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Review

Beyond Antioxidant Capacity: Selected Fruit and Vegetable Interventions, Redox Mechanisms, and Cardiometabolic Evidence—A Proposed Six-Gate Critical Review

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Abstract

Chemical antioxidant-capacity assays of fruits and vegetables cannot alone establish human clinical benefit. This purposively sampled critical narrative review evaluates selected berry-derived formulations, anthocyanin interventions, high-lycopene tomato foods, and broccoli-sprout extract in relation to redox biology and cardiometabolic outcomes. It distinguishes the edible or processed intervention from human metabolite exposure, target engagement, surrogate biomarkers, and patient-important endpoints. In a freeze-dried blueberry powder trial, a null primary insulin-resistance result coexisted with a secondary endothelial signal; pooled berry blood-pressure estimates were sensitive to the confidence-interval method; and tomato and broccoli-extract studies illustrated different limitations of endpoint-specific inference. We propose an unvalidated qualitative Six-Gate Translational Evidence Framework to annotate the particular links actually measured for an individual claim, to document missing causal links without changing trial effect estimates or assigning a new certainty grade. It does not replace RoB 2, GRADE, or formal causal inference. The review does not claim systematic coverage, quantitative pooling, or proof of clinical efficacy by antioxidant mechanisms. Claims should be bounded to the intervention format, population, comparator, and outcome examined.

Keywords: fruits and vegetables; natural antioxidants; polyphenols; food matrix; bioavailability; gut microbiota; redox signaling; anthocyanins; randomized trials; translational evidence

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1. Introduction: The Antioxidant Paradox

The health appeal of fruit and vegetable antioxidants is intuitively compelling: plant foods contain redox-active molecules, oxidative stress is implicated in many chronic disorders, and food intake is repeatedly associated with favorable population outcomes. However, moving from these three observations to the claim that a compound prevents human disease by directly neutralizing radicals requires several additional causal steps. The familiar "high antioxidant capacity = high clinical efficacy" formula obscures these steps and can mislead experimental design [1,2].

The chemical activity of an extracted compound in a cell-free tube is a legitimate analytical property, but the human organism is not a dilute reaction vessel. Physiological radical fluxes, enzymatic defenses, metabolite binding, dose and clearance alter the likelihood that a dietary phytochemical can scavenge biologically important species in vivo. Redox-mediated regulation of endogenous defenses and signaling may be more credible for selected classes

and settings; even then, in vitro pathway activation is not synonymous with demonstrated human benefit [1–3].

At population scale, a meta-analysis of prospective cohort studies observed lower cardiovascular disease and mortality risk with higher fruit and vegetable consumption [4]. Another synthesis combining two large US cohorts with 24 further cohorts reported the lowest observed mortality around five daily servings, compared with two daily servings, while acknowledging the observational design [5]. Such data support dietary patterns, not a causal attribution to individual antioxidant molecules. Fruits and vegetables also provide fiber, potassium, folate, water and other components, and may displace less healthful foods.

Recent reviews address flavonoid pharmacokinetics, gut-derived metabolites, or cardiometabolic pathways [3,6,7]. The contribution attempted here is different: we restrict the central analysis to selected fruits, vegetables, their formulated foods and extracts, and mixed-format evidence syntheses and assess whether the successive links between food identity and a clinically meaningful human endpoint have actually been measured. We thus distinguish analytical quality, mechanistic plausibility and clinical certainty instead of treating them as interchangeable.

A 2026 review already integrates polyphenol bioavailability, microbial biotransformation, endothelial signaling and cardiometabolic relevance [7], while a scoping review maps clinical studies of microbial phenolic metabolites [8]. Accordingly, we do not claim priority for these themes, invent a new biological pathway or offer a validated clinical score. The proposed contribution is a six-link, claim-level audit that makes missing transitions explicit: verified food identity, matrix, internal exposure, target engagement, biomarker validity and patient-relevant outcome. Its incremental value compared with existing narrative, reporting and evidence-appraisal frameworks remains a hypothesis for external testing.

2. Review Approach and Limits of the Evidence Base

This targeted critical narrative review evaluates published evidence available through 23 October 2026 and is not a systematic review or meta-analysis. The working literature was assembled purposively from PubMed-indexed records, publisher-hosted articles and citation following, with priority given to randomized human interventions, endpoint-specific systematic reviews, large prospective cohorts, mechanistic reviews, and authoritative methodological guidance. Historical search logs, exportable database-specific results and an independently screened study set were not retained; the strategy cannot therefore be represented as a reproducible systematic search. The authors do not claim that every potentially eligible publication was identified.

Publications were selected purposively to represent convergent evidence, conflicting human results, or methodological limitations. No claim is made to database exhaustiveness, duplicate screening, formal risk-of-bias assessment, pooled effect estimation, or PRISMA compliance. Consequently, the synthesis is interpretive; the cited studies serve as representative anchors and should not be read as a complete inventory of the literature. A reproducible systematic search and formal evidence grading would require a separate protocol and documented screening record.

2.1. Scope, Evidence Selection and Reporting Transparency

The selected clinical examples concern berry-derived foods and formulations, mixed anthocyanin interventions, high-lycopene tomato foods and broccoli-sprout extract; cohorts concern broader dietary patterns, while cancer and neurodegeneration are considered only as inference boundaries. Literature discovery used PubMed-indexed records, publisher sites and citation following through 23 October 2026, focusing on fruit/vegetable intake and mortality, anthocyanins and FMD, berry blood pressure, tomato LDL-C, broccoli extract

and glycemia, and microbial metabolism. Examples were chosen to illustrate contrasting outcomes and exposure-to-endpoint limitations rather than to enumerate all eligible studies; this selection may overrepresent striking findings. No reproducible systematic search, duplicate screening, exclusion log or formal certainty grading was undertaken, and overlapping syntheses are not independent replications. Cocoa, tea, coffee and wine are outside the core evidence set; cocoa flavanols are an external calibration case, supported by the EFSA 200 mg/day vasodilation claim [9] and the FLAVIOLA trial of 100 healthy adults (450 mg twice daily for one month versus matched control; FMD difference +1.2 percentage points, 95% CI 1.0–1.4) [10], which demonstrates a measured physiological effect rather than prevention of clinical events.

Generative-AI assistance. During the preparation and revision of this narrative review, OpenAI ChatGPT (GPT-6.0; accessed on 31 October 2026) was used for methodological organization, drafting and editing scientific English, candidate-reference discovery and preparation/revision of original conceptual diagrams. The models did not generate patient-level data, randomized treatment effects, systematic-review search histories, or independently validated numerical findings. The authors are responsible for comparing clinical assertions and graphic labels with primary sources and for verifying the final figures, references and manuscript. See Acknowledgments for the product disclosure.

2.2. Logic for Comparing Heterogeneous Evidence

Comparisons were organized around four distinct inferential targets: chemical activity under a defined assay; verified uptake or biotransformation in humans; effect on a molecular or physiological marker; and change in clinically meaningful outcomes. These targets have different causal demands and must not be merged into an omnibus "antioxidant benefit" rating. When studies disagree, the default explanation to test is structured heterogeneity—population risk, baseline diet, dose, food/extract preparation, adherence, metabolite profile, observation window and endpoint definition—rather than selecting the favorable result. A null result can mean no biologically relevant effect, inadequate exposure, poor target engagement, insufficient statistical power or an inappropriate measurement; those mechanisms cannot be distinguished without complementary data.

Because trial overlap across the selected meta-analyses was not independently enumerated in a deduplicated evidence matrix, the pooled reports are discussed as contextual syntheses, not counted as independent replications. The source review authors used different populations, product forms, and endpoint definitions [11,12].

2.3. Endpoint Specificity, Overlapping Evidence and Critical Appraisal

To reduce selective mechanistic narration, this review compares (i) enrolled population and background risk; (ii) food, extract or supplement matrix and delivered dose; (iii) prespecified primary outcome, comparator and follow-up; and (iv) estimand, effect size, confidence interval and documented null findings. Secondary or exploratory results are identified as such. In particular, a within-arm mean change and its interval are not the same estimand as a between-arm test; a p value reported for the overall treatment comparison must not be attached to an arm-specific interval as though they represented one estimate. Where trials are represented in more than one meta-analysis, their findings do not count as independent replications [11–16].

The Six-Gate audit concerns missing steps between exposure and mechanism; conventional tools address different questions. RoB 2 evaluates bias in individual randomized results, GRADE rates certainty for a body of evidence and SANRA addresses the quality of narrative reviews [13,14,17]. We do not assign retrospective RoB 2 or GRADE scores without a documented systematic procedure. The ensuing comparisons are a structured interpretation of selected published findings, not a de novo evidence synthesis.

2.4. Claim-to-Source Audit and Counterexample Selection

The selected original-source checks concentrated on the randomized blueberry study [15], an anthocyanin synthesis [11], the hypertension-specific berry synthesis [12], the broccoli sprout-extract trial [18], the PR-7 tomato trial [19], the prospective cohort synthesis [5], and the 2026 Brown scoping review and its correction [8]. For these examples, interpretive attention was directed to intervention identity, study population, primary versus secondary endpoints, confidence intervals and null findings. This is a non-exhaustive selection of published studies, not PRISMA screening or a quantitative meta-analysis.

Selection rationale by inference domain: The study by Curtis et al. [15] was chosen to contrast a null primary metabolic endpoint with a secondary vascular signal under a standardized processed-food intervention; Avendano et al. [11] and Guevara Guevara et al. [12] to contrast pooled FMD and blood-pressure estimands and analytic uncertainty; the PR-7 tomato trial [19] to distinguish a visit-specific from a global time-by-treatment test; Avesani et al. [18] to anchor a null result in a high-risk extract-treated population; and the two prospective dietary syntheses [4,5] to bound observational causal claims. The corpus deliberately samples these inferential situations and does not estimate how often they arise in all fruit-and-vegetable trials.

To keep causal claims traceable, the review uses a source-linked chain: (a) identify the exact edible intervention or its derivative; (b) specify the population and comparator; (c) distinguish the prespecified primary endpoint from secondary, exploratory and post hoc analyses; (d) record whether a value is a within-arm change, between-arm contrast or repeated-measures interaction; and (e) restrict the conclusion to that estimand and its reported uncertainty. As an editorial safeguard, priority trial findings were checked against accessible original abstracts or full texts, while a complete dual-extraction systematic evidence audit is not claimed. This distinction matters because a statistically significant single-visit comparison need not coincide with a significant overall time-by-treatment interaction [15,16,19].

