Title

The Power of Plants: The Role of Phytonutrients in Vascular Health

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The Power of Plants: The Role of Phytonutrients in Vascular Health

By

MICHELLE LYNN ZUELCH
DISSERTATION

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“Last but not least, I wanna thank me.
I wanna thank me for believing in me.
I wanna thank me for doing all this hard work.
I wanna thank me for having no days off.
I wanna thank me for never quitting.”
-Snoop Dogg

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ABSTRACT

Cardiovascular disease (CVD) is the number one cause of death in the U.S. Traditional risk factors include sex, age, genetics, hypertension, dyslipidemia, diabetes mellitus, smoking, physical inactivity, sub-optimal nutrition, and overweight and obesity. In addition to these predictive risk factors, endothelial dysfunction is an early indicator of atherosclerosis. Inflammation is also involved at each step of the atherogenic process and plays a critical role in the progression of CVD. Although CVD is commonly perceived as a disease of adulthood, studies suggest that atherosclerosis begins in childhood or adolescence and culminates over the course of the lifespan. Cardiovascular risk factors, for example obesity and dyslipidemia, often develop in childhood, and are directly associated with the severity of early atherosclerotic lesions in adolescents and young adults. According to the U.S. Dietary Guidelines Advisory Committee, a healthy diet is higher in fruits, vegetables, whole grains, low-fat or nonfat dairy, seafood, legumes, and nuts and lower in red meat, refined grains and added sugars. Diets that adhere to these guidelines are deemed some of the healthiest and are associated with reduced risk of a myriad of chronic diseases. Research suggests that the observed benefits of such diets can largely be attributed to habitual consumption of plant foods or following a plant-based diet. Frequent consumption of both nuts and berries is associated with a decreased risk for the development of cardiovascular disease. Along with protein, fiber and micronutrients, nuts provide essential fatty acids, such as linoleic and alpha-linolenic acids, and bioactive phenolics, including ellagic acid and ellagitannins. Berries are also significant contributors of fiber and micronutrients to the diet, and are similarly rich in phytonutrients, including anthocyanins, flavan-3-ols, flavonols, in addition to ellagic acid and ellagitannin. The aforementioned bioactive phytonutrients may elicit

vasculoprotective effects through a variety of mechanisms, mediating the health benefits associated with a diet rich in plants. Therefore, this dissertation focuses on the role of plant-based diets and associated phytonutrients, particularly those present in walnut and strawberry, in promoting vascular health.

Chapter I provides an in-depth discussion of both the fundamental challenges and promising future directions in research with nuts and berries. New understanding of the bioactive compounds found in both nuts and berries has reinforced their role for use in personalized nutrition efforts. Chapters II, III, and IV shift into research on individual foods. Chapter II is a comprehensive review of recent literature on the effects of strawberry intake on human health. Recent advances in research related to the gut microbiome and microbial metabolism have established a new understanding of the phytonutrients in strawberries and the potential mechanisms by which strawberry intake may promote health. Chapter III details a dietary intervention trial in postmenopausal women with overweight or obesity supplementing with 40g/d walnuts for 12-weeks. This work sought to extend previous findings of improved microvascular function in a similar cohort for four weeks but did not yield the same level of improvement. Twelve weeks of 40g/day walnut intake did, however, improve lipid profile, particularly in those with hyperlipidemia at baseline. Pending metabolomic analysis will help elucidate the mechanisms underlying the described physiological changes related to walnut intake, which may be related to circulating and lipoprotein-esterified oxylipins. Chapter IV reviews clinically relevant studies on the effects of freeze-dried strawberry powder (FDSP) on vascular health, with focus on a dietary intervention trial conducted in overweight or obese adolescent males. One week of supplementation with 50 g/d FDSP improved microvascular

function only in participants with a ‘responder’ phenotype, or who experienced increases in fasting nitrate levels with FDSP intake. These results emphasize the importance of inter-individual variability and personalized approaches in nutrition. The appendices are representative of a broader body of work pertaining to cardiovascular health within the context of both the lacto-ovo-vegetarian diet and adolescent obesity. Appendix A describes a cross-sectional study evaluating vascular function in individuals following either a vegetarian or omnivorous diet containing red meat. Appendix B discusses adolescent obesity, which has increased dramatically in recent years and is coupled with increased prevalence of chronic cardiometabolic diseases. Unique consequences, such as those related to psychosocial health, and intervention opportunities are also addressed. Finally, concluding remarks and future research directions are presented in Chapter VI.

This dissertation focuses on the role of plant-based diets and associated nutrients and phytonutrients in promoting vascular health. To a significant extent, this work reflects my research interests in health promotion and chronic disease prevention through the bioactivity of phytonutrients. Plant foods provide an array of bioactive metabolites, which regulate a number of physiological processes. A particular emphasis was placed on nuts and berries, specifically, walnuts and strawberries. Work within this dissertation demonstrates the benefits and explores proposed mechanisms of plant foods in vascular health. This work also supports the importance of consideration of interindividual variability and multiomics in the novel but growing field of personalized nutrition. This information can be used to inform personalized nutrition recommendations and promote vascular health over the lifespan.

CHAPTER I

Introduction

Introduction

Cardiovascular Disease, Atherosclerosis, and Vascular Dysfunction through the Lifespan

Cardiovascular disease (CVD) is the number one cause of death in the U.S. (1). Traditional risk factors include sex, age, genetics, hypertension, dyslipidemia, diabetes mellitus, smoking, physical inactivity, sub-optimal nutrition, and overweight and obesity (1). Some of these factors, for example, diet and blood pressure, are modifiable, whereas others such as age and genetics are not. In addition to these predictive risk factors, endothelial dysfunction is an early indicator of the atherosclerotic process (2). The endothelium is a monolayer of cells lining the blood vessels. Primary function of the endothelium is to regulate vascular homeostasis via the production of vasodilators, including nitric oxide (NO), prostacyclin (PGI2), and endothelial-derived hyperpolarizing factor (EDHF) and vasoconstrictors, such as thromboxane A2 (TXA2) and endothelin-1 (ET-1) **(Figure 1)**. Loss of regulatory function by the endothelium, known as endothelial dysfunction, is a risk factor for the development of atherosclerosis, and ultimately, CVD (3, 4). Therefore, early identification of endothelial dysfunction is essential in the prevention of cardiovascular disease progression.

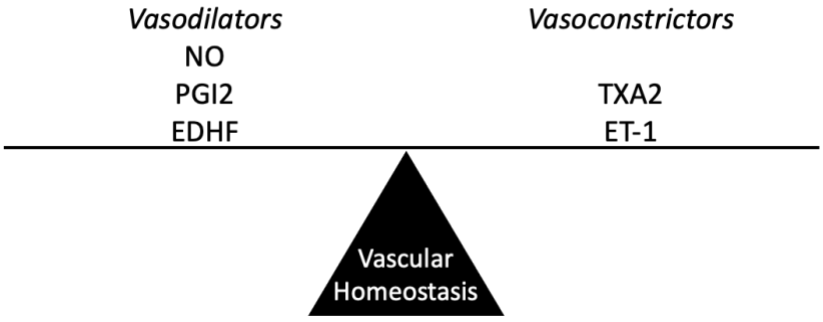

Figure 1: Vascular homeostasis is maintained via the regulation of vasodilators and vasoconstrictors

Inflammation also plays a critical role in the progression of CVD and is involved at each step of the atherogenic process. Endothelial dysfunction, vascular injury, and subsequent lipid accumulation in the vessel wall initiates an inflammatory response involving the upregulation of adhesion molecules that promotes migration of inflammatory cells to the site of the initial injury. Once in the subendothelial space, inflammatory monocytes are converted to activated macrophages (5). The activation of the macrophage inflammasome is a key step in the propagation of inflammation, stimulating the production of inflammatory cytokines that further amplify the inflammatory cascade (6). Activated macrophages take up lipids and become foam cells which, together with inflammatory cells, platelets, and vascular smooth muscle cells, form fatty streaks within the vessel (7). Fatty streaks form atherosclerotic plaques, which cause the vessel to stiffen and narrow, leading to hypertension. The plaque may also rupture and become thrombotic, leading to myocardial infarction or stoke (8).

Although CVD is commonly perceived as a disease of adulthood, studies suggest that atherosclerosis begins in childhood or adolescence, culminating over the course of the lifespan (9, 10). Findings from the Bogalusa Heart Study, a prospective, epidemiologic study of cardiovascular disease from childhood, suggest that magnitude of cardiovascular risk in youth predicts subclinical atherosclerosis and morbidity and mortality in adulthood (11, 12). Cardiovascular risk factors, for example obesity and dyslipidemia, develop in childhood and track into adulthood and are directly associated with the severity of early atherosclerotic lesions in adolescents and young adults (13). It is estimated that 70% of children and adolescents with obesity have at least one cardiovascular risk factor (14). Indeed, children with obesity have significantly impaired endothelial function and arterial elasticity (15). Evidence suggests that

obesity and dyslipidemia in youth may be primary contributors to these vascular impairments (14-18). Like obesity in adulthood, diet and lifestyle modification is a common treatment option (19).

Dietary Pattern and Vascular Health

According to the U.S. Dietary Guidelines Advisory Committee, a healthy diet is higher in fruits, vegetables, whole grains, low-fat or nonfat dairy, seafood, legumes, and nuts and lower in red meat, refined grains and added sugars (20). Diets that adhere to these guidelines, for example the Mediterranean and vegetarian diets, are deemed some of the healthiest and are associated with reduced risk of a myriad of chronic diseases (21, 22). Indeed, the benefits of each of these plant-based diets on vascular health have been well-established (23-27). Vascular effects of the Mediterranean diet include improved macro- and micro-vascular endothelial function as assessed by brachial artery flow-mediated dilation (FMD) and ischemic reactive hyperemia and cutaneous vascular conductance, respectively as well as reduced carotid intima-media thickness (IMT) (23, 24). Similarly, studies on vegetarianism report improved vascular endothelial and smooth muscle function as well as reduced carotid IMT and arterial stiffness (25-27).

The term "plant-based" is often used interchangeably to mean vegan or vegetarian, however, the definition of these diet patterns is characterized by the avoidance of certain food items, for example, meat, poultry and fish, and not necessarily by the inclusion of fruits and vegetables (28). In recognition of the fact that a vegetarian diet may not necessarily equate to one that is healthy, nutrition experts are using the term "Whole Food, Plant Based" (WFPB), to describe a plant-based diet that consists of whole, unprocessed foods and minimizes animal product (28,

29). Insufficient intakes of fruits, vegetables, whole grains, and nuts are major contributors to poor diet quality (30). Indeed, most diets that are considered healthy are rich in plant-foods, but not all exclude animal product. So, is it the avoidance of meat? Or is it the emphasis on plants? Research suggests that the observed benefits of such diets can largely be attributed to habitual consumption of plant foods or following a plant-based diet (31). Characteristics of select plant foods and potential mechanisms mediating these effects are described below.

Phytonutrients/Bioactives from Plant Foods with emphasis on Nuts and Berries

Frequent consumption of both nuts and berries is associated with a decreased risk for the development of cardiovascular disease (32-36). Along with protein, fiber and micronutrients, nuts provide essential fatty acids, such as linoleic and alpha-linolenic acids, and bioactive phenolics, including ellagic acid and ellagitannins (37, 38). Berries are also significant contributors of fiber and micronutrients to the diet, and are similarly rich in phytonutrients, including anthocyanins, flavan-3-ols, flavonols, in addition to ellagic acid and ellagitannin (38, 39). The aforementioned bioactive phytonutrients may mediate some of the health benefits associated with a diet emphasizing the intake of plants.

Essential Fatty Acids

Essential fatty acids, or those not able to be synthesized endogenously, include linoleic (LA) and alpha-linolenic (ALA) acids. Linoleic acid is an omega-6 polyunsaturated fatty acid (PUFA), while alpha-linolenic acid is an omega-3 PUFA. Dietary sources primarily include nuts, such as walnuts, seeds, and oils (40-42). Fatty fish are also rich in ALA (43). Dietary ALA is converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) while dietary LA is converted

to arachidonic acid (AA) (40). These fatty acids can be esterified to glycerol in membrane phospholipids and cleaved for participation in cell signaling where they can exert biological effects, for example, as inflammatory mediators **(Figure 2)** (44). PUFA can undergo bioactivation by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) enzymes and produce a variety of lipid products collectively referred to as oxylipins (44). A sub-class of oxylipins, eicosanoids, which includes the EPA- and AA-derived metabolites, are key regulators of inflammation **(Figure 2) (44)**.

