFs above current recommendations has shown no negative health risks in high quality RCTs, it's unclear as to why previous DGAs encouraged shifts in protein intake towards limiting high quality, nutrient dense ASPFs. It's essential to evaluate the evidence to establish a healthy range of protein intake and to substantiate whether or not limiting ASPFs is warranted and/or has unintended consequences.

An alternative approach that may be more strongly supported by the totality of evidence is the replacement of refined grains with PSPFs like beans, peas, and lentils. Given their nutrient dense profile (e.g., excellent source of fiber, complex carbohydrates, & folate, etc.; good source of protein) nutrient dense PSPFs *complement* but do not replace the nutrients provided in ASPFs (i.e., excellent source of protein, vit B12, zinc, good source of heme iron, etc.). By including high quality, nutrient dense ASPFs as the primary source of protein, followed by nutrient dense PSPFs as a replacement for nutrient-poor refined grains, a higher-protein, lower-carbohydrate dietary pattern can be achieved which likely improves nutrient adequacy, weight management, and overall health.

The most investigated outcomes related to higher-protein, lower-carbohydrate dietary patterns include optimizing skeletal muscle strength and mass, weight loss, and body composition. Weight loss in general, and more specifically the relative percentage of body fat to lean tissues, are major predictors of long-term health, affecting functional mobility and cardiometabolic function. Since 2000, there have been over 500 RCTs exploring the impact of increased dietary protein for health, most of which were focused on obesity prevention and/or treatment. Unanimously, these studies found no risk of adverse outcomes and most illustrate benefits of increased dietary protein for weight management. It's also important to note that the majority of these studies increase dietary protein through the inclusion of additional, high quality ASPFs.

In response to the growing evidence and public interest surrounding 'low carb', 'high protein', and 'ketogenic' diets, the 2020 Dietary Guidelines Advisory Committee (DGAC)

sought to examine the relationship between dietary patterns that vary in macronutrient distribution and health - with higher-protein diets being one of the patterns [5]. The committee concluded that an evidence grade was 'Unassignable' due to methodological limitations and inconsistent results. However, the protocol and inclusion criteria developed by the 2020 DGAC may have contributed to the inconsistencies and lack of available evidence. Specifically, the protocol included only energy-balance trials where at least one macronutrient needed to be *outside* of the respective Acceptable Macronutrient Distribution Range (AMDR). Thus, all energy-restriction trials were excluded as well as trials that varied protein and carbohydrate content *within* the AMDRs. Given that the majority of U.S. adults have overweight/obesity, including energy restriction trials is both appropriate and perhaps should be the primary goal. This is supported by the inclusion of energy restriction studies within the 2025 DGAC [6]. Further, since there is a fundamental need to meet all nutrient requirements for health and well-being, it's unclear as to why the 2020 DGAC protocol excluded studies that met nutrient requirements but provided flexibility *within* the AMDRs.

An initial, critical review of the current literature identified 18 systematic reviews and meta-analyses (SRMAs) on the topic of increased dietary protein and obesity (**Appendix A**). Of these, 16/18 (89%) reported improved weight management (i.e., greater weight loss, greater fat loss, less weight re-gain, &/or greater fat-free/lean mass preservation) following diets that included protein >0.8 g/kg body weight per day. In addition, cardiometabolic risk factor outcomes were evaluated in some studies and benefits, such as improved glucose regulation, blood pressure, and reduced blood triglycerides, were reported in some, but not all, analyses with increased dietary protein. Collectively, the SMRAs provide support for the recommendation to consume protein above 0.8 g/kg body weight per day to promote weight management in adults. However, prior evidence synthesis has yet to provide clarity as to what quantity of dietary protein and thus quantity of protein foods is needed to elicit this response. Furthermore, because of the physiological and behavioral differences among adults, a specific protein intake is likely not a single value but a range that accommodates diverse individuals with varying energy needs, health status, weight management goals, dietary preferences, and dietary conditions. Thus, we developed the following questions:

List of Questions

What is the healthy range of dietary protein:

a) **for weight management?**
b) **for nutrient adequacy?**

Scope & Considerations

The overarching goal of this report is to establish a healthy range of protein achieved with food-based recommendations. It is not designed, in any way, to evaluate or propose changes to the DRI (nutrient-based) protein requirements.

Given the diversity in nutrient density, protein quality, and protein quantity of foods within the protein food group and across other food group categories, it would be impossible to systematically explore this question from a 'protein food' approach. As an initial step, the DRIs for protein were incorporated into the analytical framework. Since dietary protein has no UL, only the RDA was used to establish the minimum nutrient protein intake. Next, we examined whether consuming protein above the RDA is beneficial for health promotion and disease prevention through weight management and nutrient adequacy outcomes. While previous food pattern modeling within the DGAs have included a range of protein intakes above the RDA, the protein amounts were not informed by systematic review of health outcomes or scientific evidence. Thus, the questions within this report were created to establish an evidence-based healthy range of protein to promote weight management and nutrient adequacy and translate this range into protein food-based recommendations.

Related to this topic is the designation of "higher" vs. "lower" intake. In general, these qualifiers refer to amounts that are above or below the current DRI or DGA recommendations and not referring to habitual consumption in the U.S.

From the onset of the first DGA in 1980, nearly every DGA thereafter included a key statement of 'achieving and/or maintaining a healthy weight' with most including strategies that establish healthy weight, promote weight loss, and/or prevent unhealthy weight (re)gain when appropriate. Weight management is generally defined as ability to maintain a healthy weight (and body composition) through long-term lifestyle and behavioral strategies, including a healthy dietary pattern. Since most Americans experience overweight/obesity and associated chronic diseases, it's critical that current recommendations include strategies to achieve and maintain a healthy weight (and body composition) across all life stages which will improve health, well-being, and decrease the risk of chronic diseases.

Previous DGAC committees relied heavily on epidemiological, observational evidence where individual food groups are difficult, if not impossible, to disentangle from the overall dietary and lifestyle patterns. The committees built dietary models based on consumption data and these observational studies to simulate dietary patterns that could potentially achieve nutrient goals. Alternately, this report focuses on evaluating the evidence available from RCTs that tightly controlled daily food intake and provided more specific information about the healthy range of protein and protein foods that promote weight management, including healthy weight and body composition, and nutrient adequacy.

Protein ounce equivalents (oz-eq) is a consumer translation tool introduced in the 2005 DGA as a way to standardize protein units across protein foods and has been used

continuously since then. The report highlights how the current protein oz-eq tool mischaracterizes protein nutrition and recommends a more valid version for future menu modeling or dietary pattern development. For the current report, the Reference Amounts Customarily Consumed (RACCs) were incorporated as an alternative approach. RACCs are established from consumption data, represent common serving sizes, and are included on all food labels. Although RACCs are not evidenced-based, recommended quantities, they can be incorporated into food pattern modeling and dietary patterns to convey recommended amounts for consumers (i.e., 1 serving of x, 2 ½ servings of y, etc.).

The phrase 'healthy range of protein' will be incorporated throughout this report with the intent to identify a range of protein intake that can be translated into a 'healthy, higher-protein dietary pattern' and recommended for health promotion and disease prevention. Aligning with the characteristics of the 'healthy' terminology within the past DGAs, the 'healthy range of protein' includes nutrient (and protein)-dense forms of foods and beverages, while staying within calorie limits.

Methods

The first step in examining the scientific evidence on increased dietary protein and weight management was to establish a definition of higher dietary protein. For this analysis, higher dietary protein is a diet containing:

- ≥ 1.2 g pro/kg body weight
- 20-35% of daily energy as protein.

The criteria were set at these levels since the DGA dietary patterns are designed to meet the minimum protein requirements (of 0.8 g pro/kg body weight per day) and contain 15-18% of daily intake as protein.

We developed a protocol to complete a rapid systematic review (**Appendix B**) that included an analytical framework and inclusion and exclusion criteria to guide identification of the most relevant and appropriate RCTs to use in answering the question. To clarify, the analytical framework outlined core elements of the rapid systematic review. The inclusion and exclusion criteria were selected before the literature review to operationalize the elements of the analytical framework, and specify what made a study relevant for the systematic review question.

A literature search was conducted to identify all potentially relevant articles, and those articles were screened based on the criteria selected in the protocol. For each included article, data were extracted and risk of bias assessed. The body of evidence was synthesized to answer the question and grade the strength of evidence using pre-established criteria for risk of bias, consistency, directness, precision, and generalizability. Finally, recommendations were developed.

For this rapid systematic review, the population of interest was adults (ages 19+ years). The intervention was higher-protein diets, containing ≥1.2 g pro/kg body weight or ≥20% of daily energy as protein. The comparator included protein intakes between 0.8-1.1 g pro/kg of body weight or between 10-18% of daily energy as protein. Studies were excluded if the intervention had <130 g carbohydrates; were not matched in energy to the comparator (control); were isolated amino acid or protein supplement studies; had dietary fat intake >35% of daily energy; or had protein intakes similar to the comparator (i.e., intakes <5% differential). Studies of at least 12 weeks in duration were included. Energy restriction and energy balance trials were all included.

Primary outcomes included measures of: body weight, fat mass, and lean/fat-free mass. Secondary outcomes included distribution of fat mass, BMI, and waist circumference.

When establishing inclusion and exclusion criteria, the standard criteria for publication status, language of publication, country, and study participants were utilized. Participants were included if they were healthy or had overweight/obesity, diabetes, and/or cardiovascular disease risks but were excluded if they were taking weight loss medication or had bariatric surgery previously. Studies were included if they were published any time after 1950.

The first step in examining the scientific evidence on higher dietary protein and nutrient adequacy was to establish a definition of higher dietary protein. For this analysis, higher dietary protein is a diet containing:

- ≥ 1.2 g pro/kg body weight
- 20-30% of daily energy as protein.

In addition, protein foods were defined as any food categorized as a protein food within the DGA/USDA Protein Food group. These include protein subcategories of animal-protein source foods (ASPF: meats/poultry/eggs and seafood) and PSPFs (PSPF: nuts, seeds, & soy products and (currently proposed) beans, peas, and lentils). Dairy was also included within these analyses since many dairy foods (e.g., Greek yogurt, milk, cottage cheese) are higher in protein.

No standardized definitions of high quality, nutrient density, protein density, or protein quality exist as it related to protein foods. However, since these are important characteristics that shape food-based recommendations, they are defined in the following manner:

> *Protein density: The amount of protein relative to the total calories of the food. A food in which ≥40% of the calories are from protein is considered a (higher) protein-dense food.*
>
> *Protein quality: This refers to the capacity of a food to meet the EAA requirements (and is based on the EAA composition and bioavailability). A food in which ≥40% of the total protein is comprised of EAAs is considered a (higher) protein-quality food.*
>
> *High quality protein food: A food that has higher protein density and higher protein quality.*
>
> *Nutrient dense protein food: A food that is within the USDA Protein Foods or Dairy Foods categories and contains protein in addition to other micronutrients and/or fiber at a level that qualifies that component (beyond protein) to be a "good source" (10-19% of the Daily Value) or "excellent source" (20% or more of the Daily Value) while being calorie efficient (i.e., providing significant nutrients with fewer calories).*
>
> *Animal source protein foods (ASPFs): Foods that are derived from animal products and include meat, poultry, eggs; seafood; and dairy products. All ASPFs that are unprocessed or minimally processed are generally considered high quality, nutrient dense foods due to their high protein, high EAA, and micronutrient content.*
>
> *Plant source protein foods (PSPFs): Foods that are derived from plants and include pulses (beans, peas, lentils), legumes, nuts, seeds, and soy. All PSPFs that are unprocessed or minimally processed are generally considered nutrient dense due to their micronutrient and fiber content. Although these foods contain protein, the protein density and quality of most of these foods are generally not at*

the level that signifies a 'higher quality protein food.' The exception includes (some) soy products.

Minimally processed ASPF or PSPF: Protein foods that have not been modified to contain added fats, oils, sugars , breading, sodium preservatives, etc.

Given the lack of RCTs to answer this question, a Narrative Review was completed.

A literature search was conducted to identify all potentially relevant articles, and those articles were screened based on pre-specified criteria. The body of evidence was synthesized to answer the question and recommendations for future research were developed.

For this narrative review, the population of interest were children and adolescents (ages 2-18 y); adults (ages 19+ y); and older adults (ages 65+ y). The exposure was higher-protein diets, containing ≥1.2 g pro/kg body weight; ≥ 20% of daily energy as protein; or protein foods. The comparator included protein intakes between 0.8-1.1 g pro/kg of body weight and between 10-18% of daily energy as protein; or the removal/elimination of a protein food from the diet. Studies were excluded if the intervention had <130 g carbohydrates; were not matched in energy to the comparator (control); were isolated amino acid or protein supplement studies; had dietary fat intake >35% of daily energy; or had protein intakes similar to the comparator (i.e., intakes <5% differential). Energy restriction and energy balance trials were all included.

Primary outcomes included measures of: nutrient adequacy and specific quantities of nutrients.

When establishing inclusion and exclusion criteria, the standard criteria for publication status, language of publication, country, and study participants were utilized. Participants were included if they were healthy or had overweight/obesity, diabetes, and/or cardiovascular disease risks but were excluded if they were taking weight loss medication or had bariatric surgery previously. Studies were included if they were published any time after 1950. No study duration was included for this question.

Results

For this rapid systematic review, 574 original research articles and 31 systematic reviews & meta-analyses were screened. Of those, 249 were assessed for eligibility and 30 papers were included in the final analyses (See PRISMA Flow Diagram, **Appendix C**).

The following is a summary of the characteristics of the 30 studies [7-36] included within the analysis (For more detail, see Evidence Tables, **Appendix D**):

- 2,042 participants were included
- Protein intakes within the intervention were:
    - Range: 20-35% of daily energy; Avg.: 28±4%
    - Range: 1.2-1.6 g/kg; Avg.: 1.34 ± 0.1 g/kg

    - Range: 86-149 g; Avg.: 114 ± 14 g
- Protein intakes within the Control were:
    - Range: 12-23% of daily energy; Avg.: 17±3%
    - Range: 0.8-1.0 g/kg; Avg.: 0.84±0.1 g/kg
    - Range: 54-103 g; Avg.: 71 ± 10 g
- Carbohydrate intakes within the Intervention were:
    - Range: 37-55% of daily energy; Avg.: 44±4%
    - Range: 130-280 g; Avg.: 183±40 g
- Carbohydrate intakes within the Control were:
    - Range: 48-62% of daily energy; Avg.: 55±4%
    - Range: 160-395 g; Avg.: 230±57 g
- Fat intakes within the Intervention were:
    - Range: 22-33% ; Avg.: 29±2%
- Fat intakes within the Control were:
    - Range: 21-35%; Avg.: 28±3%
- Intervention:
    - Study Durations
        - ✓ Energy Restriction, Range: 10-52 weeks; Avg.: 19±12 weeks
        - ✓ Energy Balance, Range: 4-104 weeks; Avg.: 33± 28 weeks
    - Energy Status:
        - ✓ Energy Restriction (only): 22/30 (73%)
        - ✓ Energy Restriction + Weight Maintenance: 13/30 (43%)
    - Protein Food Types:
        - ✓ >80% protein as animal-protein source foods: 30/30 (100%)
- # studies with the following outcomes:
    - Weight: 30/30 (100%)
    - Body Fat: 25/30 (83%)
    - Fat-free/Lean-mass: 22/30 (73%)
    - BMI: 12/30 (40%)
    - Waist Circumference: 12/30 (40%)

Of the 30 studies included in the analysis, 21 (68%) reported a significant effect of higher vs. lower dietary protein on at least one primary weight management outcome, whereas the remaining 32% did not. Further, 14 (45%) reported a significant effect of higher vs. lower dietary protein on two or more weight management outcomes.