Negative results were deliberately retained when they create an informative boundary: the neutral HOMA-IR primary outcome in the blueberry trial [15], the nonsignificant pooled resting systolic blood-pressure estimate in a hypertension-specific berry synthesis [12], and the null glycemic findings in the kidney-disease broccoli-extract trial [18]. These are not selected to establish that fruit or vegetable bioactives lack value; they counterbalance an otherwise selective focus on positive biological markers. A future systematic synthesis would require registered eligibility criteria, database-specific searches, deduplication, independent study selection, full-text screening logs and formal risk-of-bias procedures; none should be retroactively claimed for the present narrative review [13,14,17].

3. What Constitutes an Antioxidant in an Edible Fruit or Vegetable?

The label “natural antioxidant” covers different physicochemical and biological entities. Vitamin C is a water-soluble essential nutrient and electron donor with established physiological functions. Carotenoids are lipophilic pigments whose absorption depends strongly on release from plant structures, micellarization and co-consumed lipid. Polyphenols comprise chemically heterogeneous flavonoids, phenolic acids and other molecules

whose conjugated and microbial metabolites frequently differ from the native food compounds. Glucosinolate-derived isothiocyanates, including sulforaphane from cruciferous vegetables, are better conceptualized as electrophilic stress-response modulators than as simple stoichiometric radical scavengers [1,3,6,20–23].

Figure 1 displays four chemically distinct families: a water-soluble nutrient, lipid-soluble pigments, polyphenols (with anthocyanins as a named subclass), and glucosinolate-derived electrophilic products. It illustrates differences in food sources and exposure requirements, not an efficacy ranking.

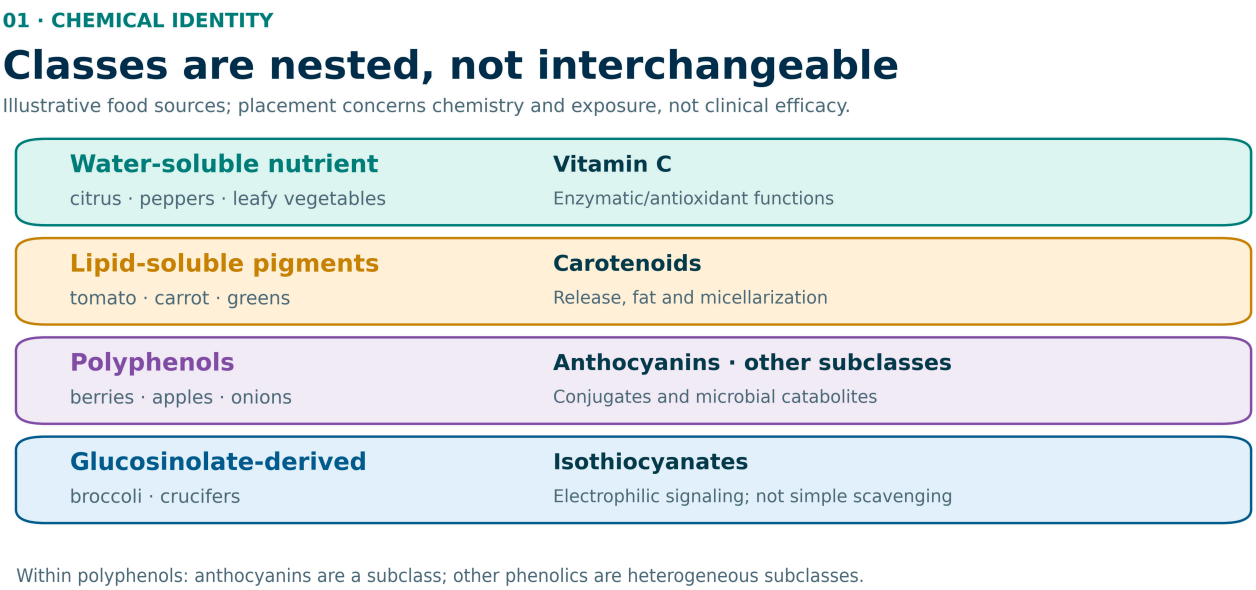

Figure 1. Chemical families relevant to the selected edible plant interventions. Anthocyanins are a subclass of polyphenols rather than a family parallel to all polyphenols; isothiocyanates are glucosinolate-derived electrophiles rather than direct radical-scavenging equivalents. Food examples are illustrative, and no clinical efficacy ranking is implied.

Table 1 maps frequently studied edible sources to their candidate pathways without implying disease prevention by any single food. Because content changes with genotype, agronomy, ripening, storage, preparation, cultivar and portion size, names such as "blueberry" or "broccoli" alone are insufficient descriptions of interventions [24,25].

Class-Specific Mechanisms Require Class-Specific Exposure Models

Water-soluble nutrients, lipophilic carotenoids and polyphenol-derived catabolites do not share a single distribution volume or exposure timeline. Vitamin C participates in essential biochemical reactions; it should not be discussed as if it were interchangeable with polyphenols that undergo extensive glucuronidation, sulfation and microbial degradation. Carotenoid absorption depends on plant-cell architecture, lipid-containing meals and host transport, whereas sulforaphane availability depends partly on the conversion of glucoraphanin and subsequent metabolism [3,20,21,23,24]. Consequently, a food-composition table cannot be interpreted as a rank-ordered list of expected clinical efficacy. Dose normalization should distinguish edible grams, estimated molecular equivalents and directly measured plasma or urinary species.

Table 1. Food-specific chemical classes and constraints on translation (conceptual synthesis; pathways are candidates unless human target engagement has been established). NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2.

Edible Source	Representative Bioactives	Biological Considerations	Key Limitation
Berries; purple fruits	Anthocyanins, phenolic acids	Vascular signaling; microbial catabolites; NO-related responses	Plant pigments do not predict circulating exposure; outcomes vary by population [11,12,15,26]
Pomegranate; some berries	Ellagitannins, ellagic acid	Microbial conversion to urolithins; person-specific metabotypes	Non-producers and producers may have different metabolite exposure [26]
Tomato; red produce	Lycopene and other carotenoids	Lipid-phase handling; altered absorption with processing/fat	Blood concentration does not isolate a unique mechanism [19,23]
Carrot; leafy vegetables	β-carotene, lutein, zeaxanthin	Matrix disruption and micellarization	Whole-food inference cannot be replaced by high-dose supplements [23,27]
Broccoli; other crucifers	Glucosinolates → isothiocyanates	Electrophilic signaling; candidate Nrf2 response	Myrosinase activity, precursor conversion and cohort context matter [18,21,28]
Citrus; peppers; green vegetables	Vitamin C, assorted phytochemicals	Micronutrient adequacy; antioxidant and enzyme cofactor biology	High-dose vitamin supplementation is not equivalent to food-based benefit [20]

4. Why Chemical Antioxidant Capacity Is Not Clinical Evidence

DPPH, ABTS, FRAP and oxygen radical absorbance capacity (ORAC) assays answer questions about chemical reaction capacity under specified test conditions. The measured number is conditional on solvent, extraction, reaction time, substrate, pH and analytical design. The ORAC method itself can be valuable for defined food analytical comparisons; its limitations arise when a chemically valid result is promoted as proof of protection against a human disease [2,22].

Mechanistic plausibility improves when an experiment uses molecular species actually detected in humans after consumption, at physiologically attainable concentrations, and when the temporal sequence from exposure to target response is documented. Testing a parent flavonoid at high micromolar doses in cells may be informative for discovery but cannot substitute for experiments involving its circulating glucuronides, sulfates or microbial products. Biological context also matters: physiological reactive oxygen species can participate in signaling, so generalized suppression of oxidation is not necessarily desirable [1–3].

Redox regulation may involve electrophile sensing through Kelch-like ECH-associated protein 1 (KEAP1) and nuclear factor erythroid 2-related factor 2 (Nrf2), modulation of inflammatory signaling, and endothelial nitric oxide (NO) availability. However, the causal weight of these mechanisms is compound- and population-specific. Nrf2 target-gene induction in cultured cells demonstrates a mechanistic possibility; a clinical claim would require exposure verification, pathway-specific evidence in humans, and an independent physiological endpoint. Inflammatory biomarkers, lipid oxidation and endothelial function represent distinct biological constructs and should not be conflated.

Redox-Homeostasis Claims and the Reverse-Translation Problem

Reactive oxygen and nitrogen species have physiological functions in signal transduction and host defense. Therefore, evidence that a molecule lowers one oxidation-related

readout is not automatically evidence that global ROS reduction is beneficial. Mechanistic reports should identify the oxidant species, compartment, kinetics and baseline pathophysiology; otherwise "antioxidant" risks becoming an unfalsifiable umbrella term. At low physiological exposure, nucleophilic/electrophilic signaling and adaptation may be more plausible for some food-derived molecules than stoichiometric quenching of all relevant radicals [1,2]. Nevertheless, pathway activation itself is not sufficient proof of patient-level benefit.

There is also a reverse-translation hazard: an in vitro concentration is chosen because it produces an attractive signal, the signal is linked retrospectively to a pathway, and a food containing a chemically related parent compound is then marketed as preventative. A stronger sequence would measure human metabolite kinetics first, confirm that the relevant conjugate or microbial catabolite reaches the tissue, reproduce the response at realistic unbound concentrations, and prospectively test whether intervention-induced exposure predicts the prespecified human endpoint. This procedure helps distinguish a coherent mechanism from a post hoc biological narrative [3,29].

5. The Food-to-Phenotype Continuum

Figure 2 isolates an exposure-to-outcome measurement continuum, highlighting where intervention composition, digestive accessibility, internal dose, pathway assays and phenotype must be established. Stages 1–4 correspond to G1–G4; the phenotype stage distinguishes G5 measurement validity from G6 patient-important relevance.

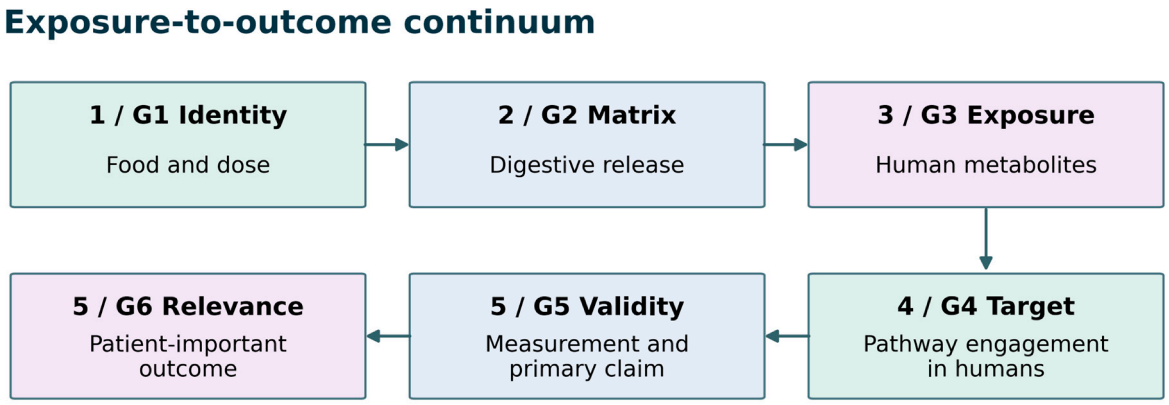

Figure 2. Five-stage food-to-phenotype continuum: stages 1–4 correspond to G1–G4, and stage 5 separates G5 measurement validity from G6 patient-important relevance. Arrows represent hypotheses to test, not established causal links. Host factors can influence several transitions.