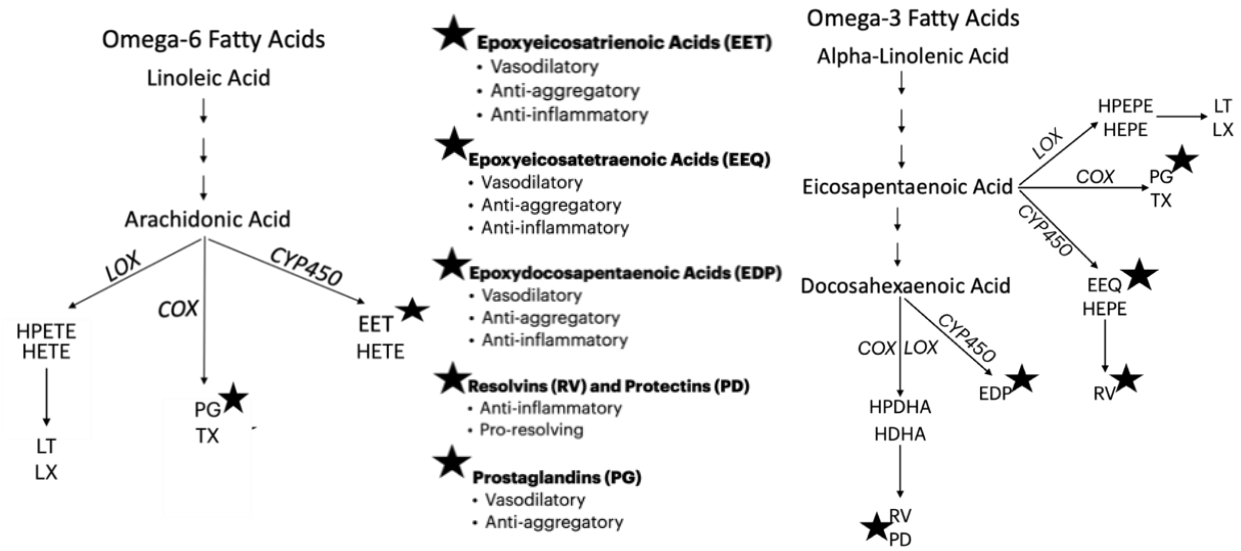

Figure 2: Eicosapentaenoic, Docosahexaenoic, and Arachidonic Acid-Derived Oxylipins and associated cardioprotective mechanisms

The cardioprotective benefits of ALA are well known: dietary omega-3 fatty acids are consistently associated with improved cardiovascular outcomes and reduced cardiovascular mortality (45-47). In contrast to that of ALA, the health impact of LA is less known, controversial even. It is important to appreciate that not all actions of omega-6 or AA-derived eicosanoids are pro-inflammatory, as was previously thought. Recent research suggests that some are actually involved in resolving inflammation and may be considered cardioprotective

(48-50). For example, while prostaglandin E2 is generally regarded as a pro-inflammatory mediator, it also acts as an inhibitor of pro-inflammatory cytokine production, such as tumor necrosis factor-α (TNF-α) (51, 52). The potential for cardiovascular benefit can be attributed to AA-derived metabolites, namely prostacyclin (PGI2) and epoxyeicosatrienoic acid (EET), produced via the metabolism of AA by COX and CYP, respectively **(Figure 2)**. Eicosanoids, particularly 14,15-EET, have been shown to exert anti-inflammatory and vasodilatory effects (53-55), while prostacyclin has both vasodilatory and anti-aggregatory properties through activation of the cAMP pathway (49) **(Figure 3)**

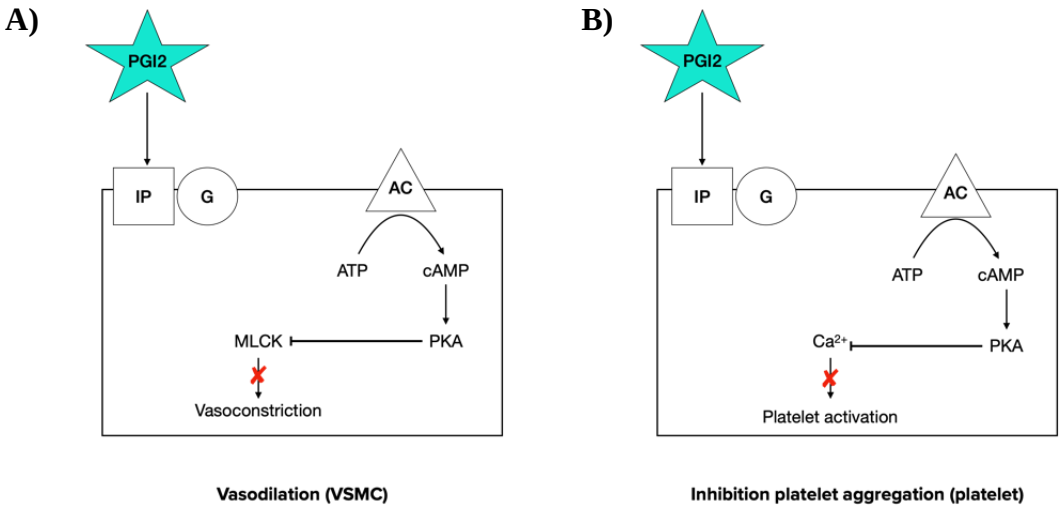

Figure 3:
3a) Prostacyclin-induced vasodilation via GCPR binding in the VSMC.
Prostacyclin acts on smooth muscle cells through prostacyclin receptor (IP) mediation, which leads to an increase in cyclic adenosine monophosphate (cAMP) concentration. cAMP dephosphorylates and thereby inhibits MLCK-induced contraction, resulting in vasodilation.
3b) Prostacyclin-induced inhibition of platelet aggregation in the platelet.
cAMP activates protein kinase A (PKA) which has several substrates in platelets, one of them being the inositol triphosphate (IP 3) receptor. Phosphorylation of this receptor by PKA inhibits calcium release from the dense tubular system (DTS), resulting in decreased intracellular calcium and inhibiting platelet activation.
Key: *PGI2: prostaglandin I2 (prostacyclin); IP: prostaglandin I2 receptor; G: G-protein-coupled receptor; AC: Adenyl cyclase; ATP: adenosine triphosphate; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; MLCK: myosin light chain kinase; VSMC: vascular smooth muscle cell*

Epoxyeicosatrienoic acid has vasodilatory, anti-aggregatory, and anti-inflammatory properties, the former of the two through its' capacity as an endothelial-derived hyperpolarizing factor (EDHF) (56, 57). Through hyperpolarization, EET stimulates vasodilation of vascular smooth muscle cells (VSMC) **(Figure 4)**. In addition to VSMC, EET hyperpolarizes platelets, which inhibits their adhesion to endothelial cells (58). The latter, anti-inflammatory effect, is through EET capacity to act as a peroxisome proliferator–activated receptor γ (PPAR-γ) agonist and inhibits the pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway (59).

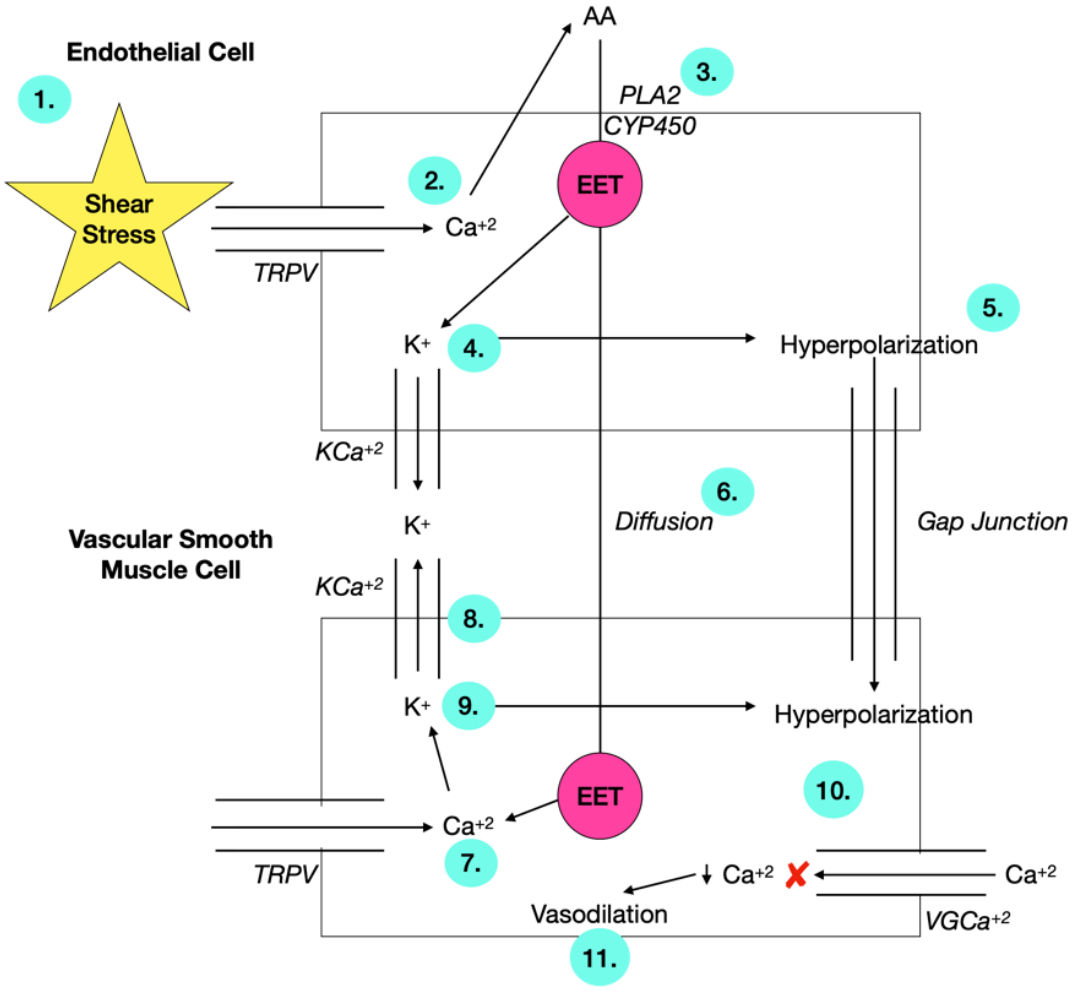

Figure 4: EETs as an EDHF; vasodilation via hyperpolarization

1) Stimulus (ie. shear stress) 2) Ca^{+2} influx through TRPV channels 3) Cleavage of AA by PLA2 and conversion to EET by CYP450 4) EET stimulates K+ efflux and subsequent hyperpolarization of endothelial cell 5) Hyperpolarization is transferred to VSMC via gap junctions 6) EETs to VSMC via diffusion and possibly GPCR 7) EETS stimulate Ca^{+2} influx through TRPV channels 8) Increased intracellular Ca^{+2} activates/opens KCa^{+2} channels 9) K^{+} efflux via KCa^{+2} channels causes hyper polarization 10) Change in membrane potential (more negative) closes $VGCa^{+2}$ channels and leads to decreased intracellular Ca^{+2} 11) Vasodilation
Key: *EET: Epoxyeicosatrienoic acid; EDHF: Endothelial-Derived Hyperpolarization Factor; KCa+2: Calcium-dependent potassium channel; TRPV: Transient receptor potential channel; VGCC: Voltage-gated calcium channels; GPCR: G-protein-coupled receptor*

Polyphenols/Phenolics

Bioactive phytonutrients, such as anthocyanins, flavan-3-ols, flavonols, ellagic acid (EA), and ellagitannin (ET), each provide unique, protective physiological properties **(Figure 5).**

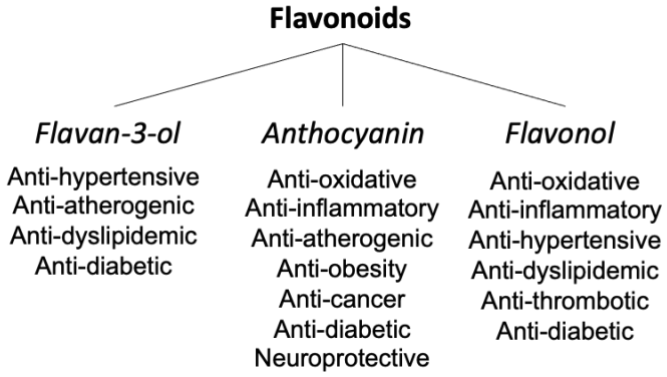

Figure 5: Proposed health benefits of select flavonoids

Ellagic Acid and Ellagitannins

Ellagic acid (EA) and ellagitannins (ET) are phenolic acids and hydrolyzable tannins, respectively, that have gained considerable interest in recent years due to their potential association with health and disease status (60). Dietary sources include berries, such as strawberry and raspberry, and walnut (38). Ellagitannins are hydrolyzed in the gastrointestinal tract to EA and further metabolized by gut microbes into urolithins (60, 61). To date, 13 different/unique urolithins have been identified (60). The urolithin metabolism pathway was only

recently full elucidated and showcased production occurring predominantly in the distal colon, emphasizing the involvement of the gut microbiome (62). The ability, or lack thereof, of an individual's gut microbiota to produce certain urolithins is known as their urolithin metabotype, or metabolic phenotype, (UM): UM-A is distinctly urolithin-A-producing, UM-B is urolithin B- and isourolithin A-producing in addition to urolithin A, and UM-O is non-urolithin-producing (63). Current data suggests that the microbial composition associated with UM-A may be cardiometebolically protective, while that associated with UM-B may be related to gut dysbiosis and increased risk for disease (63-66). Indeed, UM-A is associated with levels of apolipoprotein A and HDL-cholesterol, whereas UM-B is associated with apolipoprotein B, total cholesterol, LDL, VLDL, and oxidized LDL-cholesterol (64). The microbial composition associated with UM-0 has shown reduced bacterial abundance and diversity when compared with urolithin-producing metabotypes (66). Urolithins, particularly Uro-A, may confer health benefits (67-69). In vitro and animal studies suggest potential anti-inflammatory, -oxidative, -atherogenic, -cancer, -diabetic, -obesogenic and -dyslipidemic properties (60, 70-72). Through inhibition of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway, urolithins downregulate mitogen-activated protein kinase (MAPK) and NF-κB, resulting in decreased inflammation, cell adhesion, angiogenesis, and migration **(Figure 6a)** (72-76). Urolithins also activate the adenosine monophosphate-activated protein kinase (AMPK) pathway, which yields positive effects on lipid storage/obesity and dyslipidemia **(Figure 6b)** (68, 72, 77-80). Finally, urolithins defend against oxidative damage by activating the Nrf2 pathway and stimulating antioxidant response elements (AREs) **(Figure 7)** (72, 81-83). Recent clinical trials in humans, although limited, report anti-aging effects through improved mitochondrial function and mitophagy (84-86).