In the studies that imposed a reduction in calories, 14 (63%) reported a significant effect of higher vs. lower dietary protein on at least one weight management outcome. In the studies that imposed energy balance following weight loss, 10 (77%) reported a significant effect of higher vs. lower dietary protein on at least one weight management outcome.

In summarizing each outcome, 11/30 (37%) reported greater weight loss or greater weight loss maintenance following a higher vs. lower protein diet. Concerning changes

in body composition, 16/25 (64%) reported greater reductions in body fat or less body fat re-gain following a higher vs. lower protein diet, whereas 8/22 (37%) reported greater preservation of lean/fat-free mass loss following a higher vs. lower protein diet. BMI and waist circumference were measured in fewer studies and only a small percent (n=3, 17%) and (n=5, 28%), respectively, reported reductions following higher vs. lower protein diet.

In carefully reviewing the quality of each study (based on sample size/power calculations; study duration; quality of measurement (of included outcomes); compliance measures; dropouts; and intervention applicability, the majority of studies (21/30, 70%) were rated as moderate to high quality with high generalizability and low to moderate risk of bias.

Importantly, none of the studies reported better weight management with lower protein diets (≤1.1 g/kg body weight) vs. higher protein diets.

As shown in the Summary of Findings table (**Appendix E**), the GRADE concerning the effects of higher protein consumption on the primary outcomes (i.e., changes in weight, fat mass, and fat free/lean mass) was rated as moderate to high. The overall GRADE concerning the effects of higher protein consumption on secondary outcomes (i.e., changes in BMI and Waist Circumference) was considered low. Some of the considerable strengths of this rapid review was the evidence derived strictly from RCTs. Further, the strengths of the individual studies included the fairly large sample sizes (e.g., n=256) and the implementation of interventions over longer periods of time (e.g., up to 2 y). In addition, most of the trials included tightly controlled feeding designs which provided foods to be consumed throughout the intervention. Lastly, the majority of studies included DXA as the standard for body composition assessments. Some of the significant study limitations include the lack of power analyses and thus risk of Type 2 errors; unreported/unadjusted compliance; higher dropout rates in some studies; within-study inconsistencies of findings across all outcomes; and the potential for publication bias.

To answer the question of a healthy range of protein for nutrient adequacy, we included varying approach strategies.

We first sought to assess evidence supporting the 1.2-1.6 g/kg body weight range discussed in KQ1a. Two studies [15, 33] in the rapid systematic review reported micronutrient intakes. Both studies incorporated increased dietary protein through increased milk consumption in females with overweight/obesity who were below the estimated average requirement (EAR) for calcium. Nutrient adequacy for calcium was met with the inclusion of at least 4 servings of dairy/day within the higher-protein dietary patterns compared to the lower-protein patterns. Vitamins D and A were also higher with the inclusion of additional dairy, highlighting the nutrient density of select protein-rich foods. However, outside of these data, none of the other studies within the rapid systematic review compared the nutrient density of the diets to assess the contribution of protein-rich foods to increasing nutrient adequacy for key nutrients of

concern/underconsumption. Regardless of the inability to answer the question through our previous search of RCTs focused on weight management, a number of survey and modeling studies established the important contribution of protein-dense foods to nutrient adequacy in the U.S.

The most important factors to consider in foods represented within the protein food group are protein density and protein quality. From a serving size comparison (**Table 1**), there is a clear distinction between meat/poultry/seafood; eggs; and PSPFs in terms of energy, total protein, and EAA density. Further, the foods within the plant-protein category are also very distinct.

Table 1: Energy & Macronutrient Composition of Protein Foods within the Protein Food Group using Reference Amounts Customarily Consumed (i.e., Common Serving Sizes, RACCs)

Protein Food	Amount		Calories (kcal)	CHO (g)	FAT (g)	PRO (g)	PRO Density (%)	Total EAA (g)	EAA/ protein (%)
	RACC[40]	(g)							
Animal Protein*									
Chicken Breast	3 oz	85	132	0	3	27	83	12	44
Beef Sirloin	3 oz	85	156	0	5	26	67	11	40
Salmon	3 oz	85	129	0	5	21	65	9	41
Egg	1 egg	50	72	1	5	6	35	3	44
Plant Protein*									
Tofu	½ cup	124	94	2	6	10	43	4	41
Kidney Beans	½ cup	90	116	21	0	8	27	3	39
Lentils	½ cup	120	114	20	0	9	32	3	36
Split Peas	½ cup	100	117	21	0	8	28	3	37
Peanut Butter	2 tbsp	32	188	7	16	8	17	2	26
Almonds	1 oz	28	162	6	14	6	15	2	30
Sunflower Seeds	1 oz	28	174	6	16	5	11	2	38

Modified from: Gwin et al., 2021 [37] ; Forester et al., 2025 [38]; and Park et al., 2021 [39]
CHO: carbohydrates; PRO: protein; EAA: essential amino acids; *cooked

As stated previously, to standardize units to assist consumers and health professionals to meet protein requirements using a variety of foods, the DGA published a tool known as “protein ounce equivalents” (**Table 2**). Although this concept has been utilized throughout the iterations of the DGAs, the protein ounce equivalents are not equivalent in energy, total protein, and EAA density [37-39]. In fact, the plant protein foods provide less than ½ the equivalent protein, with the exception of tofu, and 3- to 4-fold less EAAs.

Table 2: Energy & Macronutrient Composition of Protein Foods within the Protein Food Group using USDA Protein Ounce Equivalents

Protein Food	Amount (Oz Eq)	Amount (g)	Calorie (kcal)	CHO (g)	FAT (g)	PRO (g)	PRO DENSITY (%)	Total EAA (g)	EAA/ protein (%)
Animal Protein*									
Chicken Breast	1 oz	28.3	44	0	1	9	82	4	44
Beef Sirloin	1 oz	28.3	52	0	2	9	69	4	40
Salmon	1 oz	28.3	43	0	2	7	65	3	41
Egg	1 egg	50	72	1	5	6	33	3	44
Plant Protein*									
Tofu	2 oz	56.7	43	1	3	5	47	2	41
Kidney Beans	2 oz	56.7	72	13	0	5	28	2	39
Lentils	2 oz	56.7	65	11	0	5	31	2	36
Split Peas	2 oz	56.7	67	12	0	5	30	2	37
Peanut Butter	1 tbsp	16	94	4	8	4	17	1	26
Almonds	½ oz	14.2	82	3	7	3	15	1	30
Sunflower Seeds	½ oz	14.2	88	3	8	2	9	1	38

Modified from: Gwin et al., 2021 [37]; Forester et al., 2025 [38]; and Park et al., 2021 [39]
CHO: carbohydrates; PRO: protein; EAA: essential amino acids; *cooked

To test whether the incorporation of the 'protein ounce equivalents' concept when applied to whole protein foods elicits physiological or metabolic differences, the following studies were completed [39, 41]. In healthy adults, two ounce equivalents of different ASPFs and PSPFs were consumed, on separate days. Dietary EAAs and postprandial plasma EAAs were measured along with whole body net protein balance using stable isotope tracer methodology. As expected, dietary EAAs varied across sources (when matched for protein ounce equivalents) with ASPFs having greater EAAs vs. PSPFs (all, $P<0.05$). These differences also translated into greater postprandial EAA bioavailability following the consumption of ASPFs compared to PSPFs which was observed in both studies (all, $P<0.05$). In addition, as shown in Park, et al. [39], the consumption of protein ounce equivalents of ASPFs elicited greater whole-body net protein balance vs. protein ounce equivalent-matched PSPFs ($P<0.05$). In summary, these data highlight that 'protein ounce equivalents' are not metabolically equivalent. This tool can be revised but must consider total protein and EAA density, including the limiting amino acid, and be corrected for bioavailability to reflect true serving size equivalents.

To assess the magnitude of differences in protein, and more specifically EAA content, density and adequacy when comparing PSPFs vs. ASPFs, dietary patterns varying in protein sources were modeled to include the following: omnivore, vegetarian, vegan energy-matched, and vegan protein-matched [42]. All patterns met total protein and EAA (minimum) requirements. However, all three dietary patterns met EAA requirements only when the diets were protein-matched at 1.3 g/kg body weight which is proposed for healthy aging. But, when matching total protein intake, the vegan protein-matched pattern included 300 additional calories further confounding the interchangeability.

In another modeling study using the Dutch National Food Consumption Survey (DNFCS 2019-2021), protein quantity and bioavailability were assessed within flexitarian, pescatarian, vegetarian and vegan dietary patterns and compared to an omnivorous control diet which contained ~62% of protein from ASPFs [43]. The vegan diet, containing 100% of protein from PSPFs, had a 50% reduction in bioavailable protein compared to the control, whereas the other patterns had a 5% reduction in bioavailable protein. Further, with the simulated vegan diet, over 80% of older adults had protein intakes below the EAR compared to the control diet of only ~9% not meeting requirements. The diets that included some ASPFs (i.e., flexitarian, pescatarian, and vegetarian) had EEA inadequacies ranging from 14-18%.

Using three cycles of NHANES (2013-2018) survey data, diets were assessed for total protein and protein quality based on the percent of PSPFs consumed [44]. As the proportion of PSPFs increased in the diet, the amount of total protein and quality of protein decreased (**Table 3**). This occurred as a result of higher-quality ASPFs being replaced with lower-quality PSPFs. In fact, the top protein source included in the diets containing mostly plant-proteins sources was wheat/grains at almost 50%. This is worth noting since wheat/grains are typically considered to contain the lowest quality protein (i.e., wheat gluten). This study also illustrated that in order to meet the minimum protein requirement (of 0.8 g/kg body weight), 50-75% of the protein in the diet had to come from ASPFs. Further, in order to achieve higher-protein diets (i.e., 1.2 g/kg body weight) proposed in this report, at least 75% of protein intake was needed from ASPFs.

Table 3: Total daily protein intake and protein across defined levels of plant-protein source food intake

	Defined Levels of Plant Protein Intake (% of total protein intake)			
	<25%	≥25% to <50%	≥50% to <75%	≥75%
Corresponding Animal Protein Intake (%)	>75%	≤75% to >50%	≤50% to >25%	≤25%
Protein Quantity				
Total Protein (g)	100 ± 1	80 ± 1*	63 ± 1*	46 ± 3*
Protein (g/kg body weight)	1.2 ± 0.02*	1.00 ± 0.01*	0.81 ± 0.02*	0.62 ± 0.04*
Protein (g/ideal kg body weight)	1.4 ± 0.02*	1.17 ± 0.01*	0.93 ± 0.02*	0.69 ± 0.04*
Protein from Animal (g)	82 ± 1	52 ± 1*	27 ± 1*	7 ± 1*
Protein from Animal (g/kg body weight)	1.0 ± 1.02	0.65 ±0.01*	0.33 ± 0.01*	0.08 ± 0.01*
Protein from Animal (g/ideal kg body weight)	1.2 ± 0.02	0.76 ±0.01*	0.38 ± 0.01*	0.10 ± 0.01*
Protein from Plant (g)	17 ± 1	28 ± 1*	37 ± 1*	39 ± 1*
Protein from Plant (g/kg body weight)	0.22 ± 0.003	0.35 ± 0.003*	0.48 ± 0.01*	0.54 ± 0.03*
Protein from Plant (g/ideal kg body weight)	0.25 ± 0.003	0.41 ± 0.003*	0.54 ± 0.01*	0.59 ± 0.03*
Protein Quality				
Indispensable Amino Acid Score(IAAS)	1.17 ± 0.002	1.10 ± 0.002*	0.98 ± 0.006*	0.86 ± 0.013*
Protein Digestibility Corrected Amino Acid (PDCAAS) Score	0.91 ± 0.001	0.86 ± 0.002*	0.77 ± 0.004*	0.68 ± 0.010*

Modified from (Marinangeli et al., 2023 [44]); data as mean ±SEM; *vs. <25%, P<0.01

Beyond serving as the foundational source of protein and EAAs in the diet, protein foods provide significant quantities of other nutrients (i.e., vitamins and minerals) needed to meet nutrient requirements and promote diet quality [45]. Below is a summary of existing evidence from NHANES data within various life stages comparing nutrient adequacy when specific protein foods are consumed within a dietary pattern (**Table 4**).

These data highlight the important contribution of protein foods to overall nutrient density and improving nutrient adequacy beyond just providing additional protein and EAAs. Further, many ASPFs are considered staple foods in the U.S. diet and serve as ‘carrier foods’ that ‘bring along’ other food groups and their respective nutrient packages to improve overall diet quality. For example, although beef contains a trivial amount of calcium and folate, both are increased in beef consumers, potentially as a result of being consumed with dairy or grain products, etc. Similarly, vit B12 is not found in beans, yet a dietary pattern containing beans is higher in vit B12. This may be due to the ‘carrier food’ concept or the overall pattern being healthier.