5.1. Food Matrix, Preparation and Co-Ingestion

The same nominal carotenoid dose need not generate the same systemic exposure from different preparations. In a controlled randomized Latin-square feeding study ($n = 29$), participants consumed identical vegetable salads with 3, 8 or 20 g of canola oil, soybean oil or butter; 20 g of co-consumed lipid generally supported greater postprandial carotenoid absorption than 3 or 8 g, with lipid amount more influential than source [23]. This is direct evidence for a food-matrix/co-ingestion effect on exposure, not evidence for fewer cardiovascular events. Mechanical disruption or heating may change release of some carotenoids while degrading other heat-sensitive compounds; simplistic raw-versus-cooked efficacy rankings are unjustified [24].

For broccoli, glucosinolate conversion to isothiocyanates depends on enzyme availability and food preparation. A randomized crossover human study specifically administered cooked broccoli together with raw daikon radish, so its absorption results should not be cited as an effect of broccoli alone [21]. For polyphenols, processing can change glycoside profiles, polymerization, and accessibility to digestive enzymes or microbiota. Intervention reports should state species, cultivar if known, edible weight, thermal treatment, water loss, co-ingested lipid, chemically measured dose, storage conditions, and whether added conversion-supporting foods such as radish were used [21,24,25].

5.2. Human Exposure Is a Metabolite Problem

Polyphenol bioavailability cannot be reduced to a single percentage. Parent compounds, phase-II conjugates and microbial catabolites follow different trajectories and may have different targets. Williamson's 2025 synthesis emphasizes the extensive characterization of food-polyphenol metabolism [3]. The gut microbiota can convert unabsorbed polyphenols into smaller molecules that circulate systemically, while also shaping luminal and intestinal responses [6]. Consequently, plasma metabolomics and urinary excretion should be measured alongside the food intervention whenever a metabolite-dependent mechanism is proposed.

Ellagitannins provide a particularly instructive example. Intestinal microorganisms transform ellagic acid-related substrates into urolithins; distinct microbial metabotypes can lead to different profiles after consumption of foods such as pomegranate and some berries [26]. Stratification by baseline microbial function may be more informative than merely grouping participants by nominal dietary polyphenol intake. Nevertheless, microbial association is not the same as proof of mediation; causal mediation analysis requires defensible temporal ordering, reliable exposure measurement and explicit assumptions.

5.3. Interactions: Synergy Must Be Tested, Not Assumed

Whole foods contain combinations of fiber, micronutrients and phytochemicals that may modify absorption or biological responses. Yet "synergy" is frequently invoked without factorial experiments capable of separating additive from supra-additive effects. Future trials should include whole-food, matched extract, matrix-controlled and, when ethical and feasible, constituent-combination arms. If fiber or sugar differs substantially between arms, the effect should be described as a food-intervention effect rather than attributed uniquely to antioxidant synergy.

5.4. Microbial Metabotypes: Exposure Heterogeneity Is Established More Firmly than Clinical Prediction

For ellagitannin-rich fruit foods such as pomegranate and several berries, human gut organisms can generate urolithin derivatives that are not present in the same form in the raw fruit. The urolithin producer categories (often described as metabotypes A, B and 0) therefore represent a plausible source of heterogeneity in circulating chemical exposure [26,30]. However, demonstrating a producer classification is not synonymous with establishing that an intervention reduces cardiovascular events in that class. Microbial metabolism is dynamic and can be affected by habitual diet, antimicrobial exposure, age, sampling interval and substrate dose; stable classification should be demonstrated rather than presumed.

A systematic review of human polyphenol metabotypes published online in 2025 and assigned to a 2026 journal volume synthesized 33 randomized and 12 observational studies. Its cardiometabolic findings were inconsistent and substantial evidence concerned equol, a metabolite associated with soy isoflavones rather than the edible fruit and vegetable

interventions emphasized here [31]. A 2026 scoping review of 70 studies of measured microbial-derived phenolic metabolites likewise reported heterogeneity in measurement strategies, participants and clinical endpoints [8]. Its correction, published on 24 September 2026, restored an omitted funding acknowledgment to an author; the notice does not report a reanalysis, correction of outcomes or retraction [8]. These syntheses support the need for validated exposure phenotyping, not clinical treatment selection on the basis of an unvalidated microbial metabotype.

Metabotype status may be a mediator, an effect modifier or a correlate of broader dietary behavior, depending on study design. The three concepts require different analyses. To test effect modification, prespecify the interaction between randomized treatment and baseline metabotype and report its uncertainty. To test mediation, confirm post-randomization metabolite dynamics and specify assumptions regarding mediator–outcome confounding. To test reproducibility, repeat metabolite measurements across separate diet exposures. A metabolic signature without these safeguards should be described as exploratory.

Figure 3 separates gut-mediated formation and measurable circulation of candidate compounds from the untested inference that those metabolites mediate a particular clinical response.

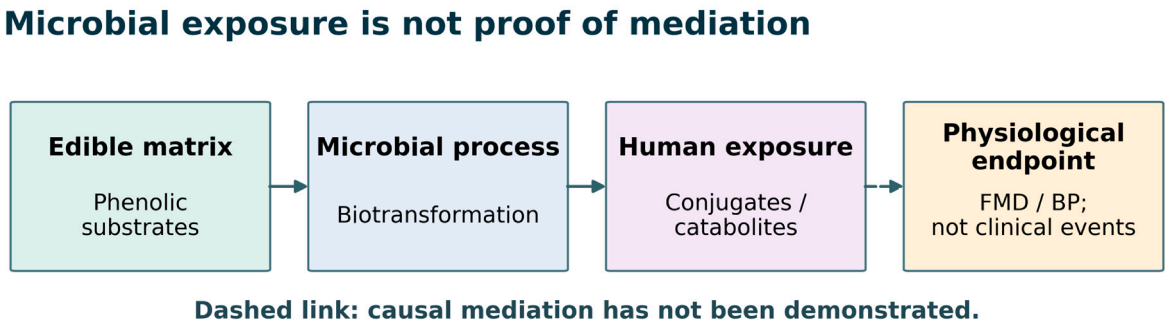

Figure 3. Microbial transformations, measured human exposure, and an unverified clinical mediation link. Solid arrows indicate a plausible metabolic route; the dashed arrow indicates that detecting circulating conjugates or catabolites does not establish their causal effect on flow-mediated dilation (FMD) or blood pressure (BP), which are physiological or surrogate outcomes rather than patient-important events. Metabotypes are exposure phenotypes, not validated clinical predictors [6,8,26,30,31].

6. Human Evidence: Promising Signals and Essential Counterexamples

6.1. Dietary Patterns and Long-Term Disease Outcomes

Higher fruit and vegetable intake is associated with favorable population outcomes across large longitudinal syntheses [4,5,32]. Wang and colleagues observed an adjusted hazard ratio for total mortality of 0.87 (95% CI 0.85–0.90) for approximately five daily servings compared with two servings in their two US cohorts [5]. The pattern is important for public health, but residual confounding, imperfect food-frequency reporting, differences in background diet and healthy-user effects constrain compound-specific causal inference. None of these cohort findings can establish that radical scavenging is the mediator.

Biomarkers of intake provide useful triangulation. However, higher plasma carotenoid or vitamin C concentrations may indicate general fruit and vegetable consumption and nutritional status rather than isolated biological efficacy [32]. Both controlled feeding and long-duration randomized outcomes studies would be needed to distinguish pathways convincingly; such trials are difficult, costly and, for some outcomes, impractically long.

6.2. Anthocyanins and Vascular Function: Endpoints Matter

A six-month double-blind RCT randomized 138 adults with metabolic syndrome; 115 completed the intervention [15]. Participants were allocated to 26 g/day milled freeze-dried blueberry powder (equivalent to 150 g fresh blueberries; 364 mg anthocyanins), a half-dose combination of 13 g blueberry powder plus 13 g matched placebo (equivalent to 75 g fresh blueberries), or 26 g/day isocaloric, carbohydrate-matched, blueberry-flavored placebo powder. These are formulated food interventions, not consumption of intact berries. The prespecified primary insulin-resistance endpoint (HOMA-IR) did not improve (overall treatment $p = 0.07$). In the full-dose arm, the published FMD change was +1.45 percentage points (95% CI 0.83–2.10); the reported between-treatment FMD test ($p = 0.003$) is not the confidence interval for an adjusted treatment-versus-placebo contrast. The actual powder matrix must be specified before the trial is invoked to support inferences about intact fruit [15].

A 2026 systematic review and meta-analysis included 18 prospective cohorts (reported across 27 cohort publications) and 65 randomized anthocyanin trials, mostly in healthy participants [11]. The Results subsection on endothelial function reports acute FMD +1.40 percentage points (95% CI 1.11–1.69; seven RCTs; Supplemental Figure 7 in Avendano et al. (2026)) [11] and chronic FMD +1.59 percentage points (95% CI 1.05–2.14; six RCTs; Supplemental Figure 10 in Avendano et al. (2026)) [11]. These differ from the abstract estimates (+1.50 and +1.41, respectively); we use the explicitly identified Results analyses rather than attach trial counts to the abstract estimates. The randomized evidence did not establish consistent improvements in blood pressure, lipids, fasting glucose or HOMA-IR. In a different target population, a 2026 meta-analysis of nine berry RCTs (547 adults with hypertension or elevated BP) found no statistically significant resting systolic BP benefit: −2.35 mmHg (conventional 95% CI −4.98 to +0.29; HKSJ 95% CI −5.41 to +0.72) [12]. For 24 h ambulatory diastolic BP (four studies; 219 participants), the −1.11 mmHg estimate had a conventional 95% CI of −2.04 to −0.17, whereas the Hartung–Knapp–Sidik–Jonkman interval (−2.23 to +0.02) included no effect. Certainty was low to very low for relevant outcomes. These syntheses differ in populations, interventions and endpoints; their estimates cannot be pooled, directly ranked or treated as independent validation of cardiovascular prevention [11,12]. The berry blood-pressure review described restricted maximum likelihood (REML) random-effects pooling with conventional confidence intervals and reported Hartung–Knapp–Sidik–Jonkman (HKSJ) intervals as sensitivity analyses [12]. For 24 h diastolic BP, the main conventional result is nominally significant (mean difference −1.11 mmHg, 95% CI −2.04 to −0.17), whereas its HKSJ sensitivity CI includes zero (−2.23 to +0.02, $p = 0.052$). We therefore characterize this inference as method-sensitive rather than selecting the HKSJ estimate as a replacement primary analysis. The publication reports prospective registration (PROSPERO CRD420251046489); the original registration text could not be retrieved for independent confirmation of which interval method was prespecified.