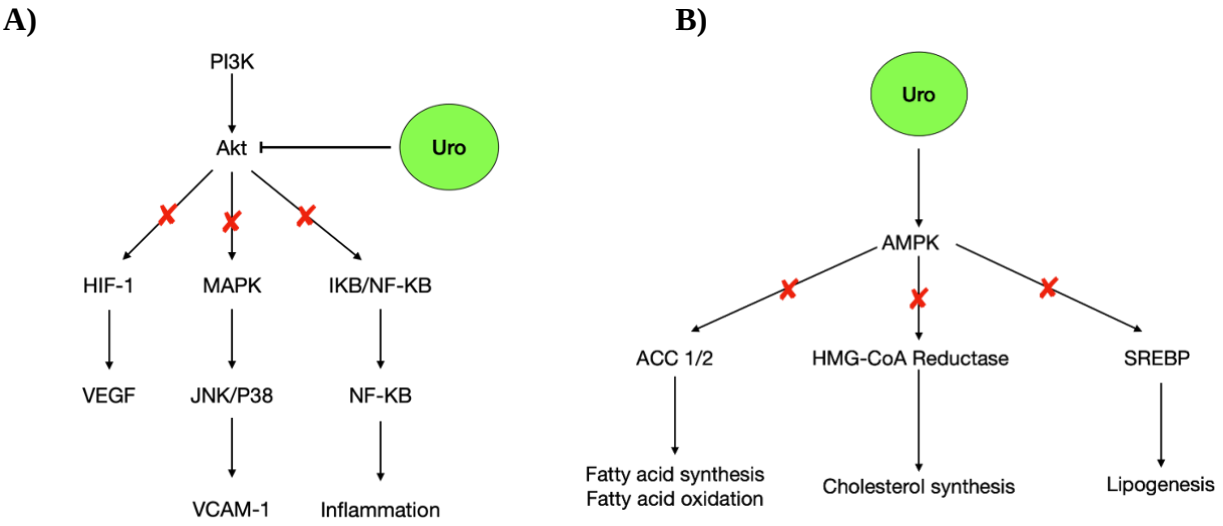

Figure 6:
6a) Urolithin-induced inhibition of the PI3K/Akt pathway
Urolithins produce an anti-inflammatory and anti-atherosclerotic effect through inhibition of the PI3K/Akt pathway, which results in decreased cell migration and adhesion, angiogenesis and inflammation.
Key: PI3K: phosphoinositide 3-kinase; Akt: protein kinase B; HIF-1: hypoxia-inducible factor-1; VEGF: vascular endothelial growth factor; MAPK: mitogen-activated protein kinase; JNK: c-Jun N-terminal kinase; p38: p38 kinase; VCAM-1: vascular cell adhesion molecule-1; IKB: inhibitory-KB kinase; NF-KB: nuclear factor kappa-light-chain-enhancer of activated B cells
6b) Urolithin-induced activation of the AMPK pathway
Urolithins activate the AMPK pathway, which yields positive effects on lipid storage/obesity and dyslipidemia through decreased synthesis of fatty acids, triglycerides, and cholesterol and increased fatty acid oxidation.
Key: *AMPK: 5' adenosine monophosphate-activated protein kinase; ACC 1/2: acetyl-CoA carboxylase 1/2; HMG-CoA reductase: 3-hydroxy-3-methylglutaryl coenzyme A reductase; SREBP: sterol regulatory-element binding protein*

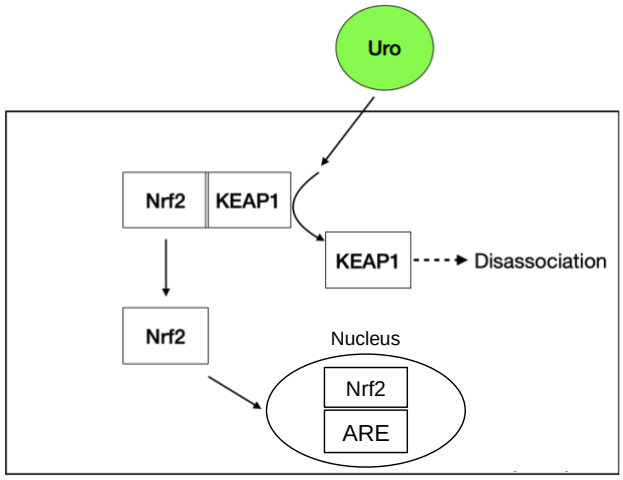

Figure 7: Urolithin-induced activation of the Nrf2 pathway
Urolithins defend against oxidative damage by activating the Nrf2 pathway and stimulating antioxidant response elements
Key: *Nrf2: nuclear factor erythroid 2-related factor 2; KEAEP1: kelch-like ECH-associated protein 1; ARE: antioxidant response element*

Anthocyanins

Anthocyanins are flavonoids that provide plants, such as grapes and berries, with their red, purple, and blue pigmentation (87). Dietary anthocyanins are present as glycosides and persist in this form in the circulation shortly following consumption, suggesting that they can be absorbed directly from the stomach. However, the main site of anthocyanin absorption is the small intestine following deglycosylation to aglycones (88, 89). Aglycones undergo phase II metabolism to form the conjugated aglycones and aglycone glycosides that are predominantly present in the circulation (88, 90). Aglycones also undergo degradation to phenolic acids and aldehydes by the colonic microbiota (88, 90). Evidence suggests that anthocyanins may have antioxidative, antiatherogenic, anticancer, antidiabetic, anti-obesity, anti-inflammatory, and neuroprotective activity (87, 88, 91). Recent mechanistic literature suggests that that certain anthocyanins, such as pelargonidin-3-glucoside (P3G) and cyanidin-3-glucoside (C3G), modulate oxidative stress and inflammation by upregulating the antioxidative nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, and by downregulating the pro-inflammatory MAPK and NF-κB pathways (92, 93).

Flavan-3-ols

Flavan-3-ols are the most commonly consumed flavonoid subgroup (94). Green and black teas, berries, and cocoa are rich sources of flavan-3-ols (95). Recently, flavan-3-ols became the first bioactive compound to have an established dietary guideline (95). The Academy of Nutrition and Dietetics recommends daily flavan-3-ol intake of 400-600 mg/d to promote cardiometabolic health (96). Once ingested, flavan-3-ols undergo extensive metabolism; first conjugation in the liver followed by microbial catabolism in the gut to a number of metabolites, which are

biologically active (97, 98). Flavan-3-ol intake is associated with significant improvements in metabolic health, including glucose metabolism and insulin sensitivity (99), but it's most profound effect is on the vasculature (95). Flavan-3-ol intake is associated with significant improvements in FMD and lipid profile and is inversely related to systolic and diastolic blood pressure and arterial stiffness (99-101). Indeed, supplementation with 500 mg flavan-3-ols/day was associated with a significant 27% reduction in death from cardiovascular disease at 3.6 year follow-up in the COcoa Supplement and Multivitamin Outcomes Study (COSMOS), a randomized clinical trial including over 20,000 participants (102). In a meta-analysis of 39 prospective cohort studies, higher intakes of the specific flavan-3-ols, catechin and proanthocyanidin, had a 25% and 17% lower risk of cardiovascular disease when compared with those with lower intake (103). Mechanisms of action, although not fully elucidated, are likely to include increased bioavailability of nitric oxide (95, 104), a potent vasodilator, as well as modulation of inflammatory cascades (105, 106).

Flavonols

Flavonols are a class of flavonoids that can confer white to pale-yellow pigment in plants (107). Flavonols are found in fruits like apples and berries as well as in green leafy vegetables and onions (38, 108). Similar to other flavonoids, flavonols undergo phase I and II metabolism following absorption and are mainly present in the circulation in their conjugated forms (109-111). Also like other flavonoids, flavonols exert a broad range of biological functions. Research shows that flavonol intake, particularly quercetin, is associated with positive cardiometabolic outcomes, having antioxidative, anti-inflammatory antihypertensive, and anti-thrombotic activity as well as promoting improved lipid and glucose regulation (109, 110, 112-115). A recent meta-

analysis including data from 18 randomized controlled trials reported that supplementation with flavonols significantly increased HDL-cholesterol and reduced total cholesterol, LDL-cholesterol, triacylglycerol, fasting plasma glucose and systolic and diastolic blood pressure (116). Potential vasoprotective mechanisms include enhanced bioavailability of NO and inhibition of cyclooxygenase-1 (COX-1), resulting in improved vascular function and reduced blood pressure and reduced platelet reactivity and inflammation, respectively (110, 116, 117). Further, flavonols suppress angiotensin-converting enzyme (ACE) activity, which is directly involved in the regulation of blood pressure (118). Flavonols may also downregulate 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase, an intermediate in cholesterol synthesis, and upregulate proteins involved in reverse cholesterol transport, such as ATP-binding cassettes, yielding a hypolipdemic effect (110, 119). Finally, flavonols elicit strong free-radical scavenging capacity via their modulation of the Nrf2 pathway (120, 121).

Cardiovascular disease, often beginning in childhood concomitant with obesity, is a significant contributor to morbidity and mortality in the U.S. (1, 10). Dietary intervention is a common and effective treatment strategy (9, 19). Evidence demonstrates that certain diets, such as the Mediterranean and Vegetarian diets, promote cardiovascular health (21, 22, 26). These plant-based diets contain biologically active polyphenolic compounds and polyunsaturated fatty acids that may elicit vasculoprotective effects through a variety of mechanisms. Walnuts and strawberries are plant foods that are rich in the aforementioned compounds of interest (38). Therefore, the following dissertation focuses on the role of plant-based diets and associated phytonutrients/bioactives, particularly those present in walnut and strawberry, in promoting vascular health **(Figure 8).**

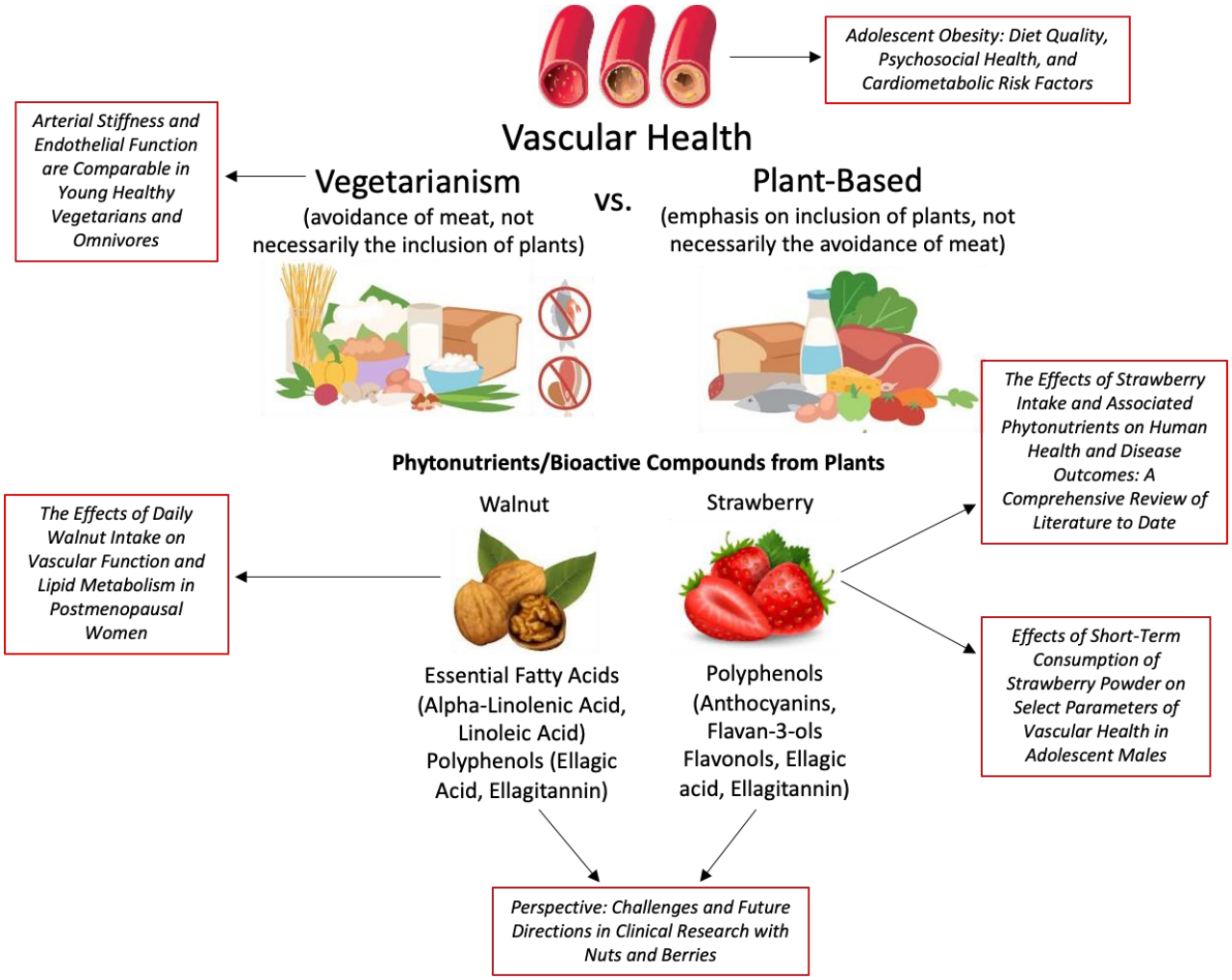

Figure 8: Dissertation Overview
A red text box indicates papers included in this dissertation that have been or intend to be published.

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CHAPTER II

Perspective: Challenges and Future Directions in Clinical Research with Nuts and Berries

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Introduction

The 2020 – 2025 Dietary Guidelines for Americans encourages the intake of a variety of plant-based foods to include nuts and berries [1]. With the goal of increasing current knowledge on nuts and berries, as well as addressing research challenges and opportunities, the *Nuts and Berries Conference: Pathways to Oxidant Defense, Vascular Function, and Gut Microbiome Changes* was held on May 5-6, 2022, at the University of California, Davis. Tree nuts and berries were selected as the focus of the conference for their unique composition, bioactivity, and multitude of associated health-promoting qualities (**Table 1**). With over fifty different edible nut species and hundreds of berry varietals, the following were selected for the purpose of the conference and this review: walnuts, almonds, hazelnuts, cashews, pecans, pistachios, strawberries, blueberries, raspberries, and blackberries.

Tree nuts and berries are significant commodities in the United States (US). The total value of tree nuts grown in California in 2021 was estimated at $8.961 billion (almonds $5.028 billion; pistachios $2.911 billion; walnuts $1.022 billion) [2]. The total value of berries grown in California in 2021 was approximately $3.667 billion (strawberries $3.023 billion; raspberries $420,700 million; blueberries $223,500 million) [2]. With over two-thirds of US tree nut and berries grown in California [2], the agricultural land-grant institution of the University of California Davis was the appropriate location to convene this conference with leading researchers, registered dietitians, community partners, and industry representatives.

Regular tree nut and berry consumption is associated with a decreased risk for the development of cardiovascular disease along with favorable effects on brain and gut health [3-7]. Tree nuts provide protein and fiber, and monounsaturated and polyunsaturated fatty acids, along with vitamins, minerals, and bioactive carotenoids, phytosterols, phenolics and flavonoids,

lignan and tannins, such as the condensed proanthocyanidins (particularly in pecans) and hydrolysable ellagitannins (particularly in walnuts) [8]. Berries are also a significant source of fiber and vitamin C, along with bioactive carotenoids, phenolics, including proanthocyanins and ellagitannins, and anthocyanins that provide berry color [9-11]. Moreover, berries provide flavan-3-ols in quantities up to 37 mg per 100 gram serving (**Table 1**), which would contribute to a recently proposed daily recommended intake level of 400-600 mg per day [12].

While research results to date have been promising, mechanisms of action in general, and for vascular and gut health specifically, have yet to be fully defined. More data are needed that can be generalized to diverse population groups, as well as for modeling of precision nutrition recommendations. This paper will review the progress and challenges of current nut and berry research and suggest future directions for the field.

Research on nuts and berries: Successes and challenges

Study design

Many different study designs have been used to assess the effects of nuts and berries on cardiometabolic health. The strengths and limitations of various clinical nutrition study designs have been addressed elsewhere [13]. A summary of the past five years of studies on nuts and berries on outcome measures of cardiovascular and gut health is presented in **Tables 2 and 3,** respectively. Eligible studies consisted of clinical human trials in children, adolescents, and adults published within the last five years (2017 – 2023), exploring associations between the consumption of nuts and berries and associated biomarkers of interest.