Table 4: Nutrient Adequacy within Dietary Patterns of Consumers vs. Non-consumers of Select Protein Foods

Nutrients	Children/Adolescents				Adults							Older Adults	Pregnant/ Lactating Women
	Beef [46, 47]	Pork [48]	Eggs [49]	Dairy [50, 51]	Beef [52, 53]	Pork [48]	Eggs [54]	Dairy [55]	Nuts [56]	Beans [57]	Plant [44]	Beef [58]	Beef [59]
Fiber									+	+	+		
Protein			+	Ø				+		+	+	+	+
Vitamin A	Ø	Ø	+	+	Ø	Ø	+	+	+	Ø	Ø	Ø	Ø
Thiamin	Ø	+			Ø	+				+	+	+	+
Riboflavin	Ø	+		+	Ø	+		+		Ø	Ø	Ø	+
Niacin	Ø	+			+	+				+	Ø	Ø	+
Vitamin B6	+	+	Ø		+	+					Ø	Ø	+
Folate	+	Ø			Ø	+			+	+	+	+	Ø
Vitamin B12	+	+	Ø	+	+	+		+		+	-	+	+
Vitamin C	Ø	Ø	Ø		Ø	+	+		+	+	+	Ø	Ø
Vitamin D	Ø	+	+	+	Ø	+	Ø	+	Ø	+	-	Ø	Ø
Vitamin E	Ø	Ø	+		Ø	+	+		+	+	+	Ø	Ø
Calcium	+	+	Ø	+	Ø	+	+	+	+	+	Ø	+	+
Phosphorus	+	+	+	+	+	+		+		+		+	+
Magnesium	Ø	+	Ø	+	+	Ø	+	+	+	+	+	Ø	Ø
Sodium	Ø	-	-	-	-	-		-	Ø	-	+	-	-
Potassium	+	+	+	+	+	+	+	+	+	+	+	Ø	Ø
Iron	+	+	+		+	+	+		+	+	+	+	+
Zinc	+	+		+	+	+		+	+	+	-	+	+
Copper	+	+			Ø	+					+	Ø	Ø
Selenium	Ø	+	+	Ø	Ø	+		+			-	+	+
Choline	+	+	+	+	+	+	+	+	Ø	+	-	Ø	Ø

The '+' indicates an improvement' '-' indicates a reduction; and 'Ø' indicates no change in nutrient adequacy when comparing consumers vs. non-consumers. A blank indicates the nutrient was not examined. The nutrients of concern or under-consumed in the U.S. are bolded.

Alternately, other studies have modeled the inclusion/exclusion of single protein foods in the American diet based on the NHANES data sets and improvements in overall diet quality and/or nutrient adequacy were identified [60-62]. For example, when adding dairy to meet the 2.5-3 servings/day, the percent of adults with calcium, magnesium, and Vit A inadequacies significantly declined and improvements in Vit D were observed [60]. In another study, adding one or two servings of beans to the typical American dietary pattern resulted in significant increases the intake of dietary fiber, potassium, magnesium, iron, folate, and choline [61]. Alternately, when removing protein-rich foods (e.g., 1 serving (i.e., 3 oz) of meat/poultry)), non-trivial reductions in protein (-23%), iron (-11%), phosphorus (-12%), zinc (-27%), copper (-11%), selenium (-21%), thiamine (10%), niacin (-21%), vit B6 (-15%), vit B12 (-28%), and choline (-22%) were observed [62]. Collectively, these analyses illustrate the contribution of even 1-2 servings of a single protein food to the overall diet nutrient density and highlight the importance of choosing a variety of high quality, nutrient dense protein foods to meet nutrient adequacy.

The previous modeling approaches included the addition or removal of specific foods. It's equally important to understand the nutrient tradeoffs when substituting one food group for another. This is highly relevant given the 2025 DGAC report to reduce and/or replace ASPFs with PSPFs [6]. The report recommended moving pulses from the vegetable food group to the protein food group and prioritizes the listing of these as 'first protein foods to choose from.' Despite this proposed recommendation, the protein and non-protein nutrient tradeoffs were not fully explored and current USDA guidance using Protein Ounce Equivalents misrepresents total protein and EAA density.

Agarwal & Fulgoni [63] modeled the replacement of current protein foods, primarily from animal sources, with PSPFs through the substitution of 6-8 oz/wk of protein foods (including ASPFs and PSPFs) with 1.5-2.0 cups/wk pulses (i.e., beans, peas, and lentils). This group also did a substitution analysis of pulses for refined grains and a combination of protein foods/refined grains substitutions. An a priori cutoff of 10% was set as meaningful change. Overall, replacing ~16-21% of currently recommended protein foods for pulses increased fiber by 10%. Other nutrients, including, but not limited to, protein, zinc, selenium, vit D, niacin, vit B12, and choline were reduced but did not meet the 10% cutoff. On the other hand, replacing ~4-14% of refined grains with pulses increased protein, fiber, iron, magnesium, phosphorus, potassium, zinc, copper, vit E, vit B6, vit B12 beyond 10%. The replacement of protein and refined grain foods with pulses led to moderate improvements which were generally less than the replacement of refined grains alone. This modeling approach doesn't discourage the replacement of some ASPFs with PSPFs but more readily encourages the replacement of refined-grain carbohydrates with PSPFs. However, protein density and protein quality were not considered when exchanging current protein foods with pulses. From a protein perspective and the need to meet the 1.2-1.6 g/kg body weight healthy range, the more appropriate substitution is through replacing refined grains with high quality, nutrient dense ASPFs, like meat/poultry/seafood and pulses, without reducing overall ASPFs.

Every protein source food has a unique nutrient profile with animal source foods providing greater amounts of protein, EAAs, calcium, vit B12, vit B6, niacin, vit D, zinc, and heme iron, whereas plant protein sources provide folate, thiamin, riboflavin, dietary fiber, etc. Given these differences, restricting or eliminating entire subgroups of protein foods (i.e., ASPFs), even if replaced with other subgroups (i.e., PSPFs), will likely create nutrient imbalances or inadequacies.

To date, two systematic reviews have been published examining the macro and micronutrient adequacy of plant-based dietary patterns containing predominately PSPFs compared to ASPFs [64, 65]. In the Bakaloudi et. al, 2021 review, 48 studies (12 cohorts and 36 cross-sectional) of 12,096 individuals following a vegan dietary pattern were included. The authors used Newcastle-Ottawa quality assessment tool to asses study quality and concluded that 44 of the 48 were classified as "good" to "very good"; however, no RCTs were included. Overall, the vegan diets were lower in daily protein intake compared to other diets, and most studies reported intakes below the protein

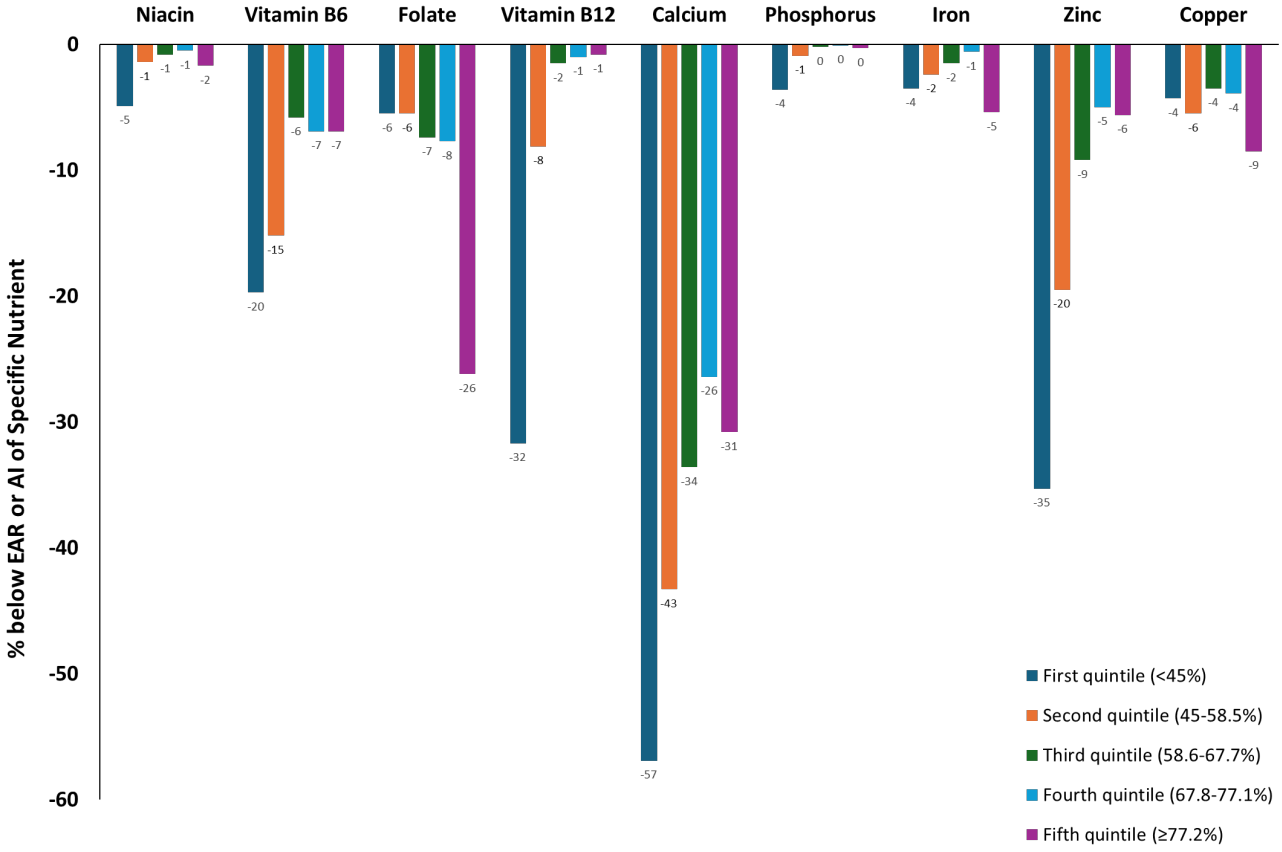
RDA. The reduced protein intake also elicited lower plasma EAAs lysine, methionine, and tryptophan concentrations. Concerning micronutrients, those following a vegan diet displayed lower intakes of riboflavin, niacin, vit B12, vit D, iodine, zinc, calcium, potassium, and selenium compared to other diets – with vit B12 and calcium being below nutrient requirements for most following vegan dietary patterns.

In the second systematic review of 121 studies by Neufingerl N, et al., 2022, comparisons included vegan and vegetarian dietary patterns vs. patterns containing meat [65]. Although protein intake was within the AMDR for all dietary patterns, vegan and vegetarian diets contained ~13% of daily energy as protein vs. diets containing meat (~16% of daily energy). Absolute protein intakes were not reported, but the amount of protein in the plant-based diets was generally be ~0.8 grams/kg body weight per day. Although dietary fiber, folate, vit C, vit E and magnesium intakes were higher in plant-based dietary patterns vs. patterns including meat, intakes and status of vit B12, vit D, iron, zinc, iodine, and calcium were generally lower. Similar to the previous review, those following a vegan diet did not meet nutrient requirements for vit B12, calcium, and iodine.; however, adults eating a dietary pattern containing meat had inadequate intakes of fiber, vit D, vit E, calcium and magnesium. Unfortunately, the study designs and quality of studies were not stated in this review but the evidence table implies that all studies were observational in nature. Collectively, both reviews suggest that nutrient adequacy requires a combination of plant and animal foods but refutes the idea that plant and animal protein foods are interchangeable.

Several approaches have been utilized to establish a recommended ratio of animal to protein source foods that best meets nutrient needs and promotes diet quality. Simulation studies have been completed to evaluate protein adequacy (to meet protein and other nutrient requirements) when ASPFs are replaced with PSPFs across the life stages and in men and women. Soh et al. [66] conducted a review including 23 studies of plant-based dietary patterns. Overall, the findings suggest that protein intake is generally lower when ASPFs are replaced with PSPFs. Further, the level of micronutrient inadequacy within plant-based dietary patterns is dependent on the quantity and quality of PSPFs included. Several additional simulation studies not included in the review provide useful insights. As reported in Vieux et al. [67], a minimum of ~50 g/d of total protein across all life stages and sexes is required just to meet non-protein-related nutrient requirements (e.g., calcium, vit B12, zinc, etc.). In establishing a model that considers nutrient and protein adequacy, affordability, and eating habits, the amount of animal protein ranged from 45-60% of total protein with slight fluctuations depending on life stage and sex. Anything below that range led to nutrient inadequacies. Additionally, when modeling based on higher-protein diet preferences (1.2 g/kg body weight), Grasso, et al. [68], illustrated that a minimum of 50% of total protein intake needed to come from ASPFs.

The NHANES 2015-2018 survey data in adults also supports these amounts. Protein and nutrient intakes and nutrient adequacy were assessed by quintiles of the percent of dietary protein from ASPFs in the American diet [69]. First, total protein intake was

higher as the percent of ASPFs in the diet increased. Further, more people met nutrient requirements for vit A, vit B12, choline, zinc, and calcium with higher consumption of ASPFs but also had a greater percent below the requirements for folate, vit C, and fiber (**Figure 1**). All quintiles were above the sodium recommendations (data not shown).



Modified from (Hoy, 2023 [69]); data as mean ±SEM

Figure 1: Nutrients of concern/under-consumed <%EAR or <%AI by Quintile of Animal Protein Intake, Ranging from <45% of protein as Animal Protein (Quintile 1) to ≥ 75% of protein as Animal Protein (Quintile 5) in Adults

Similar findings were also reported in Gwin, et al. [70]. This cross-sectional study included 530 healthy young adults and collected dietary intake data from food frequency questionnaires to assess protein and micronutrient intakes. As the protein density of the diet increased, most micronutrients (except for vit C) also progressively increased. Lastly, survey data in children and adolescents in Canada align with these previous studies [71]. Protein and nutrient intakes and nutrient adequacy were assessed by quintiles of the percent of dietary protein from plant source foods. Total protein, vit D, vit B12, riboflavin, niacin, vit B6, phosphorus, zinc, and potassium were higher as the percent of ASPFs in the diet increased, whereas folate, thiamine, and fiber were reduced. The authors suggest a 3:1 ratio of animal to plant protein to support nutrient adequacy.

To our knowledge, only one experimental study has been published to date examining the effects of replacing ASPFs with PSPFs on nutrient adequacy [72]. In the following RCT, 136 adults followed diets varying in the following animal (A) to plant-protein (P) food compositions for 12 weeks: Majority ASPFs: 70%/30% A/P; EVEN: 50%/50% A/P; and Majority PSPFs: 30%/70% A/P. A dose-response for vit B12 intake and vit B12 status was observed as intake of animal to plate source foods increased. Further, the pattern containing more ASPFs had higher intakes of iodine, zinc, heme iron but lower intakes of fiber, folate, total iron, and plant-source iron compared to the even distribution and the pattern with more PSPFs. However, iron and folate status were not different between groups. To summarize the findings from these studies, the data support the inclusion of the majority of protein foods as high quality nutrient dense ASPFs for nutrient adequacy. However, it's difficult to confirm the contribution of protein quality or protein source within these trials primarily due to the corresponding changes in protein quantity. In all studies presented, the total amount of dietary protein declined (between -17 to -49 g/d) with the increase in PSPFs within the dietary patterns.