6.3. Carotenoid-Rich Vegetables and the Difference Between Exposure and Efficacy

A 12-week trial randomized 100 Japanese adults with LDL-C 120–159 mg/dL (50 per arm); 74 entered the efficacy analysis (33 active, 41 placebo). The trial compared 50 g/day of semidried high-lycopene PR-7 tomato (approximately 22.0–27.8 mg lycopene/day) with a lycopene-free semidried tomato comparator [19]. Blood lycopene increased after the active food. LDL-C change was the primary outcome; at the week-12 efficacy assessment, mean LDL-C changes were −3.7 mg/dL (active) and +4.1 mg/dL (comparator), with a reported visit-specific between-group $p = 0.027$. However, the global time × food interaction across the longitudinal LDL-C measurements was not significant ($p = 0.13$). Both results come from Table 5 of the cited Nishimura et al. trial and answer different statistical questions.

Therefore, the study suggests a favorable final-visit lipid signal but does not establish a robust longitudinal trajectory, cardiovascular event prevention or an antioxidant-mediated mechanism; relevant measured oxidative markers were not significantly different [19].

6.4. Cruciferous Vegetables: Reproducibility Across Clinical Populations

Sulforaphane is a mechanistically plausible electrophilic-signaling candidate, but concentrated broccoli sprout extracts are not equivalent to ordinary portions of cruciferous vegetables. A 2017 translational investigation reported reduced hepatic glucose production and improved glycemic measures in a selected population with type 2 diabetes treated with broccoli sprout extract [28]. In 2026, a multicenter randomized placebo-controlled trial enrolled 99 people with type 2 diabetes and chronic kidney disease stages 3b–4; 91 completed the study. The intervention escalated from 50 to 150 µmol/day broccoli sprout extract (BSE), using the source's formulation-specific units, for 12 weeks. Glycemic control was the main outcome; fasting glucose, insulin and HbA1c did not differ significantly from placebo at the end of treatment or after eight weeks of follow-up [18]. The published report documents changes in insulin and other glucose-lowering medication during follow-up; these background-treatment changes and the high-risk clinical context complicate cross-trial comparisons. The BSE dose is not a measured systemic sulforaphane concentration. This null result does not demonstrate equivalence; it concerns the tested preparation and population and is not proof that broccoli foods are ineffective or that the proposed molecular pathway is necessarily false.

6.5. Cancer, Neurodegeneration and Limits of Therapeutic Language

For cancer and neurodegenerative disorders, mechanistic observations involving inflammation, oxidative injury, neuronal survival, mitochondrial function or proliferation generate hypotheses, not confirmed clinical prevention or treatment effects. Dietary-pattern associations cannot identify a singular phytochemical mechanism. The ATBC randomized study provides a notable boundary condition: high-dose β-carotene supplementation increased lung cancer incidence in male smokers [27]. This finding cannot be transferred to ordinary consumption of carrots or leafy vegetables. Subsequent preventive guidance also recommends against β-carotene or vitamin E supplements for primary prevention of cancer or cardiovascular disease in nonpregnant community-dwelling adults [33]. Food effects, supplement effects, treatment contexts and vulnerable populations must be reported separately.

A rigorous review must therefore distinguish three claim levels: food-associated population outcomes; intervention-associated surrogate biomarker changes; and proven reductions in disease events. No study should be promoted to a higher level merely because the tested food has impressive radical-scavenging performance or because a pathway is biologically attractive. Clinical interpretation also requires stating, for each example, the intervention formulation, target population, randomization/comparator, delivered dose, follow-up and endpoint; the intervention evidence table below summarizes these dimensions. In particular, FMD is a vascular surrogate, LDL-C is a lipid biomarker, and neither establishes prevented clinical events, while the 12-week broccoli extract null result is not an experiment on habitual cruciferous-vegetable intake [15,16,18,19].

Table 2 retains the distinct longitudinal cohort and high-dose supplement context; Table 3 is the principal clinical intervention table. The rows are not pooled or directly compared.

Table 2. Cohort and high-dose supplement context for interpretation of dietary antioxidant evidence. FV, fruit and vegetable; CVD, cardiovascular disease; RCT, randomized controlled trial.

Evidence/Food	Intervention Form, Design and Population	Reported Signal	Inference Boundary
FV intake [4,5]	Large prospective cohorts; meta-analyses	Lower mortality/CVD risk with higher intake	Associational; cannot isolate antioxidant-specific causality
β-carotene capsules [27]	Large RCT in male smokers	Higher lung-cancer risk	High-dose supplement harms cannot be extrapolated to foods

Table 3. Selected human intervention results, actual intervention formats and inference limits. Abbreviations: BSE, broccoli sprout extract; pp, percentage points; BP, blood pressure; CKD, chronic kidney disease; DBP, diastolic blood pressure; FMD, flow-mediated dilation; HKSJ, Hartung–Knapp–Sidik–Jonkman; HOMA-IR, homeostatic model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; RCT, randomized controlled trial; SBP, systolic blood pressure. MD, mean difference; CI, confidence interval; CVD, cardiovascular disease; T2D, type 2 diabetes; HbA1c, glycated hemoglobin. FMD 150 g-equivalent arm change/CI is not the placebo-adjusted between-group contrast.

Study/Evidence	Population and Intervention	Primary or Reported Finding	What Cannot Be Concluded
Curtis et al. [15] (RCT, 2019)	138 randomized; 115 completed; 26 g/day milled freeze-dried blueberry powder (150 g fresh-weight equivalent, 364 mg anthocyanins) versus matched powder placebo; 6 months	Primary HOMA-IR null ($p = 0.07$). FMD change within 150 g arm: +1.45 points (95% CI 0.83–2.10); between-treatment FMD comparison $p = 0.003$. No BP improvement.	The arm change/CI must not be misreported as the adjusted contrast; secondary FMD does not prove fewer clinical CVD events.
Avendano et al. [11] (synthesis, 2026)	65 RCTs overall; heterogeneous formulations; 7 acute and 6 chronic RCTs in the specified Results analyses [11]	Acute FMD +1.40 pp (95% CI 1.11–1.69); chronic +1.59 pp (1.05–2.14); Supplemental Figures 7 and 10 in [11].	No clinical-event benefit established from FMD; not specific to hypertensive patients.
Guevara Guevara et al. [12] (synthesis, 2026)	Nine berry RCTs, 547 participants with elevated BP or hypertension	Resting SBP MD −2.35 mmHg, conventional CI −4.98 to +0.29; HKSJ CI −5.41 to +0.72. 24 h DBP MD −1.11, conventional CI −2.04 to −0.17 ($p = 0.020$); HKSJ CI −2.23 to +0.02 ($p = 0.052$).	Resting SBP null; 24 h DBP significance sensitive to CI method; low/very-low GRADE certainty.
Nishimura et al. [19] (RCT, 2019)	100 randomized (50/arm); 74 in efficacy analysis (33 PR-7, 41 placebo); semidried tomato, 12 weeks	Primary LDL-C: week-12 change −3.7 vs. +4.1 mg/dL ($p = 0.027$); global interaction $p = 0.13$.	A single favorable visit does not establish a consistent longitudinal effect or prevention of CVD events.
Avesani et al. [18] (RCT, 2026)	99 included, 91 completed; T2D/CKD 3b–4; BSE 50–150 µmol/day versus placebo, 12 weeks; 8-week follow-up; insulin and other glucose-lowering therapy changes reported [18].	Main outcome: glycemic control. No significant differences in fasting glucose, insulin or HbA1c at end of treatment or follow-up.	No equivalence established; cannot extrapolate to broccoli foods or other populations.

6.6. What the Human Studies Establish—And What They Do Not

The clinical contrasts in Table 3 illustrate that endothelial function, pressure regulation and glucose or lipid metabolism are distinct constructs; a favorable surrogate cannot substitute for a patient-important endpoint. Differences in formulation, risk and background therapy constrain transportability [11,12,15,18,19].

6.7. Stress-Testing the Antioxidant Narrative Against Primary and Secondary Endpoints

Table 3 is the principal clinical-evidence table: it identifies the actual product format, relevant population, primary versus secondary endpoints, and statistical interpretation for each selected study. All numerical effects are quoted from published sources rather than newly estimated, and Table 2 remains a concise contextual overview.

The comparison shows why original endpoint labels matter. A dietary intervention may support a biomarker hypothesis while failing to support a proposed primary therapeutic effect. Published confidence intervals and the authors' certainty ratings must accompany any attempt to convert these results into advice. The clinical value of an intermediate endpoint depends on its reproducibility, measurement protocol, relation to longer-term outcomes and the causal assumptions linking intervention to marker [12,16].

6.8. Interpretation of Discordant Clinical Findings

Figure 4 displays the identified FMD Results analyses [11] and published BP estimates [12] on separate axes. The two BP intervals are alternative methods for the same effect, not independent observations; zero is marked on both axes.