Two long-term intervention trials, the PREDIMED (Prevención con Dieta Mediterránea) and the COcoa Supplement and Multivitamin Outcomes Study (COSMOS), published in 2018

and 2022, respectively, provide examples of study designs that could be useful for future planning. The PREDIMED dietary intervention trial provides the strongest evidence to date that incorporation of nuts into a healthy Mediterranean dietary pattern in individuals ages 55 – 80 years old for 4.8 years can reduce the risk of cardiovascular events (myocardial infarction, stroke and cardiovascular death) by 28% [6]. The COSMOS trial demonstrated that the daily intake of monomeric and polymeric flavanols from cocoa in older adults (men > 60 years and women > 65 years of age) reduces risk for cardiovascular morbidity and mortality [14]. Although the COSMOS study utilized a flavanol supplement compared to a whole food, it is a case study to support the need for larger trials with clinical outcomes based on the use of multi-site data of surrogate outcomes from dietary interventions that use randomized, double-blind controlled trials in crossover or parallel-arm study designs for studies of nuts or berries [15 -17].

A common study design for whole foods is the replacement of the test food with a nutritionally matched, isocaloric substitute. However, matching nutritional content can be a challenge, since food processing, such as blending berries and roasting nuts, causes a disruption to the nutrient matrix, potentially changing the bioavailability of key nutrients [18-20]. For nuts, controls often include the complete omission of the nut of interest. For berry research, a number of considerations exist that are alternative to consuming the whole food. One is the use of freeze-dried berry powders as the test product, controlled with an isocaloric powder either lower or devoid of potential bioactives. Attempts have been made to mask the control powders, but issues such as product color, texture, scent, and mouthfeel are challenging to completely match. While this approach is similar to a classical pharmaceutical trial design, blinding study personnel and participants is challenging, thus creating both performance and detection bias. Additionally, freeze-dried berry powders can have a different food matrix compared to the whole food, which

could influence outcome measures as well as limit generalizability to the whole fruit. A second approach for berry research is the encapsulation of test and control powders. This can aid in participant masking, but the total amount of test product provided can be limiting, and large intakes of control gelatin capsules have resulted in adverse effects [19, 20]. A third option can be examining two or more intake levels, with or without a true control group [21, 22]. Finally, the use of macro- and micronutrient matched gummies with similar amounts of calories, sugars and fiber, but devoid of other bioactives, is a novel option for use as a comparative control. In all of these approaches, the potential bioactivity of the control itself must be considered. For example, isocaloric control powders that are low in polyphenols may still have a considerable amount of fiber in order to obtain similar mouth feel and texture, but the fiber content may have effects on lipid metabolism and the microbiome, which could influence outcome measures.

Multiple cultivars of berries exist, some of which have differences in the content of bioactive ingredients, thus limiting comparison and extrapolation of results [21, 23]. For nuts, walnuts contain a variety of phenolic acids, catechins and flavonoids, most of which have been reported to possess bioactivity. Significant differences in the concentration of 16 phenolic compounds were identified when comparing black (*Juglans nigra L.*) and English (*Juglans regia L.*) walnuts [22, 23]. More than 50 cultivars of strawberries exist in the US. To help reduce the potential experimental variability created with the use of different cultivars, the California Strawberry Commission has produced a freeze-dried test material that utilizes a composite of genotypes to produce a powder that is characterized for its macro- and micronutrients and bioactive components [24]. The US Highbush Blueberry Council also provides a powder that is a 50/50 mixture of two cultivars (*Vaccinium corymbosum* and *Vaccinium virgatum*) [25]. A limitation of this approach is that the standardized mixture may contain varieties with reduced or

low bioactivity. However, the advantage of this approach is that the composite represents the "market basket" available to consumers and allows comparison of results from studies conducted among different research groups, and for generalizability of results to a broader berry application actually used by consumers.

In addition to cultivar differences, factors such as climate and seasonal differences due to heat, sunlight, and rainfall can contribute additional variability. Given the above, the characterization of bioactives within these foods is critical. New analytical equipment and techniques have increased the precision of food composition compared to analyses performed decades ago. Current advances in the development of nutrition databases have been reviewed elsewhere [26]. For example, databases such as that from the US Department of Agriculture (USDA) FoodCentral could be strengthened if the date of the analyses was included, along with the protocols used and the number of samples analyzed. Linking resources from repositories detailing data, such as chemical composition and bioactivity, will help both plant scientists and health professionals to make accurate and timely recommendations and guide future research.

Individual variability

Free-living populations have differences in background diets that can influence their responses to the intake of test foods, potentially creating significant variation in baseline measurements. This variability presents a challenge when elucidating clinically relevant effects, especially if unknown *a priori*, where statistical significance can be masked by combining and analyzing groups together. Interindividual variability may be mitigated by increasing sample size as well as using a crossover design, but challenges in recruitment, retention and budget constraints exist. One way to help minimize experimental variability is through a run-in period,

to identify participants who may be differentially metabolizing bioactive phenolics or with the goal of minimizing or removing potentially confounding metabolites from circulation prior to the intervention [27]. However, study designs that employ highly controlled settings, strict inclusion and exclusion criteria, extended washout periods that alter background diets, and ask participants to follow an atypical consumption pattern does not reflect "normal" life and may have limited applicability to the general population. Another useful model that also has limitations is the provision of nuts or berries in amounts and duration that are greater than normally consumed. Feeding relatively high amounts of nuts or berries for a limited period of time has been employed to demonstrate proof-of-concept and provide a basis for further exploration for changes in physiology [24], cognitive performance [28], and gut microbiome profiles [29]. Subsequent study designs must be realistic, guided by the USDA FoodCentral database for portion size. These trial designs should also use a duration that is realistically achievable by consumers, whose food purchasing behavior can be influenced by cost, access, and seasonal availability of the food. Studies using average daily portion sizes typically require intervention periods of months, which present challenges regarding participant compliance and retention, and cost of the study. In a review of 231 reports on berries and health, approximately 70% of studies used interventions of less than three months or contained less than 50 participants [30]. Meeting the challenge of conducting long-term studies using amounts of foods in a typical diet, with a representative sample of participants, requires a significant commitment of resources.

The health and functional levels of participants are other factors that influence study designs and outcomes. For example, studies on cognitive performance with both nuts and berries have assessed effects among those both with and without cognitive impairments [31- 33]. In such studies, short-term interventions may show little or no response after the addition of nuts or

berries to the diet [31]. Although the net change may not be statistically significant, this model does not address the ability of the food to prevent decline, which would require long-term testing. Further, an individual with cognitive impairments might demonstrate favorable responses compared to baseline measures following nut or berry intake but may still not reach the level of performance of a healthy individual. In both instances, neither change from baseline, nor absolute values of performance, fully capture the beneficial cognitive response [34-36].

Dietary interventions require the incorporation of foods into an individual's eating pattern which may present a number of challenges. One is the creation of boredom with eating the same food on a regular basis. Second is that the caloric load of the test nut or berry may displace the intake of other nutrient-dense foods. These factors may make compliance for the entire study duration an issue, particularly if the intervention is weeks or months in duration [37]. A third challenge involves compliance. In berry research studies, compliance is often not reported or the reported range of intake is so variable that it is hard to discern the significance of the results [38]. The use of food intake metabolite markers is an emerging tool that can help verify compliance [39].

In addition to compliance, dietary patterns are an important consideration needed for the interpretation of results because individuals do not eat a single food in the absence of other foods. Background or habitual intake is often not addressed in nutritional trials. The potential variability in habitual dietary intake of participants is often a confounding factor in nutrition research [40]. Dietary assessment methods, with 24-hour recalls, 3-day food records and food frequency questionnaires all have limitations [41]. These subjective measures may also not accurately capture the potential for nutrient-nutrient interactions that may alter polyphenolic or other bioactive components attributed to nut and berry consumption. Further complicating this

issue is the observation that study designs utilizing longer-term interventions or that require the intake of a large amount of the test food are more likely to result in overreporting food intake due to fear that participants may be dismissed from the intervention [42]. Innovations in dietary assessment methodology using “smart” eyeglasses or other image-based technologies have been proposed to address this issue [43]. Assessing the relationship between the intake of nutrients and bioactives from a whole food product to physiologic responses is difficult, as a multitude of processes are affected, including regulation of vascular function, provision of oxidant defense, and changes in gut microbiome profiles and subsequent output of secondary metabolites [7, 44]. Additionally, bioactives from nuts and berries can interact with each other as well as other dietary components to alter bioavailability and health-promoting properties [18]. For example, intake of dietary fats in conjunction with berries has been demonstrated to increase carotenoid bioavailability [18].

Results could also be confounded by dietary changes made by participants in addition to incorporation of the test nut or berry. Habitual dietary intake is often measured through food frequency questionnaires or repeated 24-hour dietary recalls. However, these subjective measures may not accurately capture the potential for nutrient-nutrient interactions that may alter polyphenolic or other bioactive components attributed to nut and berry consumption. Further complicating this issue is the observation that study designs utilizing longer-term interventions or that require the intake of a large amount of the test food are more likely to result in overreporting food intake due to fear that participants may be dismissed from the intervention [45].
Expanding the scope of populations to be studied is another key area for future research. Most clinical trials using nuts and berries have been conducted in middle-aged or older Caucasian adults with one or more cardiometabolic risk factors [8, 21, 23, 42]. Whether these results extend

to other population groups is either inferred or unknown. Future research would benefit from extending the study populations to include those from other racial and ethnic groups [9]. This is particularly important in order to address the current US National Institutes of Health (NIH) research initiative in precision nutrition and health, the "Nutrition for Precision Health powered by the All of Us Research Program" [46]. The inclusion of biological females in clinical nutrition trials is imperative, yet the current literature includes predominantly male participants [47]. Because many studies on nuts and berries focus on cardiometabolic outcomes, the unique aspects of female physiology must be considered [48]. For example, vascular function fluctuates with the phase of the menstrual cycle, which has largely been ignored in most past studies [49]. More studies are also needed in young children, as well as in young adults, up to about the age of 40 [50]. A pilot study (n=17) reported a correlation between blueberry supplementation and acute positive effects on memory and executive function (defined as significant functional improvement with statistical probability of <5% chance) in seven- to ten-year old children [51]. A large study among pregnant women-infant dyads (n=2,208) reported positive protective neuropsychological effects on long-term cognitive development in children at 1, 5, and 8 years of age when nuts were consumed during gestation [52]. Finally, translation of research results is challenging when considering socioeconomic status (SES), particularly when food items are not accessible or affordable [53, 54]. Barriers to participation in clinical research studies among those of low SES include a low interest in clinical trials, inefficient or inadequate explanation of the study in culturally appropriate terms, participants' distrust of biomedical research, and participant burden, including lack of transportation or the inability to prioritize participation in research over work obligations [55].

Study duration

Like many other dietary studies, research on nuts and berry studies often use acute (several hour) studies evaluating postprandial effects. However, either a lack of or successful demonstration of benefits does not necessarily predict a similar outcome over extended periods of intake. Depending on the outcome measure, detectable effects may take weeks or month for the intervention. Only a limited number of studies exist assessing the impact of nut or berry intake on the incidence or severity of diseases or metabolic dysfunction which requiring durations of months or years [56].

Moving forward: Precision nutrition, multi-omics, and biomonitoring

Precision nutrition evaluates an individual's unique biological characteristics such as genotype and phenotype, including DNA expression, influences of the gut microbiome, and metabolic response to specific foods or dietary patterns, as well as dietary habits and external factors influencing outcomes such as social determinants of health, to determine the most effective dietary strategies to improve health and prevent disease [57-59]. Understanding the sources of interindividual variability that contribute to metabolic heterogeneity and applying mathematical modeling and computational algorithms will be essential to refining dietary recommendations. Several recent publications comprehensively review research gaps and study design considerations in the field of precision nutrition and specifically concerning (poly)phenolic-rich plant foods [60, 61]. Precision nutrition will lead to important discoveries pertaining to interindividual responsiveness to the intake of nuts and berries. Ultimately, this information can be applied via targeted recommendations to individuals and groups for achievable and sustainable dietary intake of nuts and berries to promote optimal health.

The incorporation of biomonitoring technologies into study designs may also be used for precision nutrition. Current and emerging mobile devices can provide continuous data collection in free-living populations with minimal participant burden. The study of nuts and berries would be enhanced with the use of devices that can capture real-time physiological outputs at home that reflect normal living conditions [62]. Further collaborative efforts in the fields of bioengineering and artificial intelligence hold promise for advancing the understanding of benefits from nuts or berries.

An emerging personal biomonitoring technology is the Precision Health Toilet, which collects and evaluates human urine and stool, that are then analyzed using artificial intelligence to determine flow rate and volume of urine, as well as fecal analysis via the Bristol Stool Scale [63]. A second type of toilet seat, the Heart Seat™, has recently been approved by the US Food and Drug Administration for home use to monitor heart rate and oxygen saturation, with future plans to add sensors that monitor systolic and diastolic blood pressure [64]. Assessment of metabolites in the excreta seems like a feasible goal for future development, which may be useful, for examples in the detection of urinary and fecal metabolites that can reflect the metabolism of ellagic acid (from strawberries and walnuts) to urolithins [65] and of (-)-epicatechin (from a variety of berries and tree nuts) to gamma-valerolactone [66]. A third example is an ingestible capsule containing a biological photosensor that can detect gut inflammation [67, 68]. Bioluminescence can be monitored from bacteria that have been engineered to illuminate when they come into contact with a molecule for which they have been coded, such as urolithins from berries or lipid-sensitive metabolites from nuts. Finally, another type of ingestible capsule has recently been detailed that collects samples from multiple regions of the human intestinal tract during normal digestion. This device has been used to explore the

role of the gut microbiome in physiology and disease, with novel findings that intestinal and stool metabolomes differ dramatically [69, 70]. The ability of nut or berry intake to alter such metabolomes, and their association with changes in physiological function and health outcomes, would be an interesting area for future research. While these technologies are still in their infancy, they have promise to further precision nutrition research efforts on nuts and berries.

Research addressing the issue of "responders" vs. "non-responders" is important in understanding the metabolic discrepancies in many studies on nuts and berries. For example, platelet aggregation phenotypes can vary significantly by individual responsiveness to oxylipins, bioactive lipid mediators derived from polyunsaturated fatty acids present in nuts as well as in extra virgin olive oil [71]. Variations in circulating metabolites and microvascular function following the intake of freeze-dried strawberry powder (FDSP) has been reported [72]. Those individuals producing increased nitrate and nitrite levels showed favorable changes in function, while those showing no change in nitrate or nitrite levels did not [24, 72]. Another example is illustrated by a recent letter [73]) in response to a systemic review [74] of almond intake and inflammatory biomarkers. The letter notes that while the review included amounts of almonds ranging from 10 to 113 g/d, favorable responses only occurred at intake of <60g/d. Further, the authors note that while the review reports beneficial effects of almond intake on reduction in C-reactive protein and interleukin-6, subgroup analyses show that the effects on these two outcomes were not significant among those with obesity or who were rated as unhealthy prior to the intervention.