One remaining question is whether a diet containing higher amounts of protein foods can meet nutrient adequacy while providing the benefit of increased dietary protein as discussed above. To our knowledge, no observational or experimental studies exist that publish nutrient adequacy data. However, several modeling studies test this question. As presented in Murphy, et al. [73], the 2020 Healthy U.S.-style Eating Pattern (HUSS) was developed to include protein quantities above the current HUSS pattern of 18% of daily intake as protein. The proposed quantities planned included 20, 25, and 30% of daily energy as protein. To accommodate the additional energy when including more protein foods, refined grains and starchy vegetables were reduced first followed by solid fats and added sugars. Additionally, to achieve proposed protein amounts while maintaining cultural food preferences and protein-density, 68% of the protein foods were ASPFs with 57% from red meat and 43% from poultry for all patterns. For the 20% and 25% patterns, all micronutrient amounts were either similar to that of the 18% protein DGA-HUSS or slightly improved (e.g., choline, iron). Dietary fiber was also similar across diets and was actually 5 g higher in the 25% diet. The 30% diet was unable to be developed based on the initial constraints set by the authors. This was due primarily to the inability to stay within the carbohydrate acceptable range while also maintaining a balance of solid fat and added sugars. In a previous modeling study where those constraints were not set, a higher-protein dietary pattern of 30% of energy as protein was compared to the 2015 DGA-HUSS (of 18% energy as protein) and showed that the % RDA for all nutrients reported, except folate (which was still 93% of the RDA), improved in the pattern [74]. In the later study, 77% of daily protein was from ASPFs. These modeling approaches illustrate the ability to develop patterns that meet nutrient requirements while including higher-protein quantities and support the consideration of a higher-protein, healthy dietary pattern.

Statement of Findings (Summary of Findings Tables & Evidence to Discussion Tables (Appendices E, F, G, H))

The evidence supports a moderate to strong recommendation that protein intakes between 1.2 – 1.6 g protein/kg body weight that prioritize high quality, nutrient dense animal and plant source protein foods, including red meat, improve weight management in adults.

The evidence supports strong recommendation that protein intakes between 1.2 – 1.6 g protein/kg body weight that prioritize high quality, nutrient dense animal and plant source protein foods, including red meat, improve nutrient adequacy when included as part of a healthy dietary pattern across most life stages.

Discussion

Dietary protein is an essential nutrient, vital for growth, maintenance, health and well-being. Emphasizing the value of high quality, nutrient dense protein foods was a cornerstone of early national nutrition guidance, including USDAs food guidance. Prior to the onset of obesity, these recommendations, included a 'meat' group which recommended 2 or more servings per day (~6 oz) of beef, veal, pork, lamp, poultry, fish, and eggs and suggested secondary options such as dry beans, dry peas, and nuts [75]. However, beginning with the development of the DGAs in 1980, the emphasis has shifted towards plant-based dietary patterns to meet nutrient requirements and reduce the risk of chronic diseases; yet, nutrient adequacy and dietary patterns have not meaningfully improved and obesity and other chronic diseases have continued to rise. Even in the face of epidemic increases in obesity, type 2 diabetes, and cardiovascular disease, the DGAs continue to dismiss the value of high quality ASPFs and the associated nutrient-package these foods provide. This approach has the potential to reduce protein density, protein quality, and exacerbate the on-going health crisis in the U.S.

It's been widely known that excess body fat gain (i.e., obesity) is a root cause of all cardiometabolic conditions. As such, improvements in body composition and weight management are paramount to re-establishing health in the U.S. Increasing evidence supports the inclusion of high quality, nutrient dense protein foods at quantities higher than what is modeled in the current DGA dietary patterns as a strategy to combat obesity in the U.S. Thus, we reviewed the evidence to establish a healthy range of protein for weight management and to assess the impact of this range on nutrient adequacy.

To establish a range of protein for weight management, an umbrella review of current systematic reviews and meta-analyses was not appropriate. Further, many studies within these reviews do not meet nutrient requirements, especially for carbohydrates and fiber, or include fat intakes well-above the acceptable range. Thus, a rapid systematic review was performed to include RCTs that met nutrient requirements but included protein at quantities at 1.5 – 2.0 x the RDA and/or >20% of energy.

Additionally, since the majority of Americans have overweight/obesity, reflecting calorie consumption well above requirements, the review included studies of energy restriction, alone or in combination with energy balance.

In total, 30 RCTs were included with over 2/3 of the studies reporting an improvement in at least one weight management outcome following higher vs. lower protein dietary patterns. The certainty of evidence ranged from Low to High, depending on the outcome of interest. Most studies reported a greater reduction in body fat or less body fat re-gain. The standard mean difference in fat lost was modest (SMD: -1.31±2.21 kg) but significant. Weight loss was inconsistent across studies but was either greater or equivalent following the higher-protein diets. The inconsistencies may be a result of the greater preservation of lean mass observed with higher protein, energy restriction diets. Since about 1/3 of the studies did not include lean/fat-free mass measures, we were unable to determine whether this is partially responsible for the lack of weight loss. In addition, we chose not to analyze the energy restriction and energy balance trials separately since a number of studies didn't collect assessments following the energy restriction arm of the study. It's important to note that the RCTs were controlled feeding trials in which the prescribed control and intervention dietary patterns were matched for energy. Thus, it's possible that improvements in weight management occurred as a result of increased satiety, which has been consistently reported with higher-protein diets [76], leading to better dietary compliance and acceptance as shown in some studies. Although compliance rates were inconsistently reported in the RCTs, the macronutrient intakes reported in the Evidence Tables (Appendix C) are estimated from food checkoffs and/or dietary recalls through the studies, *suggesting* that the 1.2-1.6 g/kg body weight is an acceptable range among study participants during weight loss or weight maintenance. Dropout rates were fairly similar between those in the higher-protein vs. lower-protein dietary patterns (21±4% vs. 23±5%), providing additional support in terms of acceptance. Other potential mechanisms may include enhanced glucose metabolism and higher energy expenditure, likely a result of increased protein turnover and muscle protein synthesis, with increased dietary protein [77]. Due to time constraints, we were unable to include these outcomes within the rapid systematic review.

A number of approaches were included to assess nutrient adequacy within higher-protein dietary patterns. In total, 3 RCTs, 14 survey studies using NHANES data sets, 10 modeling studies, 2 simulation studies; and 3 systematic reviews were included in the narrative review. The quality of evidence ranged from Low to High depending on the outcomes of interest.

High quality ASPFs are required in high amounts in dietary patterns to achieve the 1.2-1.6 g/kg body weight healthy range while staying within calorie limits. High quality ASPF are also one of the most nutrient dense, calorie efficient foods within our food supply. First and foremost, compared to PSPFs, ASPFs provide greater amounts of total protein and EAAs as illustrated with the current protein ounce equivalents [37-39, 41]. Further, ASPFs elicit greater postprandial EAA bioavailability and greater whole-

body net protein balance compared to PSPFs, illustrating that protein ounce equivalents are not metabolically equivalent [39, 41]. Thus, the current protein ounce equivalents tool should not be used when menu modeling or with dietary pattern development and needs to be revised based on total protein, EAA density, especially the most limiting amino acid, and corrected for bioavailability to reflect true serving size equivalents.

High quality ASPFs also contain fairly high quantities of bioavailable nutrients of concern/under-consumed including vit B12, zinc, iron, selenium, phosphorus, vit A, and vit D, many of which are limited or absent in PSPFs. A number of studies illustrated higher nutrient adequacy when these foods are included within a dietary pattern. Most of the modeling studies in this narrative review support a recommendation to consume the majority of dietary protein from ASPFs to achieve the proposed healthy range of protein (of 1.2-1.6 g/kg body weight per day) while meeting nutrient needs and staying within calorie limits to achieve and/or maintain healthy weight. Additionally, while many PSPFs are limited or absent of nutrients in ASPFs, they do provide important complementary nutrients like dietary fiber, folate, magnesium, riboflavin, among others and contribute additional protein and thus are relevant for achieving nutritionally adequate dietary patterns that are higher in protein.

Establishing a healthy range of protein to promote weight management and nutrient adequacy has not been explored in any of the previous guidelines. In fact, many past DGAs have recommended a shift towards eating a plant-based dietary pattern through the reduction and/or replacement of ASPFs with PSPFs based on goals to reduce saturated fat and increase fiber intake while ignoring nutrient density for EAAs or micronutrients. These recommendations have the potential to reduce protein density and quality within the diet, encourage carbohydrate amounts that far exceed requirements, and increase the difficulty in establishing appropriate calorie levels for healthy weight.

The following statements were made in the past DGA/DGACs to highlight this shift:

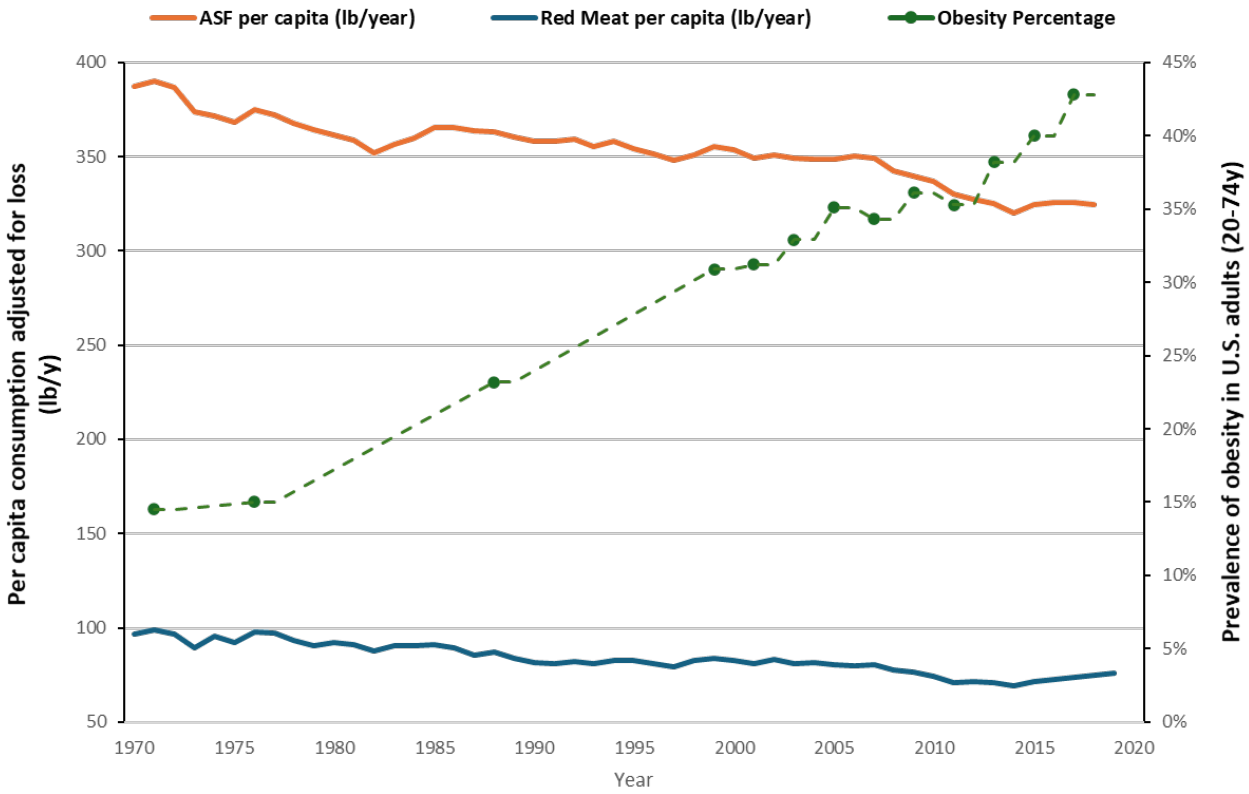
- 2015:
  “Most people would benefit from reducing consumption of red and processed meats.
- 2020:
  “Common characteristics of dietary patterns associated with positive health outcomes include lower consumption of red and processed meats.
- 2025:
  “Emphasizes dietary intakes of beans, peas, & lentils while reducing intakes of red and processed meats. “
  “Food Pattern Modeling (FPM) results provide support for exploring a flexibility that increases Beans, Peas, and Lentils and Nuts, Seeds, and Soy Products, while simultaneously decreasing Meats, Poultry, and Eggs”
  “Recommends that the Beans, Peas, and Lentils Subgroup move from the Vegetables Food Group to the Protein Foods Group to align with evidence to

encourage greater consumption of plant-based Protein Foods."
"Reorganizing the order of the Protein Foods Subgroups to list Beans, Peas, and Lentils first, followed by Nuts, Seeds, and Soy Products, then Seafood, and finally Meats, Poultry, and Eggs. This reordering of Protein Foods emphasizes the health benefits of more plant-based Protein Foods."

The approach within this report substantially differs from previous iterations of the DGACs. The available RCT data support the inclusion of more high quality, nutrient dense ASPFs, including red meat, rather than reducing ASPFs to increase PSPFs. Additional evidence in this review underscores the critical role ASPFs currently make towards ensuring nutrient adequacy in the U.S. and suggests that PSPFs, as a source of complementary protein and other essential nutrients, better serves the overall dietary pattern when it is considered a replacement for refined carbohydrates with low nutrient density. Although our question is broader than the comparison of ASPFs vs. PSPFs, Americans have historically consumed about 2/3 (i.e., 66%) of their protein from ASPFs [78]. As shown in Figure 2, ASPF and red meat consumption has steadily declined in the U.S. [79, 80]. Although a myriad of factors contributes to the rise in obesity in the U.S., overconsumption of ASPF, or specifically red meat, cannot explain the health crisis.

Past guidelines, informed primarily by observational evidence, have inappropriately implied a causal link between ASPFs, especially red meat, and chronic disease and correspondingly have used this interpretation to continuously discourage these foods in favor of PSPFs.

Numerous systematic reviews and meta-analyses have been published over the past 5 years that specifically assess the totality of evidence on ASPFs and cardiometabolic risk. Sanders et al. [81] evaluated the effects of minimally or unprocessed beef intake on CVD risk factors in adults. Twenty RCTs were included in the analyses. The average consumption of beef within these trials was 161 g/d or ~2 servings/d compared to the control of either 0 or 1 serving/d. Daily unprocessed beef intake did not affect most risk factors of cardiovascular disease. In another systematic review and meta-analysis on red meat consumption and risk factors for type 2 diabetes, 21 RCTs were examined [82]. The inclusion of red meat did not impact glycemic and insulinemic risk factors for type 2 diabetes. Similar findings were also shown in O'Connor et al. [83] with red meat consumption of ≥0.5 servings/d. Finally, with respect to red meat and obesity, Akheruzzaman et al. [84] recently published a systematic review and meta-analysis including 24 RCTs when comparing diets with/without unprocessed red meat. No effect of red meat was identified for any outcomes related to weight management and obesity (i.e., BMI, body weight, percent body fat). These analyses provide convincing evidence refuting the recommendations to limit red meat as part of a healthy dietary pattern. In the current report, most of the studies illustrate benefits, specifically for weight management and nutrient adequacy, with the inclusion of additional protein from ASPFs, including red meat.