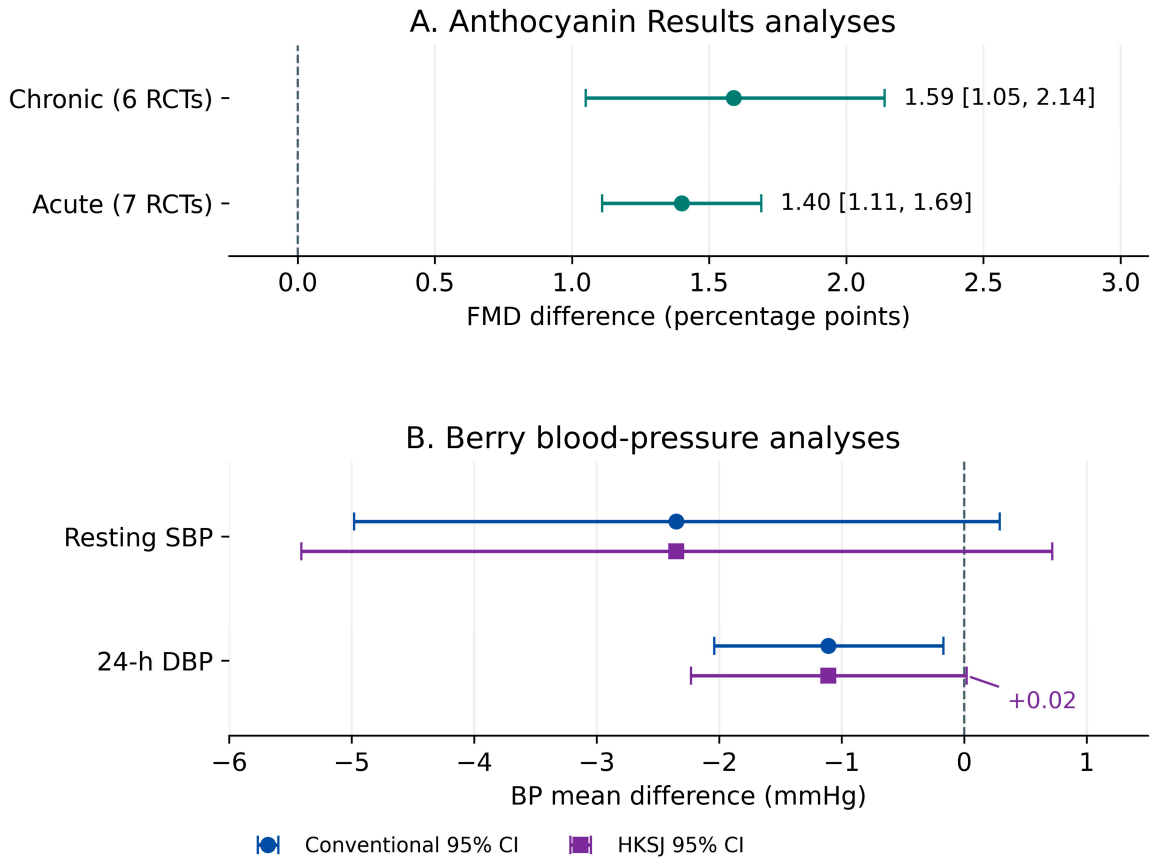

Figure 4. Published effect estimates (not a new meta-analysis). FMD: acute (7 RCTs, Supplemental Figure 7 in Avendano et al. (2026)) and chronic (6 RCTs, Supplemental Figure 10 in Avendano et al. (2026)), from the Results of [11]. BP: resting systolic (SBP) and 24 h diastolic (DBP) mean differences

from [12]. CI, confidence interval; FMD, flow-mediated dilation; REML, restricted maximum likelihood; HKSJ, Hartung–Knapp–Sidik–Jonkman; RCT, randomized controlled trial. The HKSJ upper limit for 24 h DBP is +0.02 mmHg.

7. Biomarkers: What Should a Trial Actually Measure?

Antioxidant research can fail even after recruiting human participants if the measured outcome is weakly aligned with the underlying biological question. A rise in "total antioxidant capacity" of plasma after a meal is difficult to interpret because abundant endogenous constituents and assay-specific chemistry affect the result. Conversely, urinary F2-isoprostanes measured by validated mass-spectrometric methods are established markers of lipid peroxidation, although sample timing, biological compartment and normalization influence interpretation [2,29]. Reduced lipid peroxidation is not in itself proof of clinical event prevention.

Prognostic association is not trial-level surrogate validation. A meta-analysis of prospective observational studies found that each 1-percentage-point higher brachial FMD was associated with a lower subsequent cardiovascular-event risk (adjusted RR 0.88, 95% CI 0.84–0.91); this between-person association must not be used to calculate event prevention caused by a 1-point FMD increase in a berry trial [34]. LDL-C provides a distinct example: randomized statin evidence links sustained approximately 1 mmol/L LDL-C lowering to a reduction in major vascular events, but this drug-class evidence cannot be transferred numerically to a 12-week tomato-food comparison with no significant overall LDL-C time-by-treatment interaction [35]. For Six-Gate, G5 evaluates whether a measured marker validly captures its stated construct; G6 separately asks whether patient-important effects were demonstrated for the specific intervention and population.

The more credible design prespecifies a hierarchy that separates compliance and exposure markers from target engagement and clinical outcomes. Food-weight logs or analytical intake profiles establish delivery; targeted plasma/urine metabolomics establish exposure; pathway-specific molecular assays support target engagement; endothelium-dependent flow-mediated dilation, ambulatory blood pressure, insulin sensitivity or other validated measures provide physiological endpoints. Clinical events, when realistic, remain the strongest outcome. Multiple-comparison adjustment and transparent null findings are essential [25].

Table 4 groups biomarkers by their actual biological construct, not by the generic expression "oxidative stress". Urinary F2-isoprostanes indicate lipid peroxidation under preanalytical constraints, oxidized LDL represents a lipoprotein-specific construct, and high-sensitivity C-reactive protein (hs-CRP) indexes inflammation; none alone validates an antioxidant clinical mechanism. These examples are not universal required assays.

For a candidate circulating polyphenol metabolite, G3 can be informed by timed plasma or urinary concentrations but not by parent-compound content alone. G4 requires evidence that the hypothesized pathway is engaged at a plausible unbound concentration in the relevant compartment and time window; protein binding and tissue access may matter. G5 evaluates the analytical and construct validity of the actual readout (for example, FMD or urinary F2-isoprostanes), independently of whether it proves pathway causation. A single assay can serve a target-specific and a measurement-validity role, but the evidence and limitations must be documented separately.

Table 4. Construct-specific measurement hierarchy for plant-bioactive studies (illustrative, not universal minimum requirements). Examples of assays include LC–MS, liquid chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; hs-CRP, high-sensitivity C-reactive protein; FMD, flow-mediated dilation; BP, blood pressure. Lipid peroxidation, lipoprotein oxidation, systemic inflammation and endothelial function are not interchangeable.

Layer	Example Measurement	What It Establishes	Typical Misinterpretation
Food analytics	LC-MS/HPLC-quantified food compounds; portion mass	Delivered intervention and chemical identity	Food ORAC alone treated as proof of bioefficacy
Exposure/adherence	Plasma and urinary metabolites; targeted kinetic profiles	Consumption and internal dose	Parent compound presumed main circulating effector
Mechanistic engagement	Target-tissue signaling; carefully validated transcript/protein responses	Biological plausibility of hypothesized pathway	Cell-culture Nrf2 result presented as human target engagement
Lipid peroxidation/ lipoprotein oxidation	Urinary F2-isoprostanes; oxidized LDL (assay-specific)	Distinct oxidative-damage constructs	Surrogate shift equated with prevention of clinical disease
Systemic inflammation	hs-CRP, when justified	Inflammatory response, not direct oxidative damage	Equating inflammation with oxidative damage
Functional physiology	FMD, 24 h ambulatory BP, glycemic metrics	Organ-system functional response	Short-term biomarker generalized across populations
Patient-relevant	Adjudicated clinical events; patient-centered outcomes	Clinically meaningful benefit/harm	Observational association described as randomized causation

Illustrative Biomarker Selection for Interpretable Trials

A practical hierarchy combines food analytics, internal dose, target-specific pathway readouts, and a prespecified physiological endpoint. First, analyze representative intervention batches and record edible mass, major active constituents and stability. Second, measure adherence and candidate plasma/urinary metabolites at time points aligned to plausible pharmacokinetics. Third, prefer an oxidative stress endpoint with established preanalytical safeguards, for example appropriately collected and interpreted urinary F2-isoprostanes, over the undifferentiated phrase "oxidative stress levels" [29]. Fourth, prespecify the clinically relevant endpoint and measurement method, such as blinded FMD acquisition or ambulatory blood-pressure monitoring.

The assays in the oxidative-damage and inflammation layers measure distinct constructs: urinary F2-isoprostanes indicate lipid peroxidation when supported by appropriate analytical and preanalytical controls; oxidized-LDL measures depend on assay definition; hs-CRP is a systemic inflammatory marker rather than a direct oxidative-damage assay; and FMD is a functional endothelial surrogate. None is a universal requirement, and agreement across assays must not be assumed. Where one measurement is used for both proposed target engagement (G4) and biomarker interpretation (G5), the causal and validity questions must be assessed separately [16,29].

A mechanistic discordance is scientifically meaningful. If food-derived metabolites rise without a corresponding FMD response, exposure has been verified but the proposed vascular pathway remains unsupported. If FMD changes while no candidate metabolite is detectable, the intervention may operate through another constituent or matrix feature; a polyphenol-specific attribution is premature. If both respond but ambulatory blood

pressure remains unchanged, the physiological conclusion remains limited to endothelial function. This logic allows null findings to refine mechanisms rather than simply being classified as failures.

A subsequent table provides the principal claim-language guide. Supplementary Figure S1 illustrates the same decision logic for readers requiring a visual summary; it is not a validated rating.

8. A Proposed Six-Gate Translational Evidence Framework

We propose the Six-Gate Translational Evidence Framework as a claim-level documentation audit for food-derived bioactives. Its contribution is the explicit juxtaposition of edible-intervention identity, matrix-dependent release, measured human exposure, target engagement, endpoint validity and patient-important outcomes within a single claim-specific audit. These elements are not individually novel, and assembling them into six labeled gates does not establish priority over every existing translational model. The audit has no validated scoring system, no demonstrated inter-rater reliability, no validated predictive performance and no regulatory or clinical-decision status. It complements rather than replaces RoB 2, GRADE or causal-inference study design [13,14].

The proposed gates are G1—food identity and quantified delivered dose; G2—matrix release and gastrointestinal bioaccessibility; G3—measured systemic exposure to candidate parent, conjugated or microbial species; G4—target engagement under physiologically relevant conditions; G5—measurement validity and alignment with the prespecified primary claim; and G6—patient-important outcomes, distinct from surrogate-only evidence. A gate can be unmeasured, inapplicable or contradicted; missing mechanistic proof is not equivalent to a failed intervention or a biased trial. Figure 5 visualizes the sequence, and Table 5 specifies testable questions. A causal chain is established only if competing explanations are addressed by appropriately designed studies.

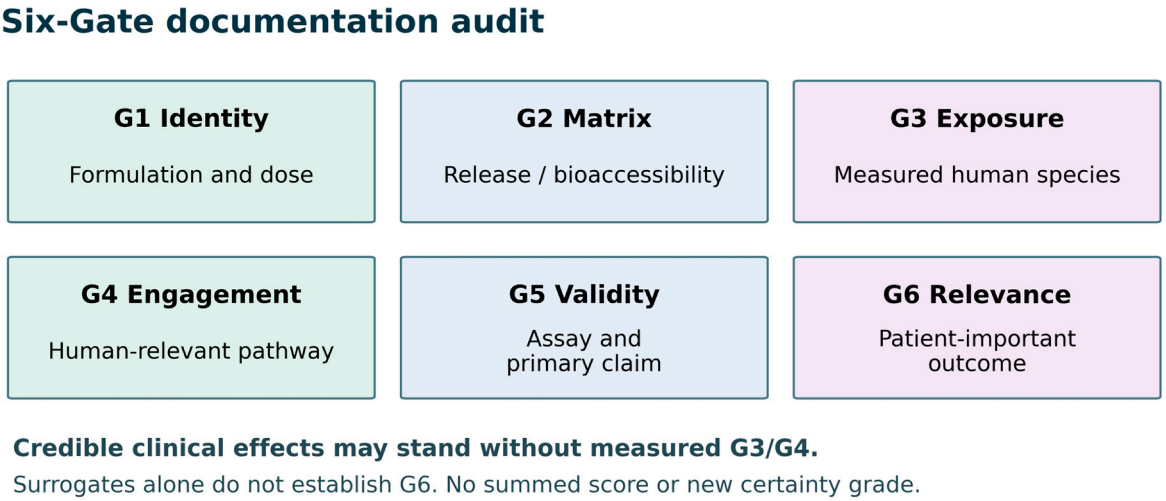

Figure 5. Six-Gate Translational Evidence Framework: G1 intervention identity and dose, G2 food matrix and bioaccessibility, G3 human metabolite exposure, G4 pathway-specific target engagement, G5 biomarker or measurement validity, and G6 patient-important relevance. Each gate is annotated descriptively, without numerical aggregation. An adequately estimated clinical effect may support a bounded outcome claim even when G3/G4 remain unmeasured; conversely, metabolite exposure or a surrogate response alone does not demonstrate clinical-event benefit.