Characterizing participants according to precision nutrition, including the use of genetic phenotyping to identify target genes that may result in "responders" and "non-responders" prior to enrollment may be helpful for clinical trials, but does not reflect responses in a free-living

population. Furthermore, in addition to physiological variations, socio-behavioral differences among individuals that may modulate responses to berries and nuts must also considered. Nonetheless, innovative precision nutrition models that can identify interindividual differences would be useful in defining mechanisms of action and potentially who would benefit the most from regular nut or berry consumption.

Plasma and serum concentrations are useful to identify the bioavailability and bioefficacy of key nutrients and phytochemicals found in nuts and berries [75]. Some compounds, such as small molecular weight polyphenols, are first absorbed in their native state in the small intestine. Other polyphenols can be biotransformed via the host microbiota to a second set of compounds that are subsequently absorbed and confer additional bioactivity beyond that obtained from the parent molecules [76, 77]. Monitoring both host and microbial metabolites in the blood and urine, and those that may accumulate in tissues of interest such as the liver and gastrointestinal epithelium, among other tissues, would be useful in understanding the dynamics of nut and berry bioactivity and specific association with site of actions [76].

Broader application of orthogonal approaches that combine untargeted with targeted metabolomic platforms and combined with the use of advanced informatics will support new understanding about the absorption, distribution, metabolism, and excretion of compounds found in nuts and berries. For example, the UC Davis West Coast Metabolomics Center conducts both targeted and untargeted assays that assess plasma microbial metabolites using a biogenic amine panel that identifies and quantifies acylcarnitines, trimethylamine N-oxide, cholines, betaines, nucleotides and nucleosides, methylated and acetylated amines, di- and oligo-peptides, and a number of microbially modified food-derived metabolites.

Some interindividual differences in response to nut or berry intake have been attributed to the composition of the gut microbiome [78]. For example, ellagitannins are polyphenolic compounds present in strawberries, raspberries and walnuts, which are metabolized by gut bacteria into an array of urolithins [29, 79]. The production of urolithins relies on the capacity of specific microbes, *Gordonibacter pamelaeae* and *Gordonibacter urolithinfaciens* [80-82]. Urolithins may decrease symptoms of chronic metabolic diseases, including inflammation and dyslipidemia [79]. Following a single intake of red raspberries, individuals with prediabetes and insulin resistance had lower concentrations of circulating urolithins compared to levels found in those who were metabolically healthy, a result related to gut microbiome composition [83]. In the same population, consuming red raspberries for 4-weeks improved hepatic insulin resistance and total and LDL-C in the pre-diabetes group, and the effects were related to decreased *R. gnavus* and increased *E. eligins*. Overall, including a practical amount of red raspberry in the diet regularly is a low-calorie dietary strategy that improves gut microbiota composition and function in individuals with pre-diabetes and insulin resistance resulting in improvements in metabolic health [84]. With a sustained emphasis on the role of gut microbiota in nutrition research, advances in our understanding of food-gut dynamics will provide new insights about the role of nuts and berries in human health and performance.

While research on a specific nut or berry provides insight into bioactivity and potential mechanisms of action, such focus also creates the potential for fragmentation, since the search for overall dietary patterns is not addressed. The composition of fruits and nuts differ at the molecular level, and a broader view assessing similarities in chemistry and health benefits is critical for translational research, as well as for messaging purposes. For example, blueberries, strawberries, pomegranate, walnuts and grapes all have reported benefits for cardiovascular

health, driven largely by the presence of similar polyphenols which are present at varying quantities in each of these foods [85-88]. While health professionals and consumers often hear messaging on a single berry or nut, the potential benefits of increasing consumption of the broader category may be obscured or lost. This challenges the ability to maintain consistent messaging and align better with translatable dietary guidance. Future interventions that combine nuts and berries with one or more other foods within a food matrix at dietary achievable doses and in more diverse populations are warranted [89-91].

To date, multi-omics technologies have provided valuable insights into exposure-disease relationships [92, 93]. Coupled with artificial intelligence, predictive modeling and continuous, personalized monitoring, these data-intensive outcomes can provide further insights about the health benefits associated with regular intake of nuts or berries. Use of highly personalized data collection devices will require secure data repositories [94]. One of the challenges of similar foods being studied in differing formats and by various research groups is the utility of the data as a combined set. Differences in test materials and experimental designs make integration of data difficult. The proper curation of combined data, whether physiologic, metabolomic, or genomic is critical to ensure that combined datasets provide synergy, statistical power, and enhanced usefulness.

Novel markers of health outcomes

The cardiometabolic benefits from regular consumption of nuts or berries are widely reported and include improved vascular function [23, 24, 95-97], reduction of cardiovascular disease risk factors [98, 99], improved insulin sensitivity [100, 101], and reduced risk of type 2 diabetes mellitus [102-104]. Antioxidant [66, 105] and anti-inflammatory [106, 107] capacity

and activity have also been noted. Metabolic outcomes may be context specific and related to the physiologic state of the individual and host microbiome composition, among other factors. Examples include findings of ellagitannin and ellagic acid rich foods (raspberries and walnuts) resulting in differential responses in healthy individuals compared to those with pre-diabetes, which are dependent on gut microbial-derived metabolite profiles (urolithin metabotype) [29, 83, 84, 108]. Many factors contribute to interindividual variability in response to diet that can extend to context-specific aspects influencing the magnitude of health benefits and reinforces the importance for further research aimed at advancing discoveries in precision nutrition. Additional health outcomes related to nut or berry intake are outlined below.

Body composition

Adding nuts or berries to the daily diet may be advantageous for weight management for several physiological reasons. One is that these foods produce feelings of satiety, helping to reduce the desire to consume calorie-rich snacks that are low in vitamins, minerals, and fibers, ultimately improving body composition over time [109]. A second possibility is due to urolithins, secondary metabolites produced from ellagitannins in nuts and berries [79]. Urolithins increase the activation of the adenosine monophosphate-activated protein kinase (AMPK) pathway, resulting in anti-obesogenic properties *in vitro* and in animal models [110, 111]. AMPK increases fatty acid oxidation and decreases triglyceride accumulation [110]. Phosphorylation of AMPK may also decrease cholesterol synthesis and lipogenesis by down-regulating 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and sterol regulatory-element binding protein expression [111, 112]. In clinical studies exploring the relationship

between food and body composition, the incorporation of nuts and berries into the diet was associated with weight loss or maintenance [113 - 115].

Brain health

Regular consumption of nuts or berries has been reported to support brain health and cognitive function, motor control, mood, and executive function at physiologically relevant intakes [116]. Middle-aged and older adults experienced improvements in balance, gait, and memory, and children experienced higher executive function and positive affect after acute and regular intake of both strawberries and blueberries [51, 117-121]. These beneficial effects may be the result of direct effects on brain signaling or indirect effects through oxidant defense and anti-inflammatory properties of polyphenols and other bioactive compounds in nuts and berry foods [122-124].

The gut-brain axis is an emerging area of research. Most studies are pre-clinical in nature using animal models, but are suggestive of a significant role of gut microbial-derived ellagitannin metabolites on brain health and neuroprotection [125, 126].

Skin health

The influence of nuts and berries on skin health and appearance is an emerging area of research [127]. Regular intake of almonds, a good source of fatty acids and polyphenols, has been associated with a significant decrease in facial hyperpigmentation and wrinkle severity [128, 129]. A walnut protein hydrolysate administered to rats exposed to ultraviolet radiation significantly reduced skin photoaging and enhanced skin elasticity [130]. Supplementation with ellagic acid, a compound found in many berries, prevented UVB-related inflammation and

collagen degradation related to skin wrinkling and aging in a murine model [131]. More human studies, using objective measures of skin wrinkles, skin elasticity and response to low-dose UVB radiation exposure are warranted. Monitoring skin responses to an ultraviolet B (UVB) radiation challenge has been used as a marker of whole-body antioxidant status in response to almond consumption [132]. The response to a UVB challenge has also been used to monitor oxidant defenses and changes in skin microbiome following the intake of pomegranate juice [133].

Eye health

Age-related macular degeneration (AMD) is the third leading cause of vision loss worldwide [134]. Anthocyanins, carotenoids, flavonoids, and vitamins C and E, found in many berries, have been shown to reduce the risk of eye-related diseases [135, 136]. Goji berries, containing the highest amount of zeaxanthin of any known food, hold particular promise since this compound binds to receptors in the macula to offer protection from blue and ultraviolet light [137]. Regular supplementation with 28g/d of goji berries for three months increased macular pigment optical density, a biomarker for AMD, as well as the skin carotenoid index [137]. Nuts may also be protective against AMD since they are a rich source of vitamin E and essential fatty acids. Regular intake of nuts has been associated with a reduced risk and slower progression of AMD in two epidemiological studies, thought to be due to the beneficial role of polyunsaturated fatty acids [138, 139].

Agricultural and administrative challenges

New cultivars

Identification of new cultivars with traits desirable for growers, processors, and consumers is a continuous effort. As researchers continue to produce new varieties by both conventional and molecular-driven approaches, assessing these varieties for nutritional value is a challenge. A combination of broad targeted and untargeted metabolomic approaches, along with defined functional phenotyping (i.e., assays relevant to bioavailability, metabolism, or health functionality) could be used for rapid screening and defining of mechanistic pathways associated with health. However, consumer preferences for new cultivars are often driven by size and appearance of the berry or nut, and flavor, rather than its nutritional value [140]. This would further confirm the need to balance improvements to nutritional profiles with enhancement of consumer driven traits, maintaining the marketable nature of the berries and nuts.

Funding and research bias

Biomedical research, particularly for clinical studies, is expensive and resource intensive. While the USDA competitive grants program offers funding for outstanding research projects, budget limitations favor animal or *in vitro* study proposals. Compelling pilot data is needed to be competitive for clinical studies funded by the USDA or NIH, so many researchers submit their initial ideas to commodity groups representing specific nuts or berries. Commodity groups represent farmers, processors, and distributors, and have been instrumental in supporting fundamental and applied research focused on their specific berry or nut.

The perception that studies funded by nut and berry commodity groups are inherently biased in favor of the test food is an issue sometimes raised by critics, journalists and the general

public. As in all nutrition research, ethical considerations regarding the structure of research questions, hypotheses, study design, outcome measures, interpretation of data, and conclusions must be rigorously considered. The food and beverage industries have played a key role in providing funds and supporting nutrition research on individual foods and beverages, including berries and nuts. While this draws scrutiny regarding scientific integrity and data reporting, collaboration between academia and industry compared to exclusive corporate funding may help offset some of these concerns. For example, in multiple reported studies, matching funds were also provided by non-industry sources, including institutional and federal agencies. In other cases, while the food industry provided the test agents, key research personnel and staff were not supported by the same funding source. The academia-industry collaboration has also led to the formation of scientific advisory committees that evaluate and recommend proposals for funding, a peer review process that helps ensure rigorous study designs, data reporting and dissemination of results. Human studies of sufficient statistical power are expensive, labor-intensive efforts requiring sophisticated and costly laboratory equipment and supplies. In order for research proposals to be competitive for funding from the USDA or NIH, pilot data is required, and for nuts and berries, the only realistic source of funding for these exploratory trials is from industry sources. Critics of industry support for nutrition research have yet to propose realistic alternatives for funding needed to generate initial data. Further, ongoing industry funding of nuts and berries research has yielded important insights into the molecular and physiological understanding of mechanisms of action. Without industry support, provided in an ethical and transparent manner, advances in our understanding of the role of nuts and berries in a healthy dietary pattern would be limited.

A risk-of-bias (ROB) study of 5,675 journal articles used in systemic reviews published between 1930 and 2015, representing a wide variety of nutrition topics, concluded that ROB domains started to significantly decrease after 1990, and particularly after 2000 [141]. Another study examined the incidence of favorable outcomes reported in studies funded by the food industry in the 10 most-cited nutrition and dietetics journals in 2018 [142]. Of the 1,461 articles included in the analysis, 196 (13%) reported industry support, with processed food and dietary supplement manufacturers supporting 68% of the studies included. Studies supported by any nut or berry commodity group were not considered due to an incidence lower than 3% of qualifying articles. Studies with food industry support reported favorable results in 56% of their articles, compared to 10% of articles with no industry involvement. The authors offer a number of suggestions to help minimize real or perceived bias, calling on research institutions to enforce strict, regularly updated, and transparent oversight in all research projects involving industry. Suggestions in support of research transparency and integrity have also been advanced from guidelines adapted from the International Life Sciences Institute North America [143]. This served as the basis for the development of consensus guiding principles in for public private partnerships developed by a group of representatives from academia, scientific societies and organizations, industry scientists, and the USDA, NIH, US Centers for Disease Control, and the US Food and Drug Administration [144]. These provisions include full disclosure of funding and confirmation of no direct industry involvement in the study design, data and statistical analyses, interpretation of the results and only minimal, if any, involvement of industry coauthor(s), often given as a courtesy to acknowledge funding and logistical support by the investigators with no intellectual involvement by the study sponsor [145]. This is in contrast to industry-initiated research, where the industry office or commodity group sets predetermined research objectives,

provides intellectual collaboration and often has input on the study design, interpretation of results and decisions regarding publication [146, 147].

While some critics may argue that repeated industry funding in support of research groups that report favorable results on a particular nut or berry shows a bias towards positive outcomes, other interpretations are also possible. First, few labs have the infrastructure, detailed methodology and analytical equipment, and trained personnel to conduct clinical studies in an efficient and timely manner. Second, registering the study on the ClinicalTrials.gov research registry also provides transparency about study design, outcome measures, and results. Industry-funded studies conducted at major universities have layers of review and accountability within their organizations to guard against malfeasance and while these layers may not focus directly on precise elements of research design and interpretation of results, faculty members at such institutions generally have a level of integrity and accountability, knowing that administrative review exists. Calls for industry funded research are often broad in scope, which allows researchers to generate proposals, research questions and hypotheses that do not have preconceived outcomes. A third consideration is that the nuts or berries under study may simply have sufficient bioactivity to produce favorable outcomes, independent of potential researcher bias.

Conclusion

Nuts and berries are an important part of a healthy eating pattern. With unique nutritional profiles, including an array of bioactive compounds and phytonutrients, nuts and berries support a variety of health-promoting qualities and are associated with improved cardiometabolic, cognitive, gut microbiome and other outcomes. Improved understanding and new insights about

nuts and berries in the human diet are predicted with advances in precision nutrition and multi-omics technologies. Nonetheless, fundamental research issues exist, including study duration, testing amounts that reflect typical use and in heterogenous populations, appropriate control groups, and funding streams. The simple question: "are nuts and berries healthy?" is best answered by "it depends" on factors discussed in this review such as in whom, how much, and how often.