*Both the per capita food availability data and the per capita loss-adjusted food availability data, despite some limitations, are useful for economic analyses because they serve as indirect measures of trends in food use.

Figure 2: Relationship between ASPF consumption, red meat consumption, and obesity prevalence in the U.S. over the past 50 years*

Due to time constraints, we were unable to apply the healthy 1.2-1.6 g/kg body weight from RCTs to other health outcomes. However, a number of points can be discussed related to increased dietary protein and kidney, bone, and diabetes risks. First, an UL for dietary protein has not been defined [4] but the acceptable range for protein is 10% to 35% of energy as protein. Currently, the average protein intake in the U.S. is approximately 15% of calories and the average intake is about 77 g/day [85]. Assuming the average calorie intake for adults range from 2000 to 2800 calories/day, the acceptable range (at 30% of energy as protein) would suggest an UL for protein between 175 and 245 g/day.

The DRIs define an UL as an intake level that exhibits risk of adverse effects. For dietary protein, risks could be measured as clinical or metabolic outcomes. Clinical outcomes have been evaluated related to kidney function, bone health, obesity, or diabetes while metabolic outcomes include urea production or amino acid degradation. Multiple systematic reviews have reported no adverse effects of increased dietary protein on renal function in healthy adults [86-88]. Multiple reviews demonstrate higher protein intake enhances bone density and strength with no adverse effects [89-91]. Studies evaluating the relationship of dietary protein to risk for type 2 diabetes have been inconsistent but epidemiology studies are often confounded with food sources,

such as processed meats and dietary patterns reflecting excess calories and low dietary fiber leading to obesity [92]. In RCTs, when dietary protein is increased to 30% of calories by isocaloric replacement of dietary carbohydrates, protein improves glycemic regulations, insulin sensitivity, and weight management [30, 93-95].

The metabolic UL for dietary protein has had limited attention. However, the existing studies suggest that daily protein intake in excess of 250 g/day poses no risk related to nitrogen or amino acid metabolism [96]. Lastly, ULs for amino acid disposal have received little attention; however, the available research indicate safe ULs for amino acids that would equate to total protein intake above 300 g/day. While an UL for dietary protein has not been established by the DRI, the published studies support a safe upper range in excess of 2.5 g/kg. Thus, promoting a healthy range of 1.2-1.6 g/kg body weight is well-within an appropriate range.

Considerations for Implementation

To meet the healthy range of protein of 1.2-1.6 g protein/kg body weight per day, daily protein intake should target 100 g protein/day or more for most adults with 50% or more of the protein coming from high quality, nutrient dense ASPFs. The RCTs and modeling studies presented in this report support these recommendations.

The language, recommendations, and modeling within the past DGA/DGACs suggest an erosion in protein quantity and quality, particularly from reduction in high-quality ASPFs. Specifically, the DGA/DGACs recommend consuming more beans, peas, and lentils while simultaneously recommending less meat, poultry, and eggs compared to what most Americans are consuming. In fact, within the 2025 DGAC report, the modeling group explored the following question: “What are the implications for nutrient intakes when proportions of animal-based Protein Foods subgroups are reduced and proportions of plant-based Protein Foods subgroups are increased” [6]. Based on these analyses, the final modeling included within the 2025 DGAC report increased beans, peas, and lentils while simultaneously reducing red meat, poultry, and eggs. However, given the contribution of key nutrients provided within ASPFs, their reduction could not be modeled or recommended at or below the 2,000 calorie level. This provides additional support for the unique value of ASPFs to improve nutrient adequacy and diet quality, especially in young people.

The rationale and justification behind the previous DGA models are questionable given the lack of experimental evidence to support this approach and the differences in nutrient composition, particularly essential amino acid content, between ASPFs and beans, peas, and lentils. As shown in Tables 1 and 2, ASPFs have 3 to 4 times more EAAs than beans, peas, and lentils. As was previously discussed, EAAs are vitally important for health, growth, and maintenance and can only be obtained through the diet. Further, beans, peas, and lentils contain 6-8 g protein/serving compared to ASPFs, like red meat and poultry, which contain between 20-27 g protein/serving. Collectively, replacing ASPFs with beans, peas, and lentils would 1) downgrade the protein and EAA density and quality of the diet and 2) reduce several under-consumed

nutrients/nutrients of concern that are offset by meat consumption (i.e., heme iron, zinc, vit B12, choline, selenium), mainly to gain dietary fiber. Given the carbohydrate (~23 g/serving) and fiber (~5 g/serving) contents of beans, peas, and lentils, a more appropriate substitution includes the replacement of nutrient poor refined grains. Similar to what is proposed in this report, the 2025 DGAC modeling group did explore the reductions in total grains from refined grain sources as well as reductions in starchy vegetables [6].

Refined grain foods, especially those containing added sugar, contribute a large proportion of carbohydrates and calories but are limited in fiber and contain lower quality protein. Thus, replacing nutrient poor refined grains for high quality, nutrient dense ASPFs would 1) increase protein and EAA density and key micronutrients found in ASPFs; 2) reduce carbohydrates to amounts that promote better glucose control; and 3) provide back some of the micronutrients that are fortified in refined grains (thiamin, riboflavin, niacin, selenium). Further, addition substitutions of refined grains with PSPFs, like beans, peas, and lentils, would 1) upgrade protein and EAA density of the diet and 2) increase dietary fiber while providing back some of the micronutrients that are fortified in refined grains (thiamin, riboflavin, niacin). To illustrate this point, **Table 5** compares the energy and nutrient compositions of the proposed substitutions using RACC Servings.

It's important to appreciate that these swaps improve overall diet quality while maintaining calorie levels. This strategy is helpful in preventing weight gain and maintaining a healthy weight. However, if weight loss is a goal, reducing calories can be achieved by further targeting nutrient poor refined grains, particularly those with added sugars.

Although the 2025 DGAC ultimately chose the replacement of starchy vegetables to improve nutrient adequacy, our modeling approach discussed below supports the replacement of refined grains instead, which more appropriately aligns with the recommendations to reduce refined carbohydrates containing added sugars [6].

Table 5: Example Food Swamps to yield more high quality, nutrient dense protein while maintaining calories & increasing nutrient adequacy

Example Food Swaps	RACC Serving Swaps (#/day)	Protein (g)	EAA (g)	Energy (kcal)	CHO (g)	Fiber (g)	Fat (g)	Iron (mg)	Zinc (mg)	Vit B6 (mg)	Vit B12 (µg)	Choline (mg)	P (mg)	Folate (mg)
Animal Source Protein Food Composite[†]	+1	+25	+10.4	+162	0	0	+6.0	+1.4	+3.1	+0.5	+1.19	+67	+198	+4
Beans, Peas, Lentils, Legumes Composite[†]	+1	+8	+3.1	+106	+14.0	+5.4	+1.6	+3.1	+0.6	+4.9	0	+22	+102	+197
Refined Grains Composite[†]	-2	-7	-2.1	-308	-61.6	-2.6	-4.2	-7.5	0	-0.4	-1.4	-12	-111	-340
Difference[‡]		+26	+11.4	-40	-47.6	+2.8	+3.4	-3.0	+3.7	+5.0	-0.21	+77	+189	-139

Example Food Swaps	RACC Swaps (#/day)	K (mg)	Ca (mg)	Vit D (µg)	Na (mg)	Mg (mg)	Copper (µg)	Selenium (µg)	Vit A (µg)	Vit E (mg)	Vit C (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)
Animal Source Protein Food Composite[†]	+1	+270	+13	+0.3	+55	+39	+0.2	+29.3	+2.3	+0.1	0	+0.23	+0.2	+7.4
Beans, Peas, Lentils, Legumes Composite[†]	+1	+245	+37	0	+158	+37	+0.1	+1.9	0	+2.0	+5.9	+0.11	+14.6	+44.3
Refined Grains Composite[†]	-2	-76	-44	-0.5	-129	-23	-0.1	-9.2	-85.4	-0.2	-2.4	-0.30	-0.2	-3.6
Difference[‡]		+439	6.00	-0.20	+84	+53	+0.2	+22.0	-83.1	+1.9	+3.5	0.04	+14.6	+48.1

CHO: Carbohydrates; P: Phosphorus; K: Potassium; Ca: Calcium; Na: Sodium; Mg: Magnesium
*Reference Amount Customarily Consumed (RACC) servings
‡Green highlights denote increases in nutrients with these swaps whereas yellow denotes reductions
†Composite for this analysis included the top 6 most commonly consumed foods in the U.S. diet
ASPF Composite Sources: Beef Composite, Pork Composite, Chicken Breast Composite, Salmon, Egg, Plain Greek yogurt; 1 RACC serving = ~ 3 oz
Pulse Composite: Black Beans, Edamame, Lentils; 1 RACC serving = ½ cup
Refine Grains Composite: White Bread (1 RACC=2 slices); Breakfast Cereal (1 RACC=1 cup), Rice (1 RACC=1 cup), Cookie (1 RACC=1 item)

We have taken these concepts and applied them to the US Healthy Eating Dietary Pattern (HUSS) within the 2020 DGA using a similar approach to that of Murphy et al. [73]. **Table 6** illustrates the RACC servings of each food group and highlights the proposed changes for a 'Higher-Protein Dietary Pattern' which includes protein quantities within the 1.2-1.6 g/kg body weight range and is higher than what was modelled in previous DGA dietary patterns. As a first step, the Higher-Protein Dietary Pattern was modeled for males ages 51-70 years and females ages 19-30 years which are representative populations consuming a 2,000 kcal diet and the nutrient goals used by USDA to assess compliance [3].

Table 6: Modified from Murphy et al (2022) to achieve a Higher-Protein Dietary Pattern based on the 2020 Healthy U.S.-Style Eating Pattern

Food groups and subgroups: *(Reported in RACC servings*)*	U.S. Habitual Intake (WWEIA, NHANES, 2021-2023)	2020 Healthy U.S.-style Eating Pattern (HUSS)	Proposed Higher-Protein Dietary Pattern
Total Fruit (RACC servings/d)	1	2	2
Total Vegetable (RACC servings/wk)	10	17 (14 ½) with (without) beans, peas, lentils, legumes	19 (9) with (without) beans, peas, lentils, legumes
Total Grains			
Whole grain (RACC Servings/d)	½	1 ½	1 ½
Refined grains (RACC Servings/d)	3	1 ½	1
Total Protein Foods			
ASPFs			
Red Meat (RACC servings/wk)	6	4	8
Poultry RACC servings/wk)	4	3 ½	6
Seafood (RACC servings/wk)	1 ½	3	4
Eggs (RACC servings/wk)	4 ½	3	3
PSPFs			
Beans, Peas, Lentils, Legumes (RACC servings/wk)	$1\frac{2}{3}$	3	10
Soy/Nuts/Seeds (RACC servings/wk)	$3\frac{1}{3}$	2 ½	2 ½
Total Dairy (RACC servings/d)	1 ½	3	3
Other Sources *(kcal/d))* *(oils, solid fats, & added sugars from sweets, desserts, salty snacks, beverages)*	~900 kcals	~450 kcals	~125 kcals

*Amounts are reported as Reference Amount Customarily Consumed (RACC) servings and were calculated from the cup and ounce equivalent values previously reported; RACC servings were used to remove the inaccuracies with protein oz equivalents and are more applicable to the US consumer.

The energy and nutrient composition of this diet is shown in **Table 7**. Similar criteria set by USDA of meeting the targets was applied to this dietary pattern [3]. Although vit D, vit E, and choline (in males) were below the RDA/AI targets, which is similar to the modeling for all 3 of the current USDA Healthy Dietary Patterns, all other nutrients met targeted goals.

This modeling approach has illustrated a few key points concerning the proposed Higher-Protein Dietary Pattern. First, including protein intakes of 1.5-2.0 times that of the RDA through the incorporation of additional servings of unprocessed and/or minimally processed ASPFs and PSPFs while simultaneously reducing nutrient poor, refined grain foods achieved nutrient adequacy while providing protein and EAAs at quantities necessary for health promotion and disease prevention. Second, nutrient dense ASPFs and PSPFs provide significant quantities of key micronutrient that allow for the substitution of refined grains, including those with fortification. Lastly, Americans are consuming 6 servings of red meat, 3½ servings of poultry, and 4½ servings of eggs (per week), totaling 14 servings/week [97]. Given the proposed Higher-Protein Dietary

Pattern includes ~5½ more servings/week than what Americans are habitually consuming, future work is needed to develop long-term RCTs to test feasibility, adherence, and acceptance of this dietary pattern in combination with cardiometabolic health outcomes. However, the majority of RCTs included in the rapid systematic review followed similar substitutions in terms of increased ASPFs with reductions in refined grains with fairly high compliance.

Table 7: Energy and Nutrient Intakes and Comparisons to Nutrient Goals for a 2000 kcal, Higher-Protein Dietary Pattern

Energy & Nutrients	Measure*	Reference Intake F: 19-30 y M: 51-70y	Intake	Meeting Recommended Intake (Yes: ✓; No: Ø)	
				F 19-30 y	M 51-70 y
Energy, kcal	%goal	2000	2000	✓	✓
Protein, g	%RDA	46;56	122	✓	✓
AMDR, %	%kcal	10-35	24	✓	✓
Carbohydrate, g	%RDA	130	249	✓	✓
AMDR, %	%kcal	45-65	50	✓	✓
Fiber, g	%AI	28	30	✓	✓
Lipid (Fat), g	N/A	N/A	62	N/A	N/A
AMDR, %	%kcal	20-35	28	✓	✓
Saturated Fats, g	%kcal	<10	8	✓	✓
Linoleic acid, g	%AI	12;14	20	✓	✓
Linolenic acid, g	%AI	1.1;1.6	2.5	✓	✓
Cholesterol, mg	Mg	<300	288	✓	✓
Calcium, mg	%RDA	1000;1200	1309	✓	✓
Iron, mg	%RDA	18;8	16	✓	✓
Magnesium, mg	%RDA	310;420	386	✓	✓
Phosphorus, mg	%RDA	700	1923	✓	✓
Potassium, mg	%RDA	2600;3400	3676	✓	✓
Sodium, mg	%CDRR	2300	1944	✓	✓
Zinc, mg	%RDA	8;11	16	✓	✓
Copper, mg	%RDA	0.9	1.7	✓	✓
Selenium, µg	%RDA	55	139	✓	✓
Vit A, µg RAE	%RDA	700;900	926	✓	✓
Vit E, mg AT	%RDA	15	9	Ø	Ø
Vit D, IU	%RDA	600	363	Ø	Ø
Vit C, mg	%RDA	75;90	129	✓	✓
Thiamin, mg	%RDA	1.1;1.2	1.8	✓	✓
Riboflavin, mg	%RDA	1.1;1.3	2.1	✓	✓
Niacin, mg	%RDA	14;16	29	✓	✓
Vit B-6, mg	%RDA	1.3;1.7	2.5	✓	✓
Vit B-12, µg	%RDA	2.4	7.9	✓	✓
Choline, mg	%AI	425;550	447	✓	Ø
Vit K, µg	%AI	90;120	138	✓	✓
Folate, µg DFE	%RDA	400	515	✓	✓

*Acceptable Macronutrient Distribution Ranges (AMDR); Recommended Dietary Allowances (RDA); Adequate Intakes (AI); 2020–2025 DGA limit for energy from saturated fat; Chronic Disease Risk Reduction (CDRR) for sodium; and cholesterol limit used in food pattern modeling exercises to support the 2020 DGA [3]; N/A: Not Applicable

In summary, the evidence presented within this report supports a recommended healthy range of dietary protein as 1.2-1.6 g protein/kg body weight for health promotion and disease prevention which can be accomplished by prioritizing high quality, nutrient & protein dense unprocessed or minimally processed animal and plant protein foods, including red meat.