Table 5. Claim-level Six-Gate questions and remedial evidence. A gate marked not assessed or not applicable does not imply a failed or biased trial. For a clinically tested outcome, the effect estimate is evaluated on its own merits irrespective of incomplete metabolite-specific mechanistic attribution. TAC, total antioxidant capacity; FMD, flow-mediated dilation; ORAC, oxygen radical absorbance capacity.

Gate	Minimum Question	Examples of Evidence Gaps (Not Equivalent to Failure)	Remedial Action
G1 Identity	Were the edible dose and composition verified?	Unspecified cultivar or extract content	Report edible mass, cultivar, batch, chromatography and stability
G2 Matrix	Were food preparation and digestive release considered?	Supplement/food assumed interchangeable	Specify processing, fats, food structure and in vitro digestion where relevant
G3 Exposure	Were plausible active metabolites observed in humans?	Cell work uses absent parent compound	Time-resolved metabolomics, bioavailability and microbiome context
G4 Engagement	Was a specified HUMAN-RELEVANT molecular target engaged at plausible free exposure in a relevant compartment?	Only high-dose cell results	Exposure-matched systems plus human pathway measures
G5 Validity	Does the assay reliably measure the named construct and align with the prespecified primary claim?	Secondary readout or time-point contrast only; assay validity may still be adequate	Validated assay, prespecified endpoint, repeat measurements
G6 Relevance	Does the outcome matter to patients?	FMD or ORAC framed as disease cure	Interpret the randomized clinical outcome independently; missing G3/G4 restrict mechanism claims, not verified G6 effects

Operational conventions (proposed; not externally validated). For each prespecified claim and each gate, use one of five evidence labels: DIRECTLY SUPPORTED when the necessary intervention-specific observation or validated measurement is reported; PARTIALLY DESCRIBED when a proxy or formulation detail exists but the gate's key causal link is untested; NOT ASSESSED when no relevant measurement is supplied; CONTRADICTED only when appropriately designed evidence directly weighs against the exact specified claim (not simply $p > 0.05$); or NOT APPLICABLE when a mechanistic gate is irrelevant to a strictly empirical efficacy question. Record the source, dose, comparator, population, time window, measurement, and an explicit reason for each label. For G5, a valid secondary assay or a visit-specific estimate that does not establish the audited longitudinal claim is PARTIALLY DESCRIBED; this is not an assertion that the assay is invalid or that a prespecified secondary endpoint was post hoc. The labels document evidence, not a new certainty grade or summed score.

Priority and conflict rules. A sufficiently credible randomized patient-important effect governs the bounded G6 efficacy conclusion even if G3/G4 mechanistic links are not measured: the mechanism remains unproven, not the observed effect invalidated. Conversely, measured metabolites at G3 with a null prespecified clinical endpoint at G6 support exposure, but not the claimed clinical benefit for that trial. A well-measured G5 surrogate with no G6 outcome allows only a surrogate-specific statement. An apparently favorable G6 result from a biased or imprecise trial still requires conventional RoB 2 and GRADE appraisal. The same measurement may inform G4 and G5 only if it independently satisfies

each gate's definition; double use never adds evidence or a numerical score. These rules are for transparent claim calibration and require external reliability testing.

A useful interpretation rule follows: a positive G5 biomarker without convincing G3 exposure cannot identify the responsible metabolite; a positive G4 molecular response without G6 events may justify mechanistic plausibility but not therapeutic efficacy. Conversely, a favorable G6 whole-food trial without a complete mechanistic chain can still justify a bounded food-based clinical conclusion, provided study quality is adequate. Translational gaps should calibrate mechanistic explanations, not erase observed outcomes.

8.1. Applying the Audit Without Turning It into an Unsupported Score

Documentation audit: incremental-value boundary. SANRA [17] evaluates whether a narrative review addresses a relevant question with adequate referencing, reasoning and literature presentation; it is a review-level quality guide. RoB 2 [13] addresses bias in individual randomized trial results, whereas GRADE [14] addresses certainty across a defined body of evidence for a particular outcome. Recent syntheses [7,8,31] already integrate polyphenol metabolism, microbial metabolites and clinical/cardiometabolic findings. Six-Gate does not replace these tools or claim novelty for those mechanisms: its narrower proposed use is to annotate, for each specific food-to-health claim, which links (food identity, matrix release, systemic exposure, target engagement, marker validity and patient-important outcome) have actually been observed, are missing, or have contrary evidence. Unlike SANRA/RoB 2/GRADE, it has not been independently validated; future studies must test inter-rater reproducibility and added value beyond simpler reporting checklists. Applying the proposed audit to the six-month blueberry trial demonstrates the difference between documented exposure and causal mechanism. G1 is directly supported by the defined blueberry dose and intervention description; G2 (matrix-derived bioaccessibility) is not fully established merely because the food form is described. G3 is supported by measured changes in circulating and urinary anthocyanin-derived phenolic metabolites, whereas G4—the necessity of a particular metabolite for vascular target engagement—remains unproven. FMD provides a measured secondary vascular surrogate (G5 PARTIALLY DESCRIBED), but G6 clinical event reductions were not tested. Moreover, the trial's primary insulin-resistance endpoint was null, a fact a narrative relying only on FMD could obscure [15]. These observations are selective examples, not a new formal study-level risk-of-bias assessment.

Comparison with established approaches. PICO describes the study question and effect estimate; ADME/PK–PD describes uptake, disposition and response; SANRA [17] appraises narrative review quality; RoB 2 [13] assesses bias in a randomized result; GRADE [14] appraises certainty across evidence for an outcome. The Six-Gate audit documents which food-to-outcome links are measured, missing or contradicted, without changing any of these assessments or effect estimates.

Worked difference versus conventional PICO description. A PICO summary of Curtis et al. [15] correctly states that standardized blueberry powder was randomized against matched placebo and that secondary FMD improved while primary HOMA-IR did not. A Six-Gate annotation adds only the explicit inferential boundary: G1 food/powder identity and dose documented; G2 processing known but digestive bioaccessibility not established; G3 circulating/urinary phenolic metabolites measured; G4 specific metabolite-to-endothelial causal engagement not demonstrated; G5 FMD observed but a secondary surrogate (PARTIALLY DESCRIBED); and G6 patient-important cardiovascular events not measured. Therefore, "the tested blueberry powder affected FMD under these conditions" is defensible, whereas "150 g intact fresh blueberries prevent cardiovascular events through

a proven anthocyanin mechanism" is not. The incremental contribution is traceability of missing causal links, not a different trial estimate or an independently validated judgment.

For broccoli sprout extract trials, the first distinction is whether the tested formulation reflects ordinary dietary broccoli or a concentrated preparation; otherwise G1/G2 may be valid within the trial but not transferable to food advice. Different glycemic responses in distinct diabetes/CKD cohorts [18,28] make population context and G6 endpoint design more important, not less. The audit explicitly allows a well-conducted positive clinical trial to stand on its observed outcome even if G4 is incompletely specified. Mechanistic incompleteness prevents confident mechanism attribution; it does not mathematically erase a randomized treatment estimate.

Table 6 supplies a language guide that prevents "antioxidant potential" from being escalated to disease-prevention wording. It is a proposed editorial instrument; neither the individual levels nor the Six-Gate structure have undergone external validity testing.

Table 6. Claim ladder for food-derived antioxidant research: minimum evidence and permitted inference (proposed editorial aid).

Claim Type	Required Minimum Evidence	Defensible Manuscript Wording
Analytical antioxidant activity	Identified sample; reproducible DPPH/ABTS/FRAP/ORAC or equivalent method	"Shows antioxidant activity under the reported assay conditions."
Human exposure	Well-specified food and timed verified plasma/urinary analytes	"Consumption increased exposure to specified metabolites."
Mechanistic response	Plausible human exposure; target-specific assay; appropriate control	"Consistent with modulation of the measured pathway."
Surrogate clinical effect	Randomized comparison; prespecified validated biomarker; effect and uncertainty	"Improved the specified biomarker in the studied population."
Disease-prevention or treatment effect	Appropriately powered trials with relevant disease outcomes and benefit-harm evaluation	"Reduced the specified event or clinical outcome under tested conditions."

8.2. Qualitative Six-Gate Applications and Compatibility with Established Quality Tools

The cases below are explicitly illustrative applications, not blinded duplicate study assessments. "Described" means a property was reported, not that it was independently verified here. Gate status must not be added or divided into a score: missing mechanistic evidence is not equivalent to an unsuccessful or biased clinical trial. For the cohort association, a patient-important outcome can be observed while causal attribution to one antioxidant remains weak.

Table 7 illustrates how the framework can locate different evidence gaps without creating a false numerical ranking of studies.

Claims audited in Table 7: (i) the characterized blueberry powder produces a clinically meaningful sustained vascular benefit attributable to its phenolic exposure; (ii) PR-7 tomato produces sustained lipid benefit attributable to lycopene-specific redox engagement. These are claims tested by the audit, not conclusions established by the trials.

The framework's most defensible use is prospective: report each proposed gate alongside the intended claim, the measurement that would test it and the observation that would disconfirm the mechanism. For clinical inference, established tools remain indispensable. An FMD effect measured with a rigorous protocol [16] is still not proof of reduced cardiovascular events; study-result risk of bias should be considered according to RoB 2 [13], and cross-study certainty according to GRADE [14].

Table 7. Illustrative Six-Gate source-by-gate annotations. Each source claim is described separately at G1–G6 as directly supported, partially described, not assessed, contradicted or not applicable; these are qualitative descriptive labels, not a score, RoB 2 assessment or GRADE certainty grade. FMD, flow-mediated dilation; HOMA-IR, homeostatic model assessment of insulin resistance; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol; pp, percentage points.