Tables and Figures

Table 1. Bioactive compounds and concentrations in select nuts and berries

Table 1.1: Flavonoids Content of Select Nuts and Berries [203]

Food Item	Class	Flavonoid	Mean Quantity (mg/100g edible portion)
Nuts			
Walnut	Anthocyanidins	Cyanidin	2.71
Almond	Anthocyanidins	Cyanidin	2.46
	Flavan-3-ols	(-)-Epicatechin	0.6
		(-)-Epigallocatechin	2.59
		(+)-Catechin	1.28
	Flavanones	Eriodictyol	0.25
		Naringenin	0.43
	Flavonols	Isorhamnetin	2.64
		Kaempferol	0.39
		Quercetin	0.36
Hazelnut	Anthocyanidins	Cyanidin	6.71
	Flavan-3-ols	(-)-Epicatechin	0.22
		(-)-Epigallocatechin	2.78
		(-)-Epigallocatechin 3-gallate	1.06
		(+)-Catechin	1.19
Cashew	Flavan-3-ols	(-)-Epicatechin	0.93
		(-)-Epicatechin 3-gallate	0.15
		(+)-Catechin	0.90
Pecan	Anthocyanidins	Cyanidin	10.74
		Delphinidin	7.28
	Flavan-3-ols	(-)-Epicatechin	0.82

		(-)-Epigallocatechin	5.63
		(-)-Epigallocatechin 3-gallate	2.30
		(+)-Catechin	7.24
Pistachio	Anthocyanidins	Cyanidin	7.33
	Flavan-3-ols	(-)-Epicatechin	0.83
		(-)-Epigallocatechin	2.05
		(-)-Epigallocatechin 3-gallate	0.40
		(+)-Catechin	3.57
	Flavonols	Quercetin	1.46
Berries			
Strawberry	Anthocyanidins	Cyanidin	1.68
		Delphinidin	0.31
		Malvidin	0.01
		Pelargonidin	24.85
		Peonidin	0.05
		Petunidin	0.11
	Flavan-3-ols	(-)-Epicatechin	0.42
		(-)-Epicatechin 3-gallate	0.15
		(-)-Epigallocatechin	0.78
		(-)-Epigallocatechin 3-gallate	0.11
		(+)-Catechin	3.11
		(+)-Gallocatechin	0.03
	Flavanones	Naringenin	0.26
	Flavonols	Kaempferol	0.50
		Myricetin	0.04
		Quercetin	1.11
Blueberry	Anthocyanidins	Cyanidin	8.46
		Delphinidin	35.43
		Malvidin	67.59
		Peonidin	20.29
		Petunidin	31.53
	Flavan-3-ols	(-)-Epicatechin	0.62
		(-)-Epigallocatechin	0.66
		(+)-Catechin	5.29
		(+)-Gallocatechin	0.12
	Flavones	Luteolin	0.20
	Flavonols	Kaempferol	1.66
		Myricetin	1.30
		Quercetin	7.67
Raspberry	Anthocyanidins	Cyanidin	45.77
		Delphinidin	1.32
		Malvidin	0.13

		Pelargonidin	0.98
		Peonidin	0.12
		Petunidin	0.31
	Flavan-3-ols	(-)-Epicatechin	3.52
		(-)-Epigallocatechin	0.46
		(-)-Epigallocatechin 3-gallate	0.54
		(+)-Catechin	1.31
	Flavonols	Kaempferol	0.06
		Quercetin	1.05
Blackberry	Anthocyanidins	Cyanidin	99.95
		Pelargonidin	0.45
		Peonidin	0.21
	Flavan-3-ols	(-)-Epicatechin	4.66
		(-)-Epigallocatechin	0.10
		(-)-Epigallocatechin 3-gallate	0.68
		(+)-Catechin	37.06
	Flavonols	Kaempferol	0.27
		Myricetin	0.67
		Quercetin	3.58

Table 1.2: Phenolic Acid Content of Select Nuts and Berries [204]

Food Item	Class	Phenolic Acid	Mean Quantity (mg/100g fresh weight)
Nuts			
Walnut	Hydroxybenzoic acids	Ellagic acid	28.5
Almond	Hydroxybenzoic acids	4-Hydroxybenzoic acid	0.00410
		Protocatechuic acid	0.26
		Vanillic acid	0.17
Hazelnut	-	-	-
Cashew	-	-	-
Pecan	-	-	-
Pistachio	-	-	-
Berries			
Strawberry	Hydroxybenzoic acids	4-Hydroxybenzoic acid 4-O-glucoside	1.53
		5-O-Galloylquinic acid	0.05
		Ellagic acid	1.24
		Ellagic acid glucoside	2.85
		5-Caffeoylquinic acid	1.93

	Hydroxycinnamic acids	Caffeoyl glucose	0.10
		Cinnamic acid	0.22
		Feruloyl glucose	0.10
		p-Coumaric acid	0.21
		p-Coumaric acid 4-O-glucoside	0.15
		p-Coumaroyl glucose	4.36
Blueberry	Hydroxybenzoic acids	4-Hydroxybenzoic acid 4-O-glucoside	0.55
		Gallic acid 4-O-glucoside	0.50
		Protocatechuic acid 4-O-glucoside	0.40
	Hydroxycinnamic acids	3-Caffeoylquinic acid	0.60
		4-Caffeoylquinic acid	0.35
		5-Caffeoylquinic acid	131.18
		5-Feruloylquinic acid	0.75
		5-p-Coumaroylquinic acid	0.35
		Caffeic acid 4-O-glucoside	0.30
		Ferulic acid 4-O-glucoside	0.55
		p-Coumaric acid 4-O-glucoside	0.95
Raspberry	Hydroxybenzoic acids	Ellagic acid	2.12
		Ellagic acid acetyl-arabinoside	0.20
		Ellagic acid acetyl-xyloside	0.36
		Ellagic acid arabinoside	2.27
		Lambertianin C	30.84
		Sanguiin H-6	76.10
	Hydroxycinnamic acids	5-Caffeoylquinic acid	0.57
		p-Coumaric acid	0.000230
		p-Coumaric acid 4-O-glucoside	0.32
Blackberry	Hydroxybenzoic acids	4-Hydroxybenzoic acid 4-O-glucoside	1.13
		Ellagic acid	43.67
		Gallic acid	4.67
		Galloyl glucose	0.27
		Protocatechuic acid 4-O-glucoside	0.43
	Hydroxycinnamic acids	3-Caffeoylquinic acid	4.53
		3-Feruloylquinic acid	0.30
		3-p-Coumaroylquinic acid	0.37

		4-Caffeoylquinic acid	0.10
		5-Caffeoylquinic acid	0.10
		Caffeoyl glucose	0.50
		Feruloyl glucose	0.43
		p-Coumaric acid 4-O-glucoside	0.27
		p-Coumaroyl glucose	0.67

Table 1.3: Carotenoid Content of Select Nuts and Berries [205]

Food Item	Carotenoid	Mean Quantity (ug/100g edible portion)
Nuts		
Walnut	b-Carotene	12
	Lutein/Zeaxanthin	9
Almond	b-Carotene	1
	Lutein/Zeaxanthin	1
Hazelnut	a-Carotene	3
	b-Carotene	11
	Lutein/Zeaxanthin	92
Cashew	Lutein/Zeaxanthin	22
Pecan	b-Carotene	29
	b-Cryptoxanthin	9
	Lutein/Zeaxanthin	17
Pistachio	a-Carotene	10
	b-Carotene	305
	Lutein/Zeaxanthin	2903
Berries		
Strawberry	b-Carotene	7
	Lutein/Zeaxanthin	26
Blueberry	b-Carotene	32
	Lutein/Zeaxanthin	80
Raspberry	a-Carotene	16
	b-Carotene	12
	Lutein & zeaxanthin	136
Blackberry	b-Carotene	128
	Lutein & zeaxanthin	118

Table 2. Intake of select nuts on cardiovascular and gut health, 2017 - 2023

Table 2.1 Walnuts

Citation	Study Design	Study Duration	Subject Characteristics	n	Nut Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Bamberger 2017 [148]	Randomized, controlled, crossover trial	8 weeks	Healthy males and females (mean age 63 years)	194	Walnut, 43 g/d	Exclusion of walnuts	↓ TC*, non-HDL-C**, LDL-C*, TG*, ApoB**
Bhardwaj 2018 [149]	Randomized, controlled, crossover trial	PP HFM	OW males and females (mean age 42 years)	27	Walnut, 60 g	Almond, 77 g	↑ FMD* ↓ sVCAM-1*
Holscher 2018 [160]	Randomized, crossover, controlled-feeding trial	3 weeks	Healthy males and females (mean age 53 years)	18	Walnut, 42 g/d	Isocaloric diet, excluding walnuts	↓ *LDL-C**
Alibabaie 2019 [150]	Randomized, controlled trial	4 weeks	Female undergraduate students (mean age 20 years)	48	Walnut, 40 g/d	Exclusion of walnuts	↓ LDL-C*, TG*
Borkowski 2019 [22]	Randomized, controlled trial	4 weeks	Hypercholesterolemic, postmenopausal females (mean age 60 years)	20	Walnut, 40 g/d	Walnut, 5 g/d	↑ lipoprotein ALA and epoxides**
Domenech 2019 [151]	Randomized, controlled trial	2 years	Healthy, elderly males and females (mean age 69 years)	236	Walnut, 30-60 g/d (15% energy)	Usual diet, excluding walnuts	↓ SysBP*
Hwang 2019 [152]	Randomized, controlled, crossover trial	16 weeks	Males and females with MetS (mean age 39 years)	84	Walnut, 45 g/d	Isocaloric snack	↑ HDL-C*
Sanchis 2019 [153]	Randomized, crossover, controlled-feeding trial	30 days	Males and females with CKD stage 3 or 4 (mean age 71 years)	13	Walnut, 30 g/d	Isocaloric diet, excluding walnuts	↓ LDL-C*, SysBP*
Tindall 2019 [154]	Randomized, crossover, controlled-feeding trial	6 weeks	OW males and females at risk of CVD (mean age 44 years)	36	Walnut, 57-99 g/d	(1) Walnut fatty acid–matched diet	↓ Central DiaBP*, Central and brachial MAP*,

						(2) oleic acid replaces ALA diet	TC**, LDL-C**, HDL-C**, non-HDL-C*, TD:HDL-C*
Abdrabalnabi 2020 [155]	Randomized, controlled trial	2 years	Healthy, elderly males and females (mean age 69 years)	625	Walnut, 30-60 g/d (15% energy)	Usual diet, excluding walnuts	↓ TG* HDL-C**
Tindall 2020 [156]	Randomized, crossover, controlled-feeding trial	6 weeks	OW males and females at risk of CVD (mean age 44 years)	34	Walnut, 57-99 g/d	(1) Walnut fatty acid–matched diet (2) oleic acid replaces ALA diet	↓ TC** non-HDL-C**, LDL-C**
Rajaram 2021 [157]	Randomized, controlled trial	2 years	Healthy, elderly males and females (mean age 69 years)	628	Walnut, 30-60 g/d (15% energy)	Usual diet, excluding walnuts	↓ TC*, LDL-C*, IDL-C*
Herselman 2022 [158]	Randomized, controlled trial	16 weeks	Healthy male and female undergraduate students (mean age 22 years)	60	Walnut, 56g/d	Usual diet, excluding walnuts	No Δ in TC or TG
Gut health							
Bamberger 2018 [159]	Randomized, controlled, crossover trial	8 weeks	Healthy males and females (mean age 63 years)	194	Walnut, 43 g/d	Exclusion of walnuts	↑*Ruminococcaceae*, * *Bifidobacteria** ↓ *Clostridium**
Holscher 2018 [160]	Randomized, crossover, controlled-feeding trial	3 weeks	Healthy males and females (mean age 53 years)	18	Walnut, 42 g/d	Isocaloric diet, excluding walnuts	↑ *Faecalibacterium**, *Clostridium**, *Dialister**, *Roseburia** ↓ *Ruminococcus**, *Dorea**, *Oscillospi*

							*ra**, *Bifidobacterium**, *SBA**
García-Mantrana 2019 [161]	Non-randomized, short-term dietary intervention trial	3 days	Healthy males and females (mean age 40 years)	27	Walnut, 33 g/d	N/A	UM-B: ↑ *Blautia**, *Bifidobacterium**, *Gordonibacter** UM-A: ↓ Lachnospiraceae* Both: ↑ *Coprococcus** and *Collinsella** ↑ SCFA*
Tindall 2020 [162]	Randomized, crossover, controlled-feeding trial	6 weeks	OW males and females at risk of CVD (mean age 44 years)	42	Walnut, 57-99 g/d	(1) Walnut fatty acid–matched diet (2) oleic acid replaces ALA diet	↑ *Roseburia**, *Eubacterium eligensgroup**, *Lachnospiraceae**, *Gordonibacter**

Table 2.2 Almonds

Citation	Study Design	Study Duration	Subject Characteristics	n	Nut Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Lee 2017 [163]	Randomized, 4-period crossover, controlled feeding trial	4 weeks	OW and obese males and females (mean age 46 years)	31	Almond, 42.5 g/d	Isocaloric diet, excluding almonds	↓ TC*, non-HDL-C*, LDL-C*, ApoB*, SysBP*, DiaBP* No Δ in FMD
Liu 2017 [164]	Randomized, controlled trial	16 weeks	Healthy males and females (mean age 26 years)	169	Almond, 56 g/d, (1) premeal or (2)	Isocaloric snack	↓ TC*, LDL-C*, non-HDL-C*

					between meals		
Bhardwaj 2018 [149]	Randomized, controlled, crossover trial	PP HFM	OW males and females (mean age 42 years)	27	Almond, 77g	Walnut, 60g	↓ sVCAM* ↑ FMD (nonsignificant)
Dhillon 2018 [165]	Randomized, controlled trial	8 weeks	Healthy males and females (mean age 18 years)	73	Almond, 56.7 g/d	Isocaloric snack	↓ TC*, HDL-C*, *LDL-C No Δ in RHI, AIx, BP
Jung 2018 [166]	Randomized, controlled, crossover trial	4 weeks	OW and obese males and females (mean age 52 years)	84	Almond, 56 g/d	Isocaloric snack	↓ TC*, LDL-C*, non-HDL-C*
Liu 2018 [167]	Randomized, controlled trial	20 weeks	Healthy males and females (mean age 27 years)	85	Almond, 56 g/d	Isocaloric snack	↓ DiaBP*, TC**, HDL-C**, LDL-C**, non-HDL-C**, TG**, VLDL-C**
Bowen 2019 [168]	Randomized, controlled trial	8 weeks	OW and obese males and females at risk for T2DM (mean age 61 years)	76	Almond, 56 g/d	Isocaloric snack	Women only: ↓ TC:HDL-C ratio*
Coates 2020 [31]	Randomized, controlled trial	12 weeks	OW and obese males and postmenopausal females (mean age 65 years)	128	Almond, 15% energy	Isocaloric snack	↓ TG*, SysBP* No Δ in ICAM-1, VCAM-1, SAC or LAC
Dikariyanto 2020 [169]	Randomized, controlled trial	6 weeks	Males and females at risk for CVD (mean age 56 years)	105	Almond, 20% of energy	Isocaloric snack	↑ FMD** ↓ LDL-C*, non-HDL-C* No Δ TG, HDL-C or BP
Dikariyonto 2020 [170]	Randomized, controlled trial	6 weeks	Males and females at risk for CVD (mean age 56 years)	105	Almond, 20% of energy	Isocaloric snack	↑ HRV*