Prioritize High quality, Nutrient dense Protein Foods as Part of a Healthy Dietary Pattern: Recommendations

- Set personalized calorie goals that will establish or maintain a healthy body weight
    - If calorie reduction is needed, do so through increased physical activity and modest reductions in daily energy between -500-750 kcal/d.
- When creating healthy meals, prioritize protein with the following recommendations:
    - Consume a variety of high quality, nutrient dense animal source protein foods, including red meat, poultry, and seafood (including fish and shellfish), eggs, and dairy.
        - Swap fried or breaded red meat, poultry, and seafood with baked, broiled, roasted, stir-fried, or grilled cooking methods.
    - Consume a variety of nutrient-dense plant source protein foods including beans, peas, lentils, and legumes, nuts, seeds, and soy.
    - For vegetarians, prioritize dairy, eggs, and beans, peas, lentils, and legumes.
    - Reduce the consumption of nutrient poor refined grains including sweets, desserts, salty snacks, and beverages.

SERVING (SVG) GOALS TO ACHIEVE THE HEALTHY RANGE OF PROTEIN INTAKE

Food Group	Protein Food Examples	Serving (srv) Recommendation	Protein per Srv[†]
Poultry and Red Meat	fresh, minimally processed* chicken/turkey thigh, breast, or drumstick; pork loin roast; beef sirloin; or ground turkey, pork, or beef	2 or more srv per day (1 srv = 3 oz cooked = 4 oz raw)	20-27 g
Seafood	fresh, minimally processed* black sea bass, catfish, crab, flounder, haddock, perch, pollock, salmon, scallop, shrimp, tilapia	3 or more srv per week (1 srv = 3 oz cooked = 4 oz raw)	18-25 g
Eggs	scrambled, omelet, hard boiled, frittata (muffin cup)	3 srv or more per week (1 srv = 1 large egg)	6 g
Dairy	whole milk, lactose-free milk, ultra-filtered milk, plain Greek yogurt, cottage cheese	3 srv per day (1 srv = 1 cup of whole milk[ǂ]) (1 srv = ¾ cup yogurt[ǂ]) (1 srv = 1 oz cheese = 1 slice) (1 srv = ½ cup cottage cheese)	Milk: 8–14 g Yogurt: 15-20 g Cheese: 6-10 g Cottage Cheese: 12–14 g
Beans, Peas, Lentils, Legumes°	Black beans, kidney beans, chickpeas, split peas, red/green lentils, edamame	1 srv or more per day (1 srv = ½ cup cooked)	6-9 g
Nuts/Seeds/Soy	almonds, pistachios, walnuts, peanuts, sunflower seeds, chia seeds, pumpkin seeds, tofu, tempeh	2.5 or more serv per week (1 srv = 1 oz nuts/seeds) (1 srv = 3 oz soy)	Nuts/Seeds: 4–7 g Soy: 8-18 g

*Sources that are baked, broiled, roasted, stir-fried or grilled and not fried or breaded
[†]Reported as grams (g)
[ǂ]No sugar added
°Excludes peanuts and soy

Eat enough of these foods each day to meet the healthy range of protein intake while choosing other nutrient dense foods from fruits, vegetables, and whole grains for a healthy dietary pattern.

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Appendix 4.10. Processed Meats & Health Risks

PROCESSED MEATS & HEALTH RISKS

Narrative Review

Donald Layman, PhD, FASN
Professor Emeritus
Department of Food Science and Human Nutrition
University of Illinois at Urbana-Champaign

Heather Leidy, PhD, FASN
Associate Professor
Department of Nutritional Sciences
Department of Pediatrics – Dell Medical School
University of Texas at Austin

Abstract

Objectives: The purpose of this report was to evaluate the experimental evidence that processed meats, including meat alternatives, increase health risks.

Methods: Given the time constraints and a lack of certainty whether randomized controlled trials exist to answer this question, an abbreviated Narrative Review was completed. A literature search was conducted to identify all potentially relevant articles, and those articles were screened based on pre-specified criteria. The body of evidence was synthesized to answer the question and recommendations for future research were developed. This narrative review, the population of interest were children and adolescents (ages 2-18 y); adults (ages 19+ y); and older adults (ages 65+ y). The exposure was processed meats. The comparator included diets limited or void of processed meats. Studies that included red and processed meats (combined) were excluded. Primary outcomes included measures of cardiovascular disease risk (i.e., blood pressure, triglycerides), obesity (i.e., weight, body composition, waist circumference, BMI), and type 2 diabetes (HbA1c, fasting glucose).

Results: Of the 74 papers screened, none of the randomized controlled trials included a processed meat vs. unprocessed meat comparison or higher intake vs. lower intake of processed meats.

Conclusions: No experimental evidence exists that processed meats, including meat alternatives, increase health risks. Since there is a lack of experimental evidence, a specific amount should not be established at this time. However, a more appropriate recommendation is to prioritize consuming unprocessed or minimally processed red meat/poultry/seafood as part of a healthy dietary pattern across all life stages.

Introduction

Nearly 50% of adults in the United States (U.S.) have cardiovascular disease (CVD) which reduces quality of life and life expectancy [1]. For the past 40 years, high-fat and high-sodium diets have been strongly linked with CVD, though the nuances of these links are still evolving. Although the role of dietary fat is more complex, a high sodium diet is clearly associated with increased blood pressure, a primary risk factor for CVD. Beginning in 1985, the Dietary Guidelines for Americans (DGAs) created the recommendation to limit dietary sodium which has been highlighted in every iteration since then. What has changed is the emphasize on which foods are thought to have the greatest impact. Table 1 below illustrates the statements made in the DGAs and which foods were included as items to limit.

Table 1: Past Dietary Guidelines for Americans statement related to processed meats

Year	Example Foods (to limit)
1985	Salty foods with cured meats being one of them
1990	Processed meat, poultry, and fish
1995	High-fat processed meats such as bacon, sausages, salami, bologna, and other cold cuts
2000	High-fat processed meats such as bacon, sausages, salami, bologna, and other cold cuts
2005	(just stated as) less processed items and those with less sodium dense
2010	Processed meats (e.g., franks, sausage, and bacon)
2015	Processed meats and poultry
2020	Red and processed meats
2025	Red and processed meats

Over time, the DGAs have expanded the recommendation from salty foods to processed meats to “red and processed meats” in an attempt to improve overall health and reduce the risk of CVD [2]. However, unprocessed meat and processed meats have very different nutrient profiles and ingredients and should not be viewed as equivalent. Regardless, the DGAs have continued to report a grade of moderate evidence that “dietary patterns that are associated with better health outcomes included higher intakes of vegetables, fruits, whole grains, low- or non-fat dairy, seafood, legumes, and nuts; moderate intake of alcohol (in adults only); and lower intakes of *red and processed meat*, refined grains, and sugar-sweetened foods and drinks.” These recommendations come from epidemiological studies, making it impossible to assess a direct effect of processed meats on health and are also limited by the inclusion of both red and processed meats in the analyses.

The previous protein questions discussed the value of high quality, nutrient dense animal source protein foods, including red meat, within a higher protein dietary pattern to promote weight management and nutrient adequacy. Within the rapid systematic review, many of the studies included a variety of animal source protein foods, including

processed and unprocessed meats. Unfortunately, these studies did not provide the proportion of these products to the overall diet, making it difficult to ascertain the contribution to the dietary pattern or the impact on health.

Current consumption of processed meat is 0.79±0.03 ounce equivalents/d in children and adolescents and 0.93±0.03 ounce equivalents/d in adults, making up about 23% and 15% of total meat/poultry/seafood consumption [3]. Although processed meats are generally recognized as low quality, 'unhealthy' foods, they do provide a number of key nutrients. For example, in a modeling study using NHANES data, one serving of processed meats was removed from dietary pattern. In doing so, non-trivial reductions in protein (−20%), phosphorus (−14%), potassium (−11%), zinc (−17%), selenium (−26%), thiamine (−14%), riboflavin (−11%), niacin (−24%), vitamin B6 (−13%), B12 (−11%), and choline (−19%) were observed [4]. Additionally, fat, cholesterol, saturated fat, and sodium also decreased (−12%, −24%, −16%, and −38%, respectively.

While the DGA recommendations have grouped fresh and processed meats together, the preponderance of evidence does not support equivalent risk or comparable mechanisms associated with red versus processed meat. Because apparent risk appears to be solely associated with processed meat, this review focused on the following question.

List of Questions

1. **What is the evidence that processed meats, including meat alternatives, increase health risks?**

Methods

The first step in examining the scientific evidence on processed meats was to establish a definition of processed meats. For this analysis, processed meats are defined as animal source protein foods that have been modified to contain added fats, oils, sugars, breading, sodium preservatives, etc.

Given the time constraints and a lack of certainty whether randomized controlled trials exist to answer this question, an abbreviated Narrative Review was completed.

A literature search was conducted to identify all potentially relevant articles, and those articles were screened based on pre-specified criteria. The body of evidence was synthesized to answer the question and recommendations for future research were developed.

For this narrative review, the population of interest were children and adolescents (ages 2-18 y); adults (ages 19+ y); and older adults (ages 65+ y). The exposure was processed meats. The comparator included diets limited or void of processed meats. Studies that included red and processed meats (combined) were excluded.

Primary outcomes included measures of cardiovascular disease risk (i.e., blood pressure, triglycerides), obesity (i.e., weight, body composition, waist circumference, BMI), and type 2 diabetes (HbA1c, fasting glucose).

When establishing inclusion and exclusion criteria, the standard criteria for publication status, language of publication, country, and study participants were utilized. Participants were included if they were healthy or had overweight/obesity, diabetes, and/or cardiovascular disease risks but were excluded if they were taking weight loss medication or had bariatric surgery previously. Studies were included if they were published any time after 1950. No study duration criteria were set for any studies.

Results

Of the 74 papers screened **(Appendix A)**, none of the randomized controlled trials included a processed meat vs. unprocessed meat comparison or higher intake vs. lower intake of processed meats.

Discussion

Potential associations between red and processed meats with CVD risks have been assessed by epidemiology studies [5-10]. The consensus from systematic reviews and meta-analyses of the epidemiology studies supports a probable association of processed meat and all-cause mortality and CVD risk but no association with red meat. The risk, while statistically significant, is weak and inconsistent with relative risk (RR) averaging approximately 1.23 across all studies (RR range 1.15 to 1.42). The systematic reviews and meta-analyses also note that the risk is not linear across all intakes but is only evident when comparing highest intake levels of processed meat (>2 oz/day) with individuals who consume processed meats occasionally or never [11]. Systematic reviews highlight the heterogeneity of the food patterns and lifestyles as confounding factors when comparing only the two extremes of processed meat intake.

While epidemiology studies suggest possible health risks associated with processed meat intake, no clear mechanism has been established to explain a causal role. Plausible mechanisms associated with consumption of processed meats include increased saturated fat, cholesterol, salt, nitrite, heme iron, polycyclic aromatic hydrocarbons, and other preservatives. A study from the Netherlands demonstrated that when data were adjusted for nitrite intake correlations to total and CVD mortality disappeared (HR = 1.10 and 1.09, respectively) [12].

The previous DGAs have included the recommendations to limit ‘red and processed meats’ to achieve a healthy dietary pattern. The previous KQ1a and b questions refute the reduction of unprocessed or minimally processed red meat. Concerning processed meats, the DGAs do not state specific minimum targets. However, taking into consideration food preferences and consumption patterns in Americans, the epidemiology data concerning processed meat and health risks, and the need to maintain appropriate calorie levels while achieving nutrient adequacy, Murphy, et al. [13]

modeled the U.S.-Style Healthy Eating Pattern to include ~5.5 ounces (~3 servings/week of processed meats (i.e., 1 serving = 2 ounces). This approach allowed for some processed foods within the dietary pattern to help with adherence and acceptance but also allowed for the achievement of all nutrient recommendations.

Since there is a lack of experimental evidence to exclude processed meats from a healthy dietary pattern, a specific amount should not be established at this time. However, a more appropriate recommendation is to prioritize consuming unprocessed or minimally processed red meat/poultry/seafood as part of a healthy dietary pattern across all life stages and to have processed versions occasionally and in moderation.

Statement of Findings (Evidence to Discussion Tables (Appendix B))

No experimental evidence exists that processed meats, including meat alternatives, increase health risks, whereas the epidemiological evidence supports only a weak recommendation but is only evident among individuals with the highest usage.

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Appendix 4.11. Life Stages with Special Considerations

LIFE STAGES WITH SPECIAL CONSIDERATIONS

A Narrative Review

Ty Beal, PhD
University of California, Santa Barbara

Introduction

These updated Dietary Guidelines for Americans (DGAs) prioritize consumption of nutrient-dense, nourishing foods while minimizing foods low in essential nutrients. This approach helps to provide more adequate diets across all populations and lowers the risk for inadequacies in life stages with special considerations. Nevertheless, there are still certain life stages that have increased nutritional requirements and require special attention.[1] This document focuses on the top priority areas requiring attention for the following life stages: infants and young children 6–23 months, adolescents 10–19 years, pregnant women, lactating women, women of reproductive age 15–49 years, and older adults ≥ 65 years.

Special Populations

Infants and Young Children

Breastfeeding alone is the ideal form of nutrition from birth through about age 6 months. Breast milk provides necessary nutrients, protective factors against disease, and other unique immunological benefits. If breast milk is unavailable, infants should be fed an iron-fortified commercial infant formula. Once an infant is developmentally ready, foods and beverages should be introduced to complement breast milk. These complementary foods and beverages are essential to meet the nutrient requirements of infants starting at about age 6 months and should be selected carefully to help meet these needs. As an infant becomes a toddler and learns to eat a variety of foods, flavors, and textures, the goal of complementary feeding becomes establishing a healthy dietary pattern and transitioning to a healthy family diet by age 2.