Case/Gate	Claim-Specific Observation [Source]	Provisional Status	What May Be Concluded
Blueberry/G1	26 g freeze-dried powder; 150 g fresh equivalent; 364 mg anthocyanins [15]	DIRECTLY SUPPORTED	Dose and actual intervention form specified
Blueberry/G2	Milled, freeze-dried matrix; mixed into foods/drinks; digestion not measured [15]	PARTIALLY DESCRIBED	Cannot infer intact-berry bioaccessibility
Blueberry/G3	Serum and 24 h urine phenolic metabolites increased [15]	DIRECTLY SUPPORTED	Measured exposure, not active-moiety proof
Blueberry/G4	No causal mediation demonstrating one metabolite drives FMD [15]	NOT ASSESSED	Mechanism attribution remains hypothetical
Blueberry/G5	Secondary FMD change +1.45 pp in full-dose arm; primary HOMA-IR null [15]	PARTIALLY DESCRIBED	Valid secondary surrogate; null primary HOMA-IR does not establish primary benefit.
Blueberry/G6	No patient-important CVD events as trial outcomes [15]	NOT ASSESSED	No trial proof of cardiovascular-event prevention
Tomato PR-7/G1	50 g/day semidried tomato vs. lycopene-free tomato control [19]	DIRECTLY SUPPORTED	Defined food and comparator
Tomato PR-7/G2	Semidried preparation specified; individual absorption kinetics not proved [19]	PARTIALLY DESCRIBED	Do not extrapolate to raw tomato
Tomato PR-7/G3	Circulating lycopene increased after food intake [19]	DIRECTLY SUPPORTED	Exposure confirmed, not pathway attribution
Tomato PR-7/G4	Specific redox target responsible for LDL-C not identified [19]	NOT ASSESSED	No specific antioxidant mechanism proved
Tomato PR-7/G5	Visit-wise LDL-C $p = 0.027$; overall time × food $p = 0.13$ [19]	PARTIALLY DESCRIBED	Primary LDL-C assessed at week 12; global trajectory remains inconclusive.
Tomato PR-7/G6	No adjudicated vascular events measured [19]	NOT ASSESSED	Cannot claim event reduction

8.3. Minimal Specification for Future Independent Validation

Before Six-Gate can be evaluated as a reproducible audit instrument, independently recruited assessors should develop a prespecified coding manual, explicitly distinguish missing from negative evidence, pilot masked duplicate classifications and report agreement with appropriate uncertainty. Discriminant validity should be tested against—not conflated with—RoB 2 and GRADE, while predictive validity requires a held-out corpus of intervention trials with prospectively defined replication outcomes. A study could have excellent metabolite characterization but an uninformative clinical endpoint; unweighted gate counts would therefore be misleading. These steps are proposed validation requirements, not results already obtained [13,14,17].

Potential failure of the model must remain visible. If completion of earlier gates does not predict better cross-study replicability, or if the tool merely reproduces reporting length and journal prestige rather than exposure quality, its claimed value would be weakened. The proposal currently provides a transparent hypothesis-generation map and no clinical efficacy score, regulatory decision rule, or hierarchy of particular fruits and vegetables.

9. Blueprint for the Next Generation of Clinical Studies

9.1. A Four-Arm Matrix-Versus-Molecule Trial

A falsifiable design would compare: (i) a characterized whole fruit or vegetable intervention, (ii) a bioactive-matched isolated preparation, (iii) a matrix-matched low-bioactive control and, where feasible, (iv) a component-plus-matrix arm. Randomization should be stratified by baseline phenotype and prespecified metabolic factors. Dietary background, energy intake, major medications and intervention palatability require control. Dose equivalence must be defined by analytically measured intake and assessed against measured systemic exposure rather than assumed from a package label.

9.2. Metabolite-Guided Rather than Parent-Compound-Only Analyses

Collect baseline stool for exploratory microbial functional profiling and identify predefined metabolites in plasma and urine at mechanistically relevant time points. Use targeted, authenticated standards when possible, record limits of detection and quantification, and distinguish exploratory mediation from causal mediation. For urolithin-producing foods, metabotype groups should be defined a priori when evidence supports the categorization [6,26]. A negative trial is informative if it establishes whether the chain failed at intake, transformation, target engagement or downstream physiology.

9.3. Prespecification, Reproducibility and Transparent Claims

Trial registration, power calculations for a single primary outcome, intention-to-treat analysis, adequate washout for crossover designs, blinded laboratory analyses and adverse-event reporting should be standard. Food-composition and metabolite data should accompany the article as reusable files. Report outcomes by prespecified subgroup cautiously and account for multiplicity. A multi-omic association cannot on its own prove a direct mechanism, and an exploratory "responder phenotype" must be externally replicated before use in precision nutrition recommendations [25].

A practical example would pair a berry intervention with 24 h ambulatory blood pressure as the primary endpoint in a well-defined hypertensive cohort; prespecified secondary outcomes could include endothelial function, LC-MS-quantified phenolic catabolites and urinary F2-isoprostanes. Because recent pooled evidence in such patients is low certainty and largely neutral for resting blood pressure [12], a carefully powered replication would provide more information than another isolated DPPH measurement. A separate broccoli intervention in metabolic disease would need chemically verified precursor-to-isothiocyanate conversion and attention to kidney function when selecting inclusion criteria [18,28].

9.4. Design Tests That Could Disprove the Favored Mechanism

A strong randomized trial should specify not only expected responses but explicit disconfirmation criteria. For a berry trial, a null change in 24 h systolic blood pressure despite verified target metabolite exposure and excellent adherence would weaken the blood-pressure claim even if endothelial function changes. An absence of metabolite exposure would instead prioritize a dose, matrix, preparation or transformation question before a large efficacy trial. A predeclared nested mechanistic substudy can distinguish the two while preserving one primary clinical endpoint.

When a claim is about synergy, design a factor that isolates it. A whole-food arm and a matched extract arm alone are insufficient if they differ in fiber, energy, sugar, palatability or bioactive delivery. Use an additive or factorial comparator framework and a prespecified statistical interaction; report whether the estimated joint effect exceeds the expected combined effect on a meaningful response scale. Without this design, "synergy" is a description of coexistence rather than an established interaction.

Finally, report sample-size justification, effect sizes with confidence intervals, missingness, preanalysis registration, clinically important thresholds and intervention-specific adverse effects. In a 2026 berry meta-analysis, apparently small 24 h diastolic pressure changes contrasted with generally null resting measures and low certainty [12]. Such patterns demand careful multiplicity accounting and external replication; they should not be converted into authoritative individual treatment advice.

9.5. Prespecified Four-Arm Test of Matrix Effects

A realistic replication program could enroll adults with elevated blood pressure after defining usual dietary intake, medication stability and exclusion criteria. Its primary question would be whether daily, analytically characterized berries change mean 24 h ambulatory systolic blood pressure versus a matched control. A four-arm parallel design could contrast (A) whole berries, (B) an anthocyanin-matched formulation, (C) a matrix- and energy-matched low-anthocyanin food control, and (D) a matched formulation with a defined matrix component, with a single proposed primary whole-food efficacy estimand: mean between-arm difference in change in 24 h ambulatory systolic BP from baseline between A (whole berries) and C (matrix/energy-matched low-anthocyanin food control) at the prespecified endpoint. A versus B, and any factorial interaction or synergy hypothesis, would be secondary and require appropriately matched components, separate power justification and multiplicity adjustment. Random allocation, allocation concealment, blinded outcome evaluation and matched energy/sugar/fiber exposures should be defined before enrollment; achieving perfect sensory blinding of whole foods may be infeasible.

The mechanistic substudy would quantify characterized anthocyanin profiles in the foods and validated phenolic catabolites in time-stamped plasma and 24 h urine using stable-isotope internal standards where suitable. Stool-based functional profiling would be exploratory; participants would not be labeled "responders" using postrandomization metabolite levels without a prespecified causal strategy. Secondary outcomes might include protocol-standardized FMD, validated lipid-peroxidation readouts and adverse events. Disconfirmation would be defined in advance: if verified exposure occurs without a change in the primary ambulatory BP endpoint, claims about BP lowering should be weakened even if FMD improves. Formal sample size requires a clinically important between-group difference and an empirically justified variance, not a guess copied from heterogeneous trials [11,12,15,16,29].

For a four-arm design, the whole-food and formulated arms cannot be assumed chemically equivalent because anthocyanin profile, physical structure, fiber, sugars and gastrointestinal kinetics may differ. An anthocyanin-matched formulation therefore tests the role of the non-anthocyanin matrix only imperfectly; predefining analytically measured exposure differences and plausible off-target ingredients is essential. Any claim of mediation would require appropriate longitudinal designs and confounder assumptions beyond randomization of food assignment. This blueprint is hypothetical and contains no estimated treatment effects or invented power calculation [3,6,23,25,30].

Figure 6 visualizes the proposed four-arm trial, with a single prespecified primary comparison and exploratory metabolite profiling rather than multiple interchangeable efficacy claims.

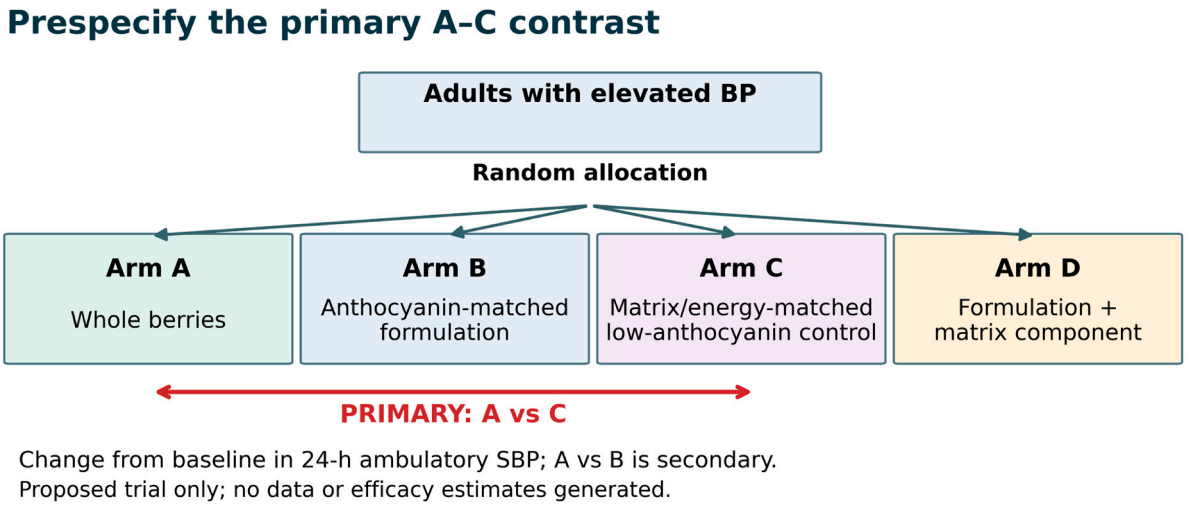

Figure 6. Hypothetical four-arm randomized berry intervention, with Arm A (whole berries) versus Arm C (matrix- and energy-matched low-anthocyanin food control) as the proposed PRIMARY comparison for change in 24 h ambulatory systolic blood pressure (SBP). Arms B and D address secondary formulation/matrix hypotheses that require matched composition and appropriately prespecified multiplicity control. No trial was implemented and no efficacy values or power estimates were generated.