Palacios 2020 [171]	Randomized, controlled, crossover trial	6 weeks	OW and obese males and females with prediabetes (mean age 48 years)	33	Almond, 85 g/d	Isocaloric snack	↑ApoA*, HDL3-C*
Gut health							
Holscher 2018 [172]	Randomized, 5-arm crossover, controlled feeding trial	3 weeks	Healthy males and females (mean age 57 years)	18	(1) Almond, 42 g/d (whole), (2) Almond, 42 g/d (roasted), (3) Almond, 42 g/d (roasted, chopped) (4) Almond butter, 42 g/d	Exclusion of almonds	Chopped: ↑ *Lachnospira**, *Roseburia**, *Oscillospira** Whole: ↑ *Dialister**
Dhillon 2019 [173]	Randomized, controlled trial	8 weeks	Healthy males and females (mean age 18 years)	73	Almond, 56.7 g/d	Isocaloric snack	↑ alpha-diversity* ↓ *Bacteroides fragilis**
Choo 2021 [174]	Randomized, controlled trial	8 weeks	OW and obese males and females at risk for T2DM (mean age 61 years)	69	Almond, 56 g/d	Isocaloric snack	↑ *Ruminococcaceae**
Creedon 2022 [175]	3-arm, parallel-design randomized, controlled trial	4 weeks	Healthy males and females (mean age 28 years)	79	Almond, 56 g/d	Isocaloric snack	↑ SCFA (butyrate) * No Δ in bifidobacteria

Table 2.3 Hazelnuts

Citation	Study Design	Study Duration	Subject Characteristics	n	Nut Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							

Adamo 2018 [176]	Randomized, controlled trial	2 weeks	Healthy males and females (mean age 26 years)	61	(1) Peeled hazelnut paste, 30 g/d (2) Unpeeled hazelnut paste, 30 g/d	(1) Snack with peeled hazelnut paste, 30 g/d (2) Snack with cocoa powder, 2.5 g/d (3) Snack with peeled hazelnut paste, 30 g/d, and cocoa powder, 2.5 g/d	↑ HDL-C*, PSV* ↓ LDL-C*, TC:HDL**, LDL:HDL**
Deon 2018 [177]	Randomized, controlled trial	8 weeks	Children and adolescents with hyperlipidemia (mean age 12 years)	66	(1) Roasted, peeled, hazelnut, 15-30 g/d (2) Roasted, unpeeled hazelnut, 15-30 g/d	Dietary advice for hyperlipidemia	↓ LDL-C** ↑ HDL:LDL** No Δ in BP
Di Renzo 2017 [178]	Randomized, controlled, crossover trial	PP HFM	Healthy males and females (mean age 31 years)	22	Hazelnut, 40 g	HFM, no hazelnuts	↓ oxLDL*
Santi 2017 [179]	Randomized, controlled, crossover trial	6 weeks	Healthy males and females (median age 55 years)	24	Hazelnut, 40 g	Standard/habitual diet, no hazelnuts	↓ LDL-C*
Tey 2017 [180]	Randomized, crossover trial	28 days	Healthy males and females (mean age 46 years)	72	Raw hazelnut, 30 g/d	Dry roasted, lightly salted hazelnut, 30 g/d	↑ HDL-C**, ApoA*, TC:HDL**, SysBP*
Guaraldi 2018 [181]	Randomized, controlled trial	8 weeks	Children and adolescents with hyperlipidemia (mean age 12 years)	60	(1) Roasted, peeled, hazelnut, 15-30 g/d	Dietary advice for hyperlipidemia	↓ Oxidatively-induced DNA

					(2) Roasted, unpeeled hazelnut, 15-30 g/d		strand breaks* No Δ in oxLDL
Michels 2018 [182]	Pre-post intervention trial	16 weeks	Healthy males and females (mean age 63 years)	32	Hazelnut, 57 g/d	Subject's respective baseline data	↓ LDL-C*, TC:HDL* No Δ in TG, HDL-C or BP
Di Renzo 2019 [183]	Pre-post intervention trial	6 weeks	Healthy males and females (mean age 52 years)	24	Hazelnut, 40 g	Subject's respective baseline data	↓ TC*, LDL-C*, TC:HDL* No Δ in BP
Gut health							
Gargari 2018 [184]	Randomized, controlled trial	8 weeks	Children and adolescents with hyperlipidemia (mean age 11 years)	15	Roasted, unpeeled hazelnut, 15-30 g/d	Normolipidemic children and adolescents	↑ Fecal acetate* No Δ in α- or β-diversity

Table 2.4 Cashews

Citation	Study Design	Study Duration	Subject Characteristics	n	Nut Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Mah 2017 [185]	Randomized, crossover, controlled feeding trial	4 weeks	Males and females with/at risk for elevated LDL-C (mean age 56 years)	51	Cashew, 28-64 g/d (11% of energy)	Isocaloric diet, excluding cashews	↓ TC*, LDL-C*, non-HDL-C*, TC:HDL*
Baer 2019 [186]	Randomized, crossover, controlled feeding trial	4 weeks	OW males and females (mean age 57 years)	42	Cashew, 42 g/d	Isocaloric diet, excluding cashews	No significant Δ in lipid profile, BP, AIx, endothelin, adhesion molecules, or clotting factors
Damavandi 2019 [187]	Randomized, crossover, controlled feeding trial	8 weeks	Males and females with T2DM (mean age 54 years)	50	Cashew, ~28 g/d (10% of energy)5	Isocaloric diet, excluding cashews	↓ LDL:HDL*

Table 2.5 Pecans

Citation	Study Design	Study Duration	Subject Characteristics	n	Nut Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
McKay 2018 [188]	Randomized, crossover, controlled-feeding trial	4 weeks	OW or obese males and females (mean age 63 years)	26	Pecan, ~42.5 g/d (15% of energy)	Isocaloric diet, excluding pecans	↓ E-selectin* ↓ TC and LDL-C trending significant No Δ in BP
Guarneiri 2021 [189]	Randomized, controlled trial	8 weeks, PP HFM	Males and females at risk for CVD (mean age 48 years)	56	Pecans, 68 g/d (1) added to diet (2) substituted for isocaloric snack	Exclusion of pecans	↓ TC*, LDL-C*, TG*, TC:HDL*, non-HDL-C*, ApoB* No Δ in BP

Table 2.6 Pistachios

Citation	Study Design	Study Duration	Subject Characteristics	n	Nut Type, Quantity	Control	Relevant Outcomes
Gut health							
Hernández-Alonso 2017 [190]	Randomized, controlled, crossover trial	4 months	Males and females with prediabetes (mean age 55 years)	39	Pistachio, 57 g/d	Isocaloric diet, excluding pistachios	↓ Gut microbiota-related metabolites (hippurate, p-cresol sulfate, dimethylamine)* and TMAO*

Includes human clinical trials that focus on only one functional food (ie. a single type of nut or berry) with outcomes of known physiologically relevant measures related to vascular function and gut health over the past five years (2017-2023). Excludes interventions using mixtures of different types of nuts or berries, nut- or berry-containing meals, and nut or berry extracts or oils. Also excludes interventions where nut or berry intake was in combination with other potentially confounding factors (ie. diet or

lifestyle modifications including physical activity and dietary counseling). *denotes statistical significance $\leq$0.05; **denotes statistical significance $\leq$0.001

AIx: augmentation index; ALA: alpha-linolenic acid; ApoA: apolipoprotein A; ApoB: apolipoprotein B; BMI: body mass index; BP: blood pressure; CHO: carbohydrate; CKD: chronic kidney disease; CVD: cardiovascular disease; DiaBP: diastolic blood pressure; FDBP: freeze-dried blueberry powder; FDRP: freeze-dried raspberry powder; FDSP: freeze-dried strawberry powder; FMD: flow-mediated dilation; G/d: grams per day; GDM: gestational diabetes; HDL-C: high-density lipoprotein cholesterol; HR: heart rate; HRV: heart rate variability; ICAM-1: intercellular adhesion molecule-1; IDL-C: intermediate-density lipoprotein cholesterol; LAC: large artery compliance; LDL-C: low-density lipoprotein cholesterol; LDL:HDL: LDL-cholesterol to HDL-cholesterol ratio; MAP: mean arterial pressure; MetS: metabolic syndrome; NCEP: National Cholesterol Education Program; NO: nitric oxide; OTUs: operational taxonomic units; OW: overweight; oxLDL: oxidized low-density lipoprotein cholesterol; PA: physical activity; PAT: peripheral arterial tonometry; Pc/wk: piece per week; PP HFM: postprandial high fat meal; PSV: brachial artery peak systolic velocity; PWV: pulse wave velocity; SAC: small artery compliance; SBA: secondary bile acids; SCFA: short-chain fatty acids; sVCAM-1: soluble vascular cell adhesion molecule-1; SysBP: systolic blood pressure; TC: total cholesterol; TC:HDL: total cholesterol to HDL-cholesterol ratio; T2DM: Type 2 Diabetes Mellitus; TG: triglycerides; TMAO: trimethylamine N-oxide; UM: urolithin metabotype; VLDL-C: very low-density lipoprotein cholesterol

Table 3. Intake of select berries on cardiovascular and gut health, 2017 – 2023

Table 3.1 Strawberries

Citation	Study Design	Study Duration	Subject Characteristics	n	Berry Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Feresin 2017 [191]	Randomized, double-blind, controlled, parallel arm trial	8 weeks	Post-menopausal females (mean age 59 years) with pre- or stage 1 hypertension	60	FDSP, 25g/d or 50g/d	Control powder	25g/d: ↓ SysBP* and PWV* 50g/d: ↑ NO metabolites*
Holt 2020 [24]	Randomized, controlled, double-blind, crossover trial	Acute (1 hour), Short-term (1 week)	Adolescent males (mean age 16 years)	25	FDSP, 50g/d	Isocaloric control powder, devoid of polyphenols	↑ plasma nitrate and nitrite** and RHI* No Δ inplatelet reactivity
Basu 2021 [192]	Randomized, controlled crossover trial	14 weeks	Males and females (mean age 53 years) with one or more characteristics of MetS	33	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ Total VLDL and chylomicrons**, small VLDL**, and total and small LDL particles** No Δ in conventional lipid profile
Huang 2021 [85]	Randomized, controlled, double-blinded, 2-arm, 2-period crossover trial	4 weeks	Males and females (mean age 53 years) with moderate hypercholesterolemia	34	FDSP, 25g/d	Isocaloric control powder	↑ FMD (treatment-by-hour effect)* ↓ SysBP*

							No Δ in lipid profile, ApoA, or ApoB
Gut health							
Ezzat-Zadeh 2021 [193]	Single arm intervention trial (placebo group omitted due to prebiotic content)	4 weeks	Males and females (mean age 30 years)	14	FDSP, 26g/d	N/A	↑ *20 OTUs** ↓ *4 OTUs** No Δ in fecal microbial metabolites or SCFA

Table 3.2 Blueberries

Citation	Study Design	Study Duration	Subject Characteristics	n	Berry Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Curtis 2019 [194]	Double-blind, randomized controlled trial	6 months	OW and obese males and females (mean age 63 years) with MetS	115	Blueberry, 75g/d or 150g/d	Control powder	↑FMD*, AIx*, HDL-C*, ApoA* No Δ PWV, BP, LDL-C or LDL:HDL ratio
Stote 2020 [195]	Double-blind, randomized, controlled Trial	8 weeks	Males with T2DM (mean age 67 years)	52	Blueberry, 22g/d FDBP	Control powder	No Δ in TC, LDL-C, HDL-C, or BP
Curtis 2022 [196]	Double blind, randomized controlled trial	Acute (1 dose)	OW and obese males and females (mean age 63 years) with MetS	45	FDBP, 26g/d (1 C whole fruit equivalent)	Isocaloric control powder	↑HDL-C*, ApoA* ↓ TC* No Δ in LDL-C, TG, FMD,

							PWV, AIx, or BP
Krikorian 2022 [197]	Randomized, controlled trial	12 weeks	OW and obese males and females (mean age 56 years)	27	FDBP, ½ C whole fruit equivalent	Control powder devoid of fiber	No Δ in lipid profile
Wang 2022 [198]	**Randomized, controlled. crossover trial**	1 week	Normal to OW males and females (mean age 26 years)	37	Blueberry, 160g/d FDBP, 20g/d	Fiber matched control capsule	No Δ in lipid profile, plasma nitrite, PWV, or BP

Table 3.3 Blackberries

Citation	Study Design	Study Duration	Subject Characteristics	n	Berry Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Solverson 2018 [199]	Randomized, controlled, crossover trial	1 week	OW or obese males (mean age 60 years)	27	Blackberry, 600g/d	Isocaloric gelatin	↑ fat oxidation* No Δ in TG

Table 3.4 Raspberries

Citation	Study Design	Study Duration	Subject Characteristics	n	Berry Type, Quantity	Control	Relevant Outcomes
Cardiovascular health							
Istas 2018 [200]	3-arm double-blind, randomized, controlled, crossover trial	Acute (24 hours)	Males (mean age 27 years)	10	Raspberry, 200 g/d or 400 g/d	Control drink devoid of polyphenols	↑ FMD** No Δ PWV, AIx, or BP
Xiao 2019 [201]	Randomized, single-blind, three-arm, 24-hour, within-subject crossover trial	Acute, postprandial	OW or obese males and females (mean age 34 years) with prediabetes and insulin resistance	32	Raspberry, 125g/d or 250g/d	Absence of raspberry with test meal	No Δ in TG