The complementary feeding period (6–23 months) is a critical window when growth and development occur more rapidly than at any other time in life. Nutrient needs—especially for iron—are proportionally higher than at any other life stage.[1] Inadequate nutrition during this period can have severe, lifelong effects on health and development. Alongside continued breastfeeding or an appropriate infant formula, infants should be offered a diverse variety of minimally processed nutrient-dense foods in developmentally appropriate textures. Nutrient-dense animal-source foods, including unprocessed red meat, organ meats, fish (including shellfish), eggs, and unsweetened dairy, as well as dark green leafy vegetables, legumes, nuts, and seeds, are especially important because infants' small stomachs require foods that deliver more nutrients in smaller portions.[1,2] Nutrient-poor foods like unfortified refined grains and ultra-processed foods like sugar-sweetened beverages should be significantly limited to avoid displacing nourishing foods that support critical growth and development and prevent excess calorie intake and risk for obesity. In contexts where nutrient-dense foods are inaccessible or insufficient, fortified products or targeted supplements under the guidance of a qualified health provider may be needed.

Adolescents

Adolescence (10–19 years) is the second fastest period of growth after infancy, marked by rapid gains in height, muscle, and bone mass as well as hormonal and cognitive development.[3] Energy, protein, and calcium needs rise during this life stage, and iron requirements increase substantially for girls due to menstrual blood losses.[1,4] Nearly 40% of U.S. adolescent girls are iron-deficient.[5] Adequate calcium and vitamin D are critical to achieving peak bone mass. Adolescents should be encouraged to consume a variety of nutrient-dense foods—especially dairy products with added vitamin D and other calcium-rich foods such as dark leafy greens, along with iron-rich foods for girls such as unprocessed red meat, organ meats, and bivalves.[1] At the same time, adolescents should minimize sugar-sweetened beverages and ultra-processed snacks, which tend to increase during this life stage in the U.S.[6] Where access to nutrient-dense foods is limited or dietary patterns place adolescents at increased risk for iron deficiency or inadequate bone accretion, fortified foods or supplementation under qualified health-care guidance may be warranted.

Pregnant Women

Pregnancy substantially increases requirements for several nutrients to support maternal health, expanding blood volume, and fetal growth. Iron and folate are top priorities: iron needs increase by 50% to meet increased erythropoiesis and reduce risk of maternal anemia, while adequate folate—including folic acid from fortified foods or supplements—is critical before conception and during early pregnancy to prevent neural-tube defects.[7] Protein, choline, iodine, and vitamin B12 also warrant attention because of their roles in fetal brain development, and omega-3 fatty acids—particularly DHA—are important for neurodevelopment.[1] Pregnant women should be encouraged to consume a diverse range of nutrient-dense foods, especially iron-rich animal-source foods (such as minimally processed lean red meat, organ meats in safe quantities, and fully cooked bivalves), folate-rich foods (such as dark leafy greens, beans, and lentils), eggs (rich in choline), dairy (rich in calcium), and seafood that is low in mercury but rich in DHA.[1,7] Where diets fall short or physiological needs cannot be met through food alone, use of prenatal supplements that provide iron, folic acid, iodine, vitamin B12, and DHA under qualified health-care guidance is recommended.[7]

Lactating Women

Lactation increases nutrient and energy needs above pre-pregnancy levels to support milk production. Adequate intake of several key micronutrients is critical because their concentration in breast milk—such as vitamin B12, iodine, vitamin D, vitamin A, and fatty acids like DHA—depends on the mother's diet and status.[1,7] Choline requirements remain elevated during lactation to support infant brain development and maternal recovery. Women who are breastfeeding should be encouraged to consume a diverse array of nutrient-dense foods, especially animal-source foods rich in vitamin B12, iodine, choline, and high-quality protein (eg, dairy products, eggs, meats, seafood low in mercury but rich in DHA), together with folate-rich legumes, dark leafy

greens, and vitamin-A-rich foods such as orange-fleshed vegetables and liver in safe amounts.[1] Where diets are limited—particularly among women with low intake of animal-source foods (eg, vegans or vegetarians)—supplementation or fortified foods providing iodine, vitamin B12, vitamin A, vitamin D, or DHA under qualified health-care guidance may be warranted.[7]

Non-pregnant, Non-lactating, Women of Reproductive Age

Women of reproductive age have elevated iron requirements compared with men due to regular menstrual blood losses, and iron-deficiency anemia remains common in this group in the U.S. and globally.[1,8] Adequate iron intake—especially from heme iron-rich animal-source foods such as minimally processed lean red meat, organ meats, and fully cooked bivalves, along with iron-fortified plant foods and vitamin C-rich fruits and vegetables to enhance non-heme iron absorption—is essential to prevent iron deficiency and support health across the reproductive years. All women planning pregnancy should ensure adequate folate intake, including folate/folic acid from fortified foods or supplements, before conception and in early pregnancy to reduce the risk of neural-tube defects.[7] Where dietary patterns limit iron intake—such as in women with low consumption of animal-source foods—or folate intake, fortified foods or supplementation under qualified health-care guidance may be warranted. Other micronutrients of concern for subsets of women in this age group include iodine (for those not consuming dairy or iodized salt) and vitamin B12 (for those following strict vegetarian or, especially, vegan diets).[1]

Older Adults

Older adults experience declines in energy needs but stable or higher requirements for key nutrients, including protein, vitamin B12, vitamin D, and calcium.[1] Age-related reductions in stomach acid and intrinsic factor increase the risk for vitamin B12 malabsorption,[9] while adequate vitamin D and calcium remain critical for maintaining bone mass and reducing fracture risk.[4] Adequate dietary protein (at least 1.2 g/kg) is essential to help preserve muscle mass and strength and reduce the risk of sarcopenia.[10] Older adults should be encouraged to prioritize nutrient-dense foods such as dairy products fortified with vitamin D, minimally processed lean meats, seafood, eggs, legumes, nuts, seeds, and high-fiber whole plant foods, while limiting foods that are high in calories but low in nutrients. Where intake or absorption of nutrients such as vitamin B12, vitamin D, calcium, or protein is inadequate, fortified foods or supplements under qualified health-care guidance may be warranted.[1,4]

Conclusions and Implications for the DGAs

These updated DGAs emphasize eating patterns rich in nutrient-dense foods to improve diet quality and reduce risk of nutrient inadequacy across the population. This life-stage review highlights that while this approach benefits everyone, certain groups have higher physiological requirements or face barriers to meeting them through food alone—notably infants and young children 6–23 months, adolescents, women of reproductive

age, pregnant and lactating women, and adults ≥ 65 years. Prioritizing animal-source foods, dairy products with added vitamin D, seafood low in mercury but rich in DHA, legumes, nuts, seeds, fruits, and vegetables—especially dark leafy greens and orange-fleshed varieties—together with fortified foods where needed—can help close key nutrient gaps in these groups. Attention to iron, folate/folic acid, vitamin B12, iodine, vitamin D, calcium, choline, DHA, and protein remain essential to support healthy growth, development, reproduction, and aging. These considerations should inform food-based guidance, fortification and supplementation policies, and nutrition-security efforts so that the DGAs better address both the general population and those with increased nutritional needs across the life course.

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Appendix 4.12. Special Considerations for Vegan and Vegetarian Diets

SPECIAL CONSIDERATIONS FOR VEGAN AND VEGETARIAN DIETS

A Narrative Review

Ty Beal, PhD
University of California, Santa Barbara

Introduction

Vegan and vegetarian diets are addressed as special populations with unique considerations. Vegan diets are those including no animal products, and vegetarian diets, for our purposes, are those including plant-source foods and eggs and dairy (i.e., lacto-ovo vegetarian). Between 4–7% of U.S. adults consume a vegetarian diet,[1,2] and 1% consume a vegan diet.[2] Individuals may choose to consume a vegan or vegetarian diet for ethical, health, religious, or environmental reasons. While healthy vegan and vegetarian diets can be protective against noncommunicable diseases,[3] they can also increase risk for certain nutrient deficiencies, especially during critical life stages.[4]

To assess the unique considerations for individuals consuming vegan or vegetarian diets, we will conduct a rapid evidence synthesis to address the following research questions:

1. What nutritional challenges do vegans and vegetarians face?
2. What can vegans and vegetarians do to ensure adequate nutrition?

We first address the challenges, evidence, and strategies to ensure adequate nutrition on vegan and vegetarian diets focusing on essential nutrients of concern. Then we address unique considerations during critical life stages. We highlight key evidence gaps and limitations and conclude with implications for the Dietary Guidelines for Americans (DGAs).

Key Nutrients: Challenges, Evidence, and Strategies

Food pattern modeling from the 2025 Scientific Committee suggests potential shortfalls in several nutrients among vegetarian and vegan patterns.[5] Several age-sex groups in the healthy vegetarian dietary pattern had less than the RDA for vitamins D and E, choline, and iron (Figure 1).[5] Several age-sex groups in the healthy vegan dietary pattern had less than the RDA for vitamins A, D, E, B6, and B12, riboflavin, niacin, choline, calcium, iron, magnesium, phosphorus, potassium, zinc, and protein (Figure 2).[5] Additionally, omega-3 fatty acids (EPA/DHA) and iodine warrant special attention as they are limited in plant-source foods, though not shown in these models. Protein intake for vegetarians is typically between 64–70 g/day and for vegans is between 60–64 g/day.[6] This means that about one-third of vegans are below the RDA for protein, let alone the optimal intake, and if accounting for bioavailability, this proportion would increase further.[7]

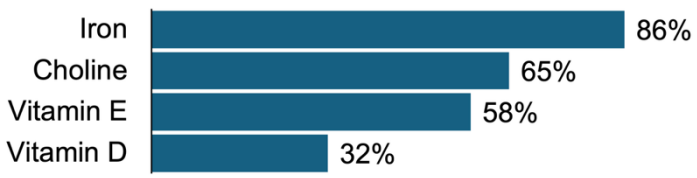

Figure 1. Percentage of RDA for women aged 31–50 years on a modelled healthy vegetarian dietary pattern.

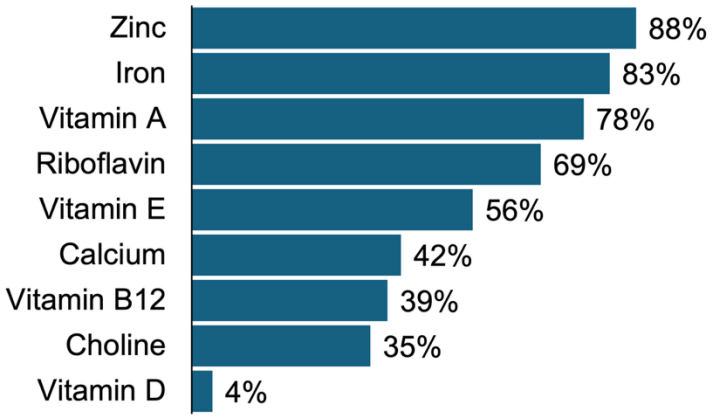

Figure 2. Percentage of RDA for women aged 31–50 years on a modelled healthy vegan dietary pattern.

We identify nutrients of concern by triangulating evidence on food pattern modeling, observational data, and intervention data, in the sections below. We discuss each followed by recommended dietary strategies to ensure adequate nutrition.

Vitamin B12

Vitamin B12 is absent in nearly all unfortified plant-source foods, with a rare exception for certain seaweeds.[8] Vitamin B12 deficiency can lead to megaloblastic anemia, neurological damage, and elevated homocysteine levels associated with cardiovascular risk.[9] While vegetarians can get vitamin B12 from eggs and dairy, vegans must get vitamin B12 from fortified foods or supplements. Food pattern modelling found healthy vegetarian diets could meet 100% of the RDA for vitamin B12 for all age-sex groups, but vegan diets could not meet the RDA for any age-sex groups.[5] In the EPIC-Oxford cohort, half of vegans and 7% of vegetarians were deficient in vitamin B12 whereas virtually no omnivores were deficient.[10] Similarly, half of those in a macrobiotic community in New England that consistently consumed few animal-source foods were deficient in vitamin B12.[11] A randomized controlled trial (RCT) of vegan diets versus omnivorous diets found that vitamin B12 intake and biological status declined after just four weeks on a vegan diet.[12]

For vegetarians to maintain adequate vitamin B12 status without fortified foods or supplements requires regular consumption of eggs and dairy. For vegetarians with inadequate consumption of eggs and dairy or strict vegans, daily supplementation rather than weekly supplementation has been demonstrated in a RCT to provide adequate vitamin B12 status.[13] While vegetarians and vegans could benefit from consuming more foods fortified with vitamin B12, supplementation is a more reliable way to ensure adequacy.[14]

Iron

Iron is available in plant-based foods in a form called non-heme iron (2–20% absorbed), which is about half as bioavailable on average as heme iron (15–35% absorbed),[15]

found exclusively in animal tissue. Iron deficiency can lead to iron deficiency anemia, impaired cognitive function, reduced work capacity, compromised immune function, and adverse pregnancy outcomes including preterm delivery and low birth weight.[16] About 34% of women of reproductive age in the U.S. have absolute iron deficiency.[17] Vegan and vegetarian diets can make it difficult to maintain adequate iron status due to three factors: low or no intake of heme iron, high intake of phytate and other anti-nutrients that hinder iron absorption, and absence of animal-source foods, like meat, that enhance non-heme iron absorption.[18] However, physiological adaptations can allow for increased non-heme iron absorption when individuals have been on a plant-based diet long-term.[19] Food pattern modelling found that healthy vegan and vegetarian diets were inadequate in iron for women of reproductive age, especially pregnant women.[5] A systematic review and meta-analysis of observational studies and intervention trials found that vegans and vegetarians consistently have lower iron stores than omnivores.[20]

Vegans and vegetarians can improve their iron status by consuming iron-rich plant foods like beans and lentils, paired with vitamin C-rich foods and consumed separately from tea, coffee, and calcium supplements to enhance absorption. Considering one third of women in the U.S. are currently iron deficient,[17] relying on iron-fortified foods may not be reliable enough for vegan and vegetarian women. Iron status also varies substantially depending on the individual. Vegan and vegetarian women should monitor their iron status and supplement only if needed, as unnecessary iron supplementation can cause gastrointestinal distress, oxidative stress, and may increase infection risk in iron-replete individuals.[16]

Zinc

Zinc is widely available in plant and animal-source foods, but most zinc in plant sources such as beans, nuts, seeds, and whole grains is bound to phytate, which hinders absorption. Zinc deficiency can impair immune function, delay wound healing, cause growth faltering in children, and increase susceptibility to diarrhea and respiratory infections.[21] Since eggs and dairy are good sources of zinc, food pattern modeling found that healthy vegetarian diets are adequate in zinc for all age-sex groups.[5] However, for healthy vegan diets, most age-sex groups were inadequate in zinc.[5] Based on actual diets of young adults in Germany, 67% of vegans and 24% of vegetarians were zinc deficient versus 11% of omnivores.[22] Daily oral zinc supplementation resolved deficiency in vegans and vegetarians, indicating the efficacy of the intervention.[22] Vegetarians should consume eggs and dairy regularly as well as beans, lentils, nuts, and seeds, to prevent zinc deficiency without supplementation. Vegans should consume beans, lentils, nuts, and seeds, as well as zinc-fortified foods and consider oral supplementation if needed. Soaking, sprouting, and fermenting legumes, nuts, seeds, and grains improves zinc bioavailability.