10. Challenges, Boundary Conditions and Research Agenda

First, chemically diverse compounds should not be reduced to a generic class effect. Anthocyanin-rich foods, lycopene-containing tomatoes, vitamin C-rich vegetables and glucosinolate-rich crucifers differ in dose ranges, target hypotheses and exposure kinetics. Second, food matrices, geographical provenance, processing methods and storage create systematic variation in intervention content, even when the species name is unchanged. Third, body mass, age, diet, microbiome, chronic disease and medications may shift the net response; such moderators require powered evaluation, not post hoc storytelling [3,6,23,24].

Fourth, nutrition trials may face blinding constraints, contamination by background dietary choices, incomplete dietary adherence and surrogate endpoint dependence. Fifth, redox terminology should be mechanistically constrained: oxidation is not always pathological, Nrf2 induction is not always beneficial, and "anti-inflammatory" should denote a specified inflammatory endpoint measured with adequate reliability. Sixth, null and adverse findings deserve high visibility because they set boundaries on causal claims and distinguish a food recommendation from a drug-like therapeutic assertion [1,2,12,18,27].

This review has important limitations. Literature selection was selective and purposive rather than exhaustive; there is no preregistered review protocol, exportable all-database search history, duplicate screening, full-text exclusion log, new RoB 2 assessment, quantitative synthesis or independent GRADE certainty appraisal. Therefore, a PRISMA flow diagram, pooled estimates and claims of comprehensive coverage are inappropriate. Several included syntheses may reuse the same primary clinical trials, and we do not count their pooled findings as independent confirmations. A structured source-identity and priority-endpoint review was undertaken for the working bibliography, but this does not establish dual independent verification of every extracted number against all full texts. The proposed Six-Gate framework has not undergone independent reproducibility, content-validity or predictive-validity testing, and is not an evidence-grading instrument [13,14,17].

Supplementary Figure S2 illustrates endpoint discordance summarized in Table 3, including the method-sensitive ambulatory DBP result. Trial overlap has not been quantified; no new estimate or formal certainty grade is implied.

10.1. Prioritized Falsifiable Research Agenda

Priority A is food standardization: assess cultivar, maturity, growing conditions, processing, co-ingested fat, matrix and batch-to-batch variability, then publish absolute quantities per edible portion. Priority B is human chemical exposure: characterize parent molecules, conjugates and microbial catabolites at timed, biologically plausible concentrations, including non-producer phenotypes [3,8,26,30,31]. Priority C is mechanistic specificity: select accessible targets, define relevant oxidant species or signaling nodes and test physiological-range molecules. Priority D is clinical reproducibility: preregister adequately powered primary outcomes, recruit relevant risk strata and replicate both positive and null findings. Priority E is interpretation and safety: systematically separate food-based recommendations from high-dose supplements and report adverse events and medication interactions [27,33].

Six-Gate makes a falsifiable methodological proposal: independently coded evidence chains with better characterized food dose, matrix and human exposure may show better endpoint replicability after adjustment for sample size, population, trial quality and reporting length. A preregistered external validation should measure masked rater agreement, test whether missing gates predict replication independently of RoB 2 and GRADE, and compare the proposed audit with simpler single-item reporting checklists. Failure to improve prediction would weaken its incremental value; no proprietary score or claim of validated originality follows from its present formulation.

10.2. Disconfirmation Criteria and Transportability of Evidence

An evidence-driven antioxidant hypothesis should prespecify what would strengthen or weaken its exact causal chain. The relevant target is a reproducible effect after a characterized human exposure, not the largest in vitro assay value. A precise, well-controlled null estimate after verified exposure can challenge a specific efficacy claim; a wide interval or poor exposure verification cannot establish no effect. Distinct failures at delivery, transformation, target engagement and clinical measurement motivate distinct follow-up experiments.

Transportability depends on food/extract formulation, dose, background risk, concomitant treatment and endpoint. A secondary FMD response in metabolic syndrome does not demonstrate prevention of cardiovascular events or BP reduction [15,16]. Likewise, the PR-7 tomato trial reported a favorable week-12 LDL-C comparison ($p = 0.027$) but a nonsignificant global time-by-food interaction ($p = 0.13$), precluding a strong longitudinal efficacy claim [19]. In hypertensive/elevated-BP berry trials, resting systolic BP was not clearly reduced (MD −2.35 mmHg; 95% CI −4.98 to +0.29; HKSJ −5.41 to +0.72), and 24 h diastolic BP sensitivity to the HKSJ adjustment (MD −1.11 mmHg; HKSJ 95% CI −2.23 to +0.02; $p = 0.052$) further qualifies interpretation [12]. Multiple inferential decisions, rather than the sign of a point estimate, determine what a study can support.

Table 8 frames examples of potentially disconfirming observations for clearly specified causal hypotheses. A nonsignificant comparison with wide confidence intervals does not establish absence of effect; rigorous disconfirmation requires an interpretable exposure, appropriate control and adequately precise estimate. This hypothetical matrix is not a registered trial protocol.

Table 8. Proposed disconfirmation matrix for fruit- and vegetable-derived bioactive claims (illustrative; no clinical grading implied). FMD, flow-mediated dilation; BP, blood pressure; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power; ABPM, ambulatory blood-pressure monitoring; LDL-C, low-density lipoprotein cholesterol; CKD, chronic kidney disease; T2D, type 2 diabetes; HbA1c, glycated hemoglobin; CVD, cardiovascular disease.

Claim to Test	Necessary Measurement	Disconfirming Observation	Allowed Inference
In vitro antioxidant assay predicts vascular benefit	Food composition, human exposure and prespecified FMD or BP	High ORAC/FRAP result with no relevant circulating exposure or physiological benefit	Analytical activity only; no inferred clinical benefit [1,2,22]
Anthocyanins lower ambulatory BP	Characterized whole food, adherence, timed metabolites, 24 h ABPM	Verified exposure and an adequately precise estimate excluding a clinically important BP effect; imprecise or method-sensitive results remain inconclusive.	No BP-lowering efficacy demonstrated for that protocol; FMD is separate [11,12]
PR-7 tomato produces sustained lipid benefit	Prespecified LDL-C trajectory and between-arm interaction	Favorable final visit but no consistent longitudinal between-arm pattern	Week-12 comparison of primary LDL-C ($p = 0.027$; efficacy $n = 74$ of 100 randomized); global interaction $p = 0.13$ [19].
Broccoli-sprout extract improves glycemia in CKD/T2D	Defined extract and randomization, prespecified glucose/HbA1c outcomes	Adequately precise comparison excluding a clinically meaningful glycemic effect; a nonsignificant result alone does not disconfirm the claim.	No glycemic benefit demonstrated under tested conditions; not equivalence or a whole-food inference [18,28].
Microbial metabotype determines clinical responders	Repeated metabolites, baseline metabotype interaction, preregistered primary outcome	Metabotype stratification does not reproducibly modify randomized response	Exposure phenotype may exist without validated clinical prediction [8,26,30,31]
FMD change proves event prevention	Prospective CVD events, causal pathway, adequate power and replication	No event benefit despite reproducible surrogate change	Mechanistic surrogate cannot substitute for patient-level outcome [15,16]

11. Conclusions

In summary, chemical antioxidant-capacity measurements establish reactivity in defined assays, not reductions in human disease. Dietary exposure depends on edible or formulated product identity, processing, matrix and host metabolism; the biologically relevant circulating species must be measured rather than assumed from food composition [1–3,22–24].

The clinical examples reinforce endpoint specificity. A powder-based blueberry RCT had a null primary insulin-resistance result despite a secondary vascular signal [15]; the berry BP synthesis showed a conventionally favorable but HKSJ-sensitive 24 h DBP result [12]; the PR-7 tomato trial had a positive week-12 comparison without a significant global time-by-food interaction [19]; and broccoli-sprout extract was neutral for glycemia in a specific CKD/T2D cohort [18]. These observations cannot be pooled into a general antioxidant-treatment effect.

Prospective cohort associations with fruit/vegetable intake reflect dietary patterns and cannot isolate antioxidant-specific causality [4,5,32]. Likewise, harm from selected high-dose supplements cannot be generalized to ordinary vegetable foods [27,33].

Product format, comparator, population, primary endpoint and uncertainty define the permissible inference.

The proposed Six-Gate audit uses G1 product identity, G2 bioaccessibility, G3 human exposure, G4 target engagement, G5 measurement validity and primary-claim alignment, and G6 patient-important relevance to locate evidence gaps at claim level. The incremental contribution is documentation and traceability of missing causal links, not a different trial estimate or a new certainty grade. Its added value beyond PICO, SANRA, RoB 2, GRADE and established PK/PD reasoning is unvalidated and requires independent testing of inter-rater reliability and added predictive value [13,14,17]. Mechanistic uncertainty does not erase a credible randomized clinical effect; conversely, exposure or a favorable surrogate alone is not proof of reduced clinical events.

The selective narrative sampling deliberately includes discordant examples and may overrepresent their prominence. No reproducible systematic search, new meta-analysis, independent full-text audit of all sources, or external validation of Six-Gate was undertaken. Future studies should compare characterized foods with appropriate matrix controls, measure time-aligned metabolites, prespecify meaningful endpoints and report adverse and null results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox15121554/s1, Figure S1: claim-language decision diagram; Figure S2: selected endpoint discordance. Relevant original studies are cited in the main text [11,12,15,18,19].

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Abbreviations

ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); BP, blood pressure; CKD, chronic kidney disease; CVD, cardiovascular disease; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FMD, flow-mediated dilation; FRAP, ferric reducing antioxidant power; GRADE, Grading of Recommendations Assessment, Development and Evaluation; HbA1c, glycated hemoglobin; HPLC, high-performance liquid chromatography; KEAP1, Kelch-like ECH-associated protein 1; LC–MS, liquid chromatography–mass spectrometry; LDL-C, low-density lipoprotein cholesterol; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; ORAC, oxygen radical absorbance capacity; RCT, randomized controlled trial; ROS, reactive oxygen species; SANRA, Scale for the Assessment of Narrative Review Articles; TAC, total antioxidant capacity; USPSTF, US Preventive Services Task Force.

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