Vitamin D

Other than certain UV-exposed mushrooms, vitamin D is lacking in plant foods. Vitamin D deficiency causes rickets in children, osteomalacia in adults, and may increase risk for infections, cardiovascular disease, and diabetes.[23] About 25% of the U.S. has a moderate vitamin D deficiency,[23] and vegetarians and, especially vegans, are at increased risk.[24] Food pattern modeling of healthy vegetarian and vegan diets found no age-sex groups could meet vitamin D requirements.[5] While sunlight typically contributes to requirements, for all age-sex groups the contribution from healthy vegan diets in the food pattern modeling was close to zero compared with healthy vegetarian diets, which were around one-third of requirements, demonstrating how dependent vegans are on sunlight or supplementation.[5] Vitamin D and calcium supplementation appears to reduce the risk of fractures in vegans.[24] Current vitamin D fortification in plant-based foods is inconsistent, so vegans and vegetarians who do not obtain enough sunlight to meet requirements need to supplement to ensure adequacy.

Calcium

Calcium is available in plant and animal-source foods, although dairy is a primary source in the U.S. diet. Calcium deficiency compromises bone density leading to osteoporosis and increased fracture risk and may contribute to hypertension and cardiovascular disease.[25] Food pattern modeling found that healthy vegetarian diets could meet calcium requirements for all age-sex groups yet vegan diets fell far short of meeting calcium requirements for all age-sex groups.[5] Vegetarians should consume dairy daily to meet calcium needs. A systematic review and meta-analysis of observational studies confirms that vegans consistently have lower calcium intakes.[26] In the EPIC-Oxford cohort, vegans were at increased risk of bone fracture due to low calcium intakes.[27] Vegans should consume calcium-fortified plant-milks, tofu set with calcium, and low-oxalate leafy greens (eg, kale, collards) to increase calcium intake. Supplementation for vegans may be required to protect bone health, however, this should be done cautiously based on each individual's risk, given that excess calcium supplementation may increase risk for cardiovascular disease and kidney stones.[28]

Iodine

Iodine content in foods is highly variable and dependent upon soil iodine content. Animal-source foods, including dairy, are the most reliable dietary sources of iodine. Iodine deficiency causes goiter and hypothyroidism with associated fatigue and weight gain, and, when occurring during pregnancy and early childhood, can result in permanent cognitive impairment and developmental delays.[29] Vegetarians should consume dairy regularly to ensure iodine needs are met. Vegans in countries around the world[30] and in the U.S.[31] are at increased risk for iodine deficiency; to ensure adequacy, they should supplement daily with 150 µg iodine (no higher, to avoid excess) or regularly consume iodized salt.[32] While seaweed has high iodine content, it is highly variable, and frequent consumption could lead to excess iodine intake and associated thyroid dysfunction.[32]

Choline

Choline is available in small amounts in plant-source foods but is much more concentrated in animal-source foods, including eggs. Choline deficiency can cause fatty liver disease, muscle damage, and during pregnancy may increase risk of neural tube defects and impair fetal brain development.[33] Food pattern modeling found that healthy vegetarian and especially vegan diets were highly inadequate in choline for all age-sex groups.[5] Only 10% of Americans consume the AI for choline.[33] Vegetarians should consume eggs regularly to ensure choline needs are met. Given the low choline content in plant-source foods, vegans should consider supplementation to meet choline needs.

Vitamin A

Vitamin A in plant-source foods exists as provitamin A carotenoids, requiring conversion to active retinol, with efficiency averaging about 12:1 but varying depending on the source and individual characteristics.[34,35] Food pattern modeling found most age-sex groups consuming healthy vegetarian diets were adequate while healthy vegan diets for most age-sex groups were inadequate in vitamin A.[5] Vegetarians should consume eggs and dairy regularly to ensure vitamin A adequacy. Poor conversion efficiency, affected by genetics and nutritional status, can influence vitamin A status, especially in vegans who do not consume retinol.[34] Vegans should consume carotenoid-rich foods such as carrots, sweet potatoes, and dark leafy greens with fat to enhance absorption and ensure adequate zinc status to support conversion.[34]

Omega-3 Fatty Acids

Omega-3 fatty acids are available in terrestrial plant sources such as nuts and seeds as ALA and in animal sources (particularly fatty fish) and algae as DHA, EPA, or DPA. ALA is less bioavailable than these critical long-chain omega-3 fatty acids and is converted into them at a 10:1 ratio at best.[36] Omega-3 fatty acid deficiency, particularly EPA and DHA, may increase risk of cardiovascular disease, impair cognitive function and visual development, and contribute to inflammatory conditions.[37] While food pattern modeling found that healthy vegetarian and vegan dietary patterns are adequate in ALA for all age-sex groups,[5] vegetarians and vegans are often inadequate in omega-3 and have low status in the body.[38]

Vegetarians and vegans who prefer to consume omega-3 fatty acids primarily through ALA should, along with ensuring adequate intake of ALA, consider reducing their omega-6 fatty acid intake since omega-6 and omega-3 compete for the same metabolic enzymes.[38] To ensure adequate omega-3 status in the body and optimal health, vegetarians and vegans should monitor their omega-3 status and ideally supplement with algal forms of DHA and EPA if needed.[38]

Protein and Amino Acids

Most plant sources of protein do not contain all essential amino acids in high enough quantities to meet requirements without being combined with complementary plant proteins or animal-source foods. Protein deficiency can lead to muscle wasting,

impaired immune function, delayed wound healing, edema, and in children, stunted growth and development.[39] While frank protein deficiency is rare in the U.S., many vegans—especially older adults, adolescents, pregnant women, athletes, and those with low energy intake—may fall short of optimal protein and leucine targets without deliberate planning. There are a few plant sources of protein that do not have major limiting amino acids, such as soy[40] and mycoprotein.[41] Most plant proteins, however, are limited in at least one essential amino acid—for example beans and lentils are low in methionine while grains are typically low in lysine.[40] Food pattern modeling found that healthy vegetarian diets met protein requirements for all age-sex groups whereas healthy vegan diets fell short for several age-sex groups, including pregnant women, lactating women, and men and women aged 51 years and older.[5] The modeling only assessed overall protein adequacy, not optimal intakes (see protein review for more details), nor adequacy of essential amino acids. Vegetarians should consume eggs and dairy regularly to ensure adequacy in protein and essential amino acids. Vegans should consume diverse protein-rich plant proteins, especially soy, and combine complementary plant proteins like beans and grains in meals to ensure adequacy of protein and essential amino acids.

Life Stage Considerations

Pregnancy and Lactation

During pregnancy and lactation, nutrient requirements increase substantially, making adequacy more challenging to achieve on vegetarian and, especially vegan, diets. Iron needs increase by 50% during pregnancy,[42] yet plant-based diets provide only non-heme iron with poor bioavailability, necessitating careful monitoring of iron status and likely supplementation. Vitamin B12 is critical for fetal neurological development, and deficiency can cause irreversible damage; pregnant vegans should take daily supplements because maternal stores are often inadequate and deficiency can cause irreversible fetal neurological harm.[9] Choline requirements are 450 mg/day during pregnancy and 550 mg/day during lactation for fetal brain development,[43] yet plant sources provide minimal amounts, making supplementation often necessary for vegans. DHA supports fetal brain and visual development, with studies showing lower DHA in breast milk of vegan mothers compared to omnivores, indicating the need for algal DHA supplementation.[44] Obtaining enough calcium during lactation is particularly challenging for vegans avoiding dairy, requiring regular consumption of calcium-fortified foods or moderate doses (eg, 500 mg) of calcium supplementation.[45] Prenatal vitamins specifically formulated for vegetarians and vegans, containing at a minimum, vitamin B12, iodine, algal DHA, and, if status is low, iron, should be initiated before conception when possible.

Infancy and Early Childhood

Vegan and vegetarian diets during infancy and early childhood require careful planning to support rapid growth and neurodevelopment. Exclusively breastfed infants of vegan mothers need vitamin B12 supplementation from birth (0.4 µg/day) as deficiency can

cause developmental delays and failure to thrive.[46] Iron stores from birth deplete by 4–6 months, and plant-based complementary foods provide insufficient bioavailable iron, making iron-fortified cereals or iron supplementation essential, especially between 6–11 months.[47] Vitamin D supplementation (400 IU/day) is critical for all breastfed infants but especially those in vegan families with limited fortified food options.[48] Calcium requirements (700 mg/day for 1–3 years) are difficult to meet without dairy, requiring fortified plant milks or supplementation.[49] Growth velocity should be monitored closely as studies show vegan children may be shorter and lighter than their omnivorous peers, though usually within normal ranges.[50] Energy density can be problematic as high-fiber plant foods may cause early satiety before caloric needs are met, requiring inclusion of energy-dense foods like nut butters and avocados.[51]

Older Adults

Older adults following vegetarian and vegan diets face unique challenges due to age-related physiological changes combined with plant-based dietary restrictions. Protein requirements increase to 1.0–1.2 g/kg body weight to prevent sarcopenia,[52] yet plant proteins have lower digestibility and leucine content critical for muscle protein synthesis, making adequate intake challenging without careful planning.[53] Vitamin B12 absorption declines with age due to reduced intrinsic factor and gastric acid production, affecting 10–30% of older adults regardless of diet, but creating particular risk for vegans who lack dietary sources entirely.[54] Vitamin D synthesis from sunlight decreases with age while requirements increase to 800 IU/day, and combined with limited dietary sources in vegan diets, supplementation becomes essential.[55] Calcium absorption efficiency declines while needs remain high (1,200 mg/day) for bone health, which is particularly concerning for vegans with already lower intakes.[56] Energy intake decreases due to age-related declines in calorie needs while nutrient density needs increase, requiring strategic food choices to meet nutritional needs with limited calories. Regular monitoring of sarcopenia and nutritional biomarkers, particularly B12 and vitamin D, becomes increasingly important for older vegetarians and vegans.

Key Evidence Gaps and Limitations

While this summary document is based on a triangulation of evidence, several limitations and evidence gaps should be acknowledged when translating these findings into dietary guidance. The evidence base itself has inherent limitations: food pattern modeling relies on idealized dietary patterns that may not reflect the actual, often less-optimal, food choices of individuals; observational studies, while valuable for identifying associations in real-world populations, cannot establish causality and may be influenced by confounding lifestyle factors; and randomized controlled trials are often short-term and may not capture the long-term health effects of sustained dietary patterns. Furthermore, much of the observational data comes from specific cohorts in Europe and the U.S. that may not be fully generalizable to the diverse American population.

A primary limitation in assessing nutrient adequacy is the cross-cutting issue of bioavailability from plant sources. Direct comparisons of nutrient intake levels between

omnivores and vegans/vegetarians can be misleading, as highlighted above for non-heme iron, phytate-bound zinc, provitamin A carotenoids, and ALA-based omega-3s. More research is needed to understand the practical impact of dietary inhibitors and enhancers on the long-term nutritional status of U.S. vegans and vegetarians. Similarly, while guidance often relies on consuming fortified foods, the level and consistency of fortification in the U.S. food supply are highly variable, making it an unreliable sole strategy for achieving adequacy for nutrients like vitamins D and B12. Evidence is also limited on the real-world feasibility, adherence, and equity of relying on supplementation and fortified foods across populations.

Another limitation is the scarcity of biomarker data—particularly in nationally representative surveys such as NHANES—where vegans are underrepresented and key biomarkers (eg, choline, iodine, DHA) are not consistently measured, limiting the ability to assess nutrient status directly rather than by intake estimates. Finally, significant individual variability in nutrient absorption and metabolism presents a challenge for creating population-level recommendations. Genetic factors can influence the efficiency of converting plant-based precursors into their active forms, such as carotenoids to retinol. Likewise, an individual's existing nutrient status, particularly for iron, dictates the safety and necessity of supplementation. These evidence gaps are especially pronounced during critical life stages such as pregnancy, infancy, and older adulthood, when nutrient requirements are heightened and consequences of inadequacy are most severe. More high-quality, U.S.-based longitudinal research is needed to clarify the long-term health outcomes—beyond nutrient adequacy alone—for diverse individuals following vegan and vegetarian diets across the life course within the current American food environment.

Conclusions and Implications for the DGAs

Vegetarian and vegan diets can support health but present distinct challenges in achieving nutrient adequacy. For lacto-ovo vegetarians, shortfalls are most likely in iron, vitamin D, and choline, though these can often be met with regular egg and dairy (with added vitamin D) intake. For vegans, the risks are broader and include vitamin B12, iron, calcium, vitamin D, zinc, iodine, choline, omega-3 fatty acids (EPA/DHA), and protein. Achieving adequacy on a vegan diet requires deliberate planning, consistent use of fortified foods, and, critically, targeted supplementation—particularly vitamin B12, which is essential to prevent irreversible neurological harm.

The DGAs should therefore provide explicit, practical guidance that distinguishes between vegetarian and vegan patterns. This includes highlighting the importance of bioavailability, noting that higher intakes of non-heme iron and zinc are needed to overcome absorption inhibitors, and clarifying that supplementation is a more reliable strategy than fortified foods for nutrients like vitamin D and B12. Special emphasis is needed for vulnerable life stages—pregnancy, lactation, infancy, early childhood, adolescence, and older adulthood—where nutrient requirements are elevated and the consequences of inadequacy can be severe.

Finally, the DGAs should acknowledge key evidence gaps, including the reliance on idealized food pattern models, limited nationally representative biomarker data, especially for vegans and vegetarians, and variability in individual nutrient absorption and metabolism. Clear guidance for individuals to work with healthcare providers to monitor nutritional status for key biomarkers such as iron, vitamins B12 and D, and omega-3 can help mitigate risks. By addressing these limitations transparently, the DGAs can equip Americans choosing vegetarian and vegan diets to do so safely, while setting a research agenda to strengthen the evidence base for future updates.

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