Franck 2020 [202]	2-arm parallel-group, randomized, controlled trial	8 weeks	Males and pre-menopausal females (mean age 32 years)	48	Raspberry, 280g/day	Habitual diet	↓ SysBP*, ApoB* No Δ in lipid profile
Zhang 2022 [84]	Randomized crossover trial	4 weeks	OW or obese males and females (mean age 35 years) with prediabetes and insulin resistance	36	Raspberry, 50g/d	N/A	↓ TC*, LDL-C*, and LDL:HDL ratio*
Gut Health Outcomes							
Zhang 2022 [84]	Randomized crossover trial	4 weeks	OW or obese males and females (mean age 35 years) with prediabetes and insulin resistance	36	Raspberry, 50g/d	N/A	↑ *Eubacterium eligens**↓ Ruminococcus gnavus*

Includes human clinical trials that focus on only one functional food (ie. a single type of nut or berry) with outcomes of known physiologically relevant measures related to vascular function and gut health over the past five years (2017-2023). Excludes interventions using mixtures of different types of nuts or berries, nut- or berry-containing meals, and nut or berry extracts or oils. Also excludes interventions where nut or berry intake was in combination with other potentially confounding factors (ie. diet or lifestyle modifications including physical activity and dietary counseling). *denotes statistical significance $\leq$0.05; **denotes statistical significance $\leq$0.001

AIx: augmentation index; ALA: alpha-linolenic acid; ApoA: apolipoprotein A; ApoB: apolipoprotein B; BMI: body mass index; BP: blood pressure; CHO: carbohydrate; CKD: chronic kidney disease; CVD: cardiovascular disease; DiaBP: diastolic blood pressure; FDBP: freeze-dried blueberry powder; FDRP: freeze-dried raspberry powder; FDSP: freeze-dried strawberry powder; FMD: flow-mediated dilation; G/d: grams per day; GDM: gestational diabetes; HDL-C: high-density lipoprotein cholesterol; HR: heart rate; HRV: heart rate variability; ICAM-1: intercellular adhesion molecule-1; IDL-C: intermediate-density lipoprotein cholesterol; LAC: large artery compliance; LDL-C: low-density lipoprotein cholesterol; LDL:HDL: LDL-cholesterol to HDL-cholesterol ratio; MAP: mean arterial pressure; MetS: metabolic syndrome; NCEP: National Cholesterol Education Program; NO: nitric oxide; OTUs: operational taxonomic units; OW: overweight; oxLDL: oxidized low-density lipoprotein cholesterol; PA: physical activity; PAT: peripheral arterial tonometry; Pc/wk: piece per week; PP HFM: postprandial high fat meal; PSV: brachial artery peak systolic velocity; PWV: pulse wave velocity; SAC: small artery compliance; SBA: secondary bile acids; SCFA: short-chain fatty acids; sVCAM-1: soluble vascular cell adhesion molecule-1; SysBP: systolic blood pressure; TC: total cholesterol; TC:HDL: total cholesterol to HDL-cholesterol ratio; T2DM: Type 2 Diabetes Mellitus; TG: triglycerides; TMAO: trimethylamine N-oxide; UM: urolithin metabotype; VLDL-C: very low-density lipoprotein cholesterol

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CHAPTER III

Strawberry (Fragaria x Ananassa) Intake on Human Health and Disease Outcomes: a Comprehensive Review of the Literature to Date

Introduction

Current dietary recommendations for adults focus on consuming a healthy dietary pattern that reduces the risk for the development of chronic disease. This includes the intake of 1-2 cups of fruit per day, supported in part, by consistent observations of reduced morbidity and mortality with increased intakes of these foods (1-6). These plant-based foods contain a diverse array of phytonutrients to include essential micronutrients and vitamins important for oxidant defense, and those not traditionally classified as essential, such as the flavan-3-ols (flavanols), with research to date in support of a potential dietary recommendation towards cardiometabolic health of 400-600 mg/day (7). Foods that may help achieve this level of intake includes cocoa, tea and berries, such as strawberry (8). The historical intake of strawberry (genus *Fragaria;* family *Rosaceae*) includes populations living in ancient times in in Europe, North and South America (9). *Fragaria vesca* is a wild strawberry with documented medicinal use in ancient Rome that can also be genetically linked to the modern cultivated strawberry *Fragaria x ananassa*, which originated in Europe during the 18th century from the hybridization of the wild strawberry species, *Fragaria virginiana* of North American and *Fragaria chiloensis* of South America (10-12).

Epidemiological observations specific for strawberry intake report of reduced risk for cardiovascular disease and cancer in women consuming a single weekly serving of strawberries with the reduced risk associated with increased intake of strawberry flavonoids (13, 14). Strawberry flavonoids include anthocyanins, flavan-3-ols, and flavonols. Additional potential health promoting strawberry (poly)phenols and

phytonutrients include phenolics, ellagic acid (EA), ellagitannin (ET), fiber, micronutrients and vitamins. This combination of phytonutrients can have profound impacts on health. Indeed, as well be reviewed in this report, data from dietary intervention trials supports that strawberry intake favorably affects metabolism and systemic physiology, as well as the gastrointestinal microbiome. The latter important due to increased recognition that dietary induced change within the gastrointestinal microbiome can affect overall health through its influence on metabolism and the nervous and cardiovascular systems. In this comprehensive review, we consolidate recent human studies that examine the effects of strawberry intake on human health, and discuss potential mechanisms, and future directions.

Methods

A comprehensive literature search was conducted in English-language journals and scientific indexing databases, including PubMed and Cochrane, as well as from citations in articles that were already determined to be relevant. Papers to compile relevant studies pertaining to strawberry intake and associated nutrients and phytonutrients on human health. Relevance was defined as human studies, both experimental and observational, supplementing with whole strawberries or freeze-dried strawberry powder (FDSP), published between 2000 and 2023.

A primary search was conducted through PubMed. Several search terms were utilized: “Fragaria OR strawberries OR strawberry OR "strawberry polyphenol" OR "strawberry anthocyanin" OR "strawberry ellagitannin" OR “strawberry phenolic” OR “strawberry

phenols” AND "cardiovascular diseases" OR "heart disease risk factors" OR hypertension OR “blood pressure” OR lipid OR dyslipidemia OR hypercholesterolemia OR cholesterol OR triglyceride OR "diabetes mellitus, type 2” OR “insulin resistance” OR “type 2 diabetes” OR cardiometabolic OR “cardiometabolic disease” OR inflammation OR inflammatory OR “inflammatory markers” OR “vascular endothelium” OR “endothelial function” OR “vascular function” OR vasodilation OR “platelet reactivity” OR “platelet function” OR nitric oxide OR obesity OR “metabolic health” OR “metabolic syndrome” OR microbiota OR "gastrointestinal microbiome" OR microbiome OR "gut microbiome" OR "gut health" OR "microbial composition" OR "oxidative Stress" OR "oxidant defense" OR "oxidative damage" OR cognition OR “cognitive function” OR “cognitive health” OR phenols OR ellagic acid OR anthocyanins OR quercetin OR catechin OR ascorbic acid OR folic acid AND dietary OR diet OR consume OR "consumption" OR "intake" OR "dietary intake" OR "supplementation" OR eat OR drink OR beverage OR nutrition.

A total of 513 articles were reviewed with careful consideration in accordance with pre-determined exclusionary criteria. Research using strawberry extract or flavor, or where strawberry intake was combined with other food items, or where intake was in combination with other potentially confounding factors (e.g., diet or lifestyle modifications including physical activity and dietary counseling) was not included. Studies with outcomes pertaining to communicable disease or allergies and those that were not physiologically relevant (e.g., an outcome reporting *in vitro* total antioxidant capacity (TAC)) were also excluded.

Among the 58 articles included in this review, 45 were clinical trials and 13 were observational studies. Of the 45 clinical studies, 33 (73%) used FDSP as test product, while the remaining 12 (27%) used fresh, frozen, or pureed strawberries in their interventions. Studies varied from acute intake to short-term interventions, with study duration ranging postprandial to 6 months. Strawberry quantity ranged from 10 to 50 g/d of FDSP (equivalent to 100-500 g fresh strawberries), 200-500 g/d of fresh or frozen strawberries, and 60-500g/d of pureed strawberries.

The United States Department of Agriculture (USDA) Dietary Guidelines for Americans recommends that adults consume two servings of fruits per day to maintain health (15). A single serving of fruit is equivalent to roughly one cup of whole, halves, sliced, or pureed fruits. In the case of strawberries, one cup is equal to different weights depending on the form of the fruit (144g for whole fruits, 152 g for half fruits, 166 g for sliced fruits, and 232 g for pureed fruits) (16). While the USDA database considers 144-166 g of strawberries as one serving (15), most portion equivalences of FDSP were based on 132 g of fresh strawberries. Although the discrepancy between 132 g and 144-166 g may not be significant with one to two servings, consuming four or more servings can result in a difference of one serving.

Strawberry Phytonutrients

Strawberries contribution to a healthy dietary pattern includes the provision of a number of phytonutrients. For essential nutrients, a single serving (1 cup; 166g of sliced fruit)

provides 100 and 10%, respectively, of the daily value for vitamin C and folate, along with minerals and micronutrients, such as potassium, magnesium, calcium, iron, zinc copper and manganese **(Table 1)**. Additional phytonutrients include phenolics and polyphenols ((poly)phenols) that are secondary plant metabolites derived via the shikimate, pentose phosphate and phenylpropanoid pathways (17). The induction of this pathway by light and temperature produces metabolites that plants utilize to adapt to their environment, including protection from pathogens, invasive plant species, and oxidative damage (18, 19). Major strawberry secondary metabolites includes the phenolic acids, hydroxycinnamic and benzoic acids, and polyphenols such as flavonoids, stilbenes and tannins **(Table 1)**. Flavonoids are further categorized into six subclasses: flavones, flavanones, isoflavones, anthocyanidins, flavonols, and flavanols, with the latter three dominant in strawberry. Distinguishing features of the main categories of strawberry flavonoids are their glycosylation pattern that, as will be discussed later, can influence their absorption from the gastrointestinal tract (20, 21). Anthocyanins provide strawberries with their red pigmentation and include pelargonidin-3- glucoside (P3G) and rutinoside, and cyanidin-3-glucoside (C3G), providing strawberries with their red pigmentation (22). Strawberry flavonols include the 3-*O*-b glucuronides of quercetin, isorhamnetin and kaempferol (23). Flavanols are not present as glycosides, and in strawberry include epicatechin and catechin (23).

The seeds and fruit of strawberry also contain higher molecular weight tannins, a large structurally diverse class of polyphenols categorized into gallotannins, complex tannins, ET, and condensed tannins (24, 25), with the latter two predominant in strawberry fruit

(23, 26-28). Condensed tannins are flavanol oligomers linked from C4 of one flavanol to C6 or C8 of the other. Procyanidins are condensed tannins of epicatechin and catechin units with dimer procyanidin B1 (epciatechin – catechin), B3 (catechin – catechin) and trimer C2 consisting of 3 epicatechin units, with one analysis estimating their sum total in strawberry at approximately 15 mg/100 g fresh weight (23, 28, 29). Gallotannins are derived from gallic acid, and contain six or more galloyl units that bind to polyol-, flavanol-, triterpenoid units, while ET do not contain a glycosidically linked flavanol unit, but are formed through the oxidative formation of C-C bonds between two gallotannin polymers, forming an axially chiral monomer, hexahydroxydiphenoyl (HHDP) (24, 25, 30). The polymerization level of an ET is dependent on the number of HHDP groups present (31). The total amount of ET varies with varietal, with one recent compositional analysis of mature strawberry fruit ranging from 2.96 to 7.00 ug/g, and included monomeric pedunculagin and casuarictin, dimeric sanguiin H-6 and potentillin, and oligomeric agrimoniin along with its gallated derivative fragariin A (27, 31, 32). Furthermore, hydrolysis of the HHDP moiety of ET within a plant produces EA and related glycosides (30, 33-35). Together over 20 different ET and EA conjugates have been detected within strawberry fruit (34).

The key to understanding the potential health promoting effects of tannin is that they are not absorbed, therefore similar to fiber, their direct impact is on the gastrointestinal track and other dietary components. Total fiber consists of the sum of dietary and functional fiber with dietary fiber consisting of nondigestible carbohydrate and lignin, and functional fiber as isolated nondigestible carbohydrate that has a functional physiological

effect in humans. Fiber can be further defined through their physiological effects as fermentable, that is, fiber fermented to short chain fatty acids (SCFAs) by gut bacteria, and viscous fiber that slows transit time by thickening intestinal contents, with strawberry containing both fiber types. Individuals with diets low in fiber intake have a reduced diversity in gut microbial populations that leads to an imbalance in microbial metabolite production, and increased inflammation and permeability (17, 36).

Epidemiological Observations of Strawberry Intake

Among the 13 observational studies included in this review, 11 were cohort and 2 were cross-sectional studies. The consumption of strawberries was evaluated using different methods such as food frequency questionnaires (FFQ), which reported serving sizes in grams per day, or in tertiles, quartiles or quantiles (37-40). In some studies, the number of servings (13, 41-44) or frequency (14, 45, 46) of consumption was reported instead. Two studies from the same lab group involving participants from the Nurses' Health Study (NHS) I and II and the Health Professionals Follow-Up Study (HPFS) reported the portion size of strawberries ranging from 0 to 27 g/d (39, 40). In some studies where direct information on strawberry consumption was not available, anthocyanin concentration was used as a proxy with the assumption that strawberries and blueberries were the major contributors. The consumption of anthocyanins ranged from 8-19 mg/d and 6-22 mg/d in two studies using pooled participants from NHS I and II and HPFS with different inclusion criteria (37, 38). The studies conducted by the same lab group also used pooled data from participants in NHS I and II and HPFS and reported the average strawberry consumption in the highest quartile as approximately 27 g, which was

equivalent to a quarter of one serving based on the USDA database (144-166 g) (39, 40). However, the difference between the approximate consumption and the actual amount of strawberries or FDSP provided in the clinical studies suggests that the quantification of the intake from the FFQ may not be indicative of real-life consumption.

Several prospective cohort studies have examined the intake of flavonoid-rich foods, including strawberries, on the risk of cardiovascular diseases, including coronary heart disease (CHD) and stroke (**Table 2)**. The Japan Public Health Center-based prospective study following approximately 87,000 men and women aged 44-76 years for 13 years, reported over 4,000 cases of incident stroke and 2,000 cases of incident CHD (47, 48). After adjusting for confounding factors, consumption of strawberries was associated with significantly lower risk of stroke in women ($p<0.001$), but not in men (47). Within the same cohort, strawberry intake was inversely related to CHD risk, although this relationship was only borderline in significance (48). Additional cohorts examined the relationship between strawberry intake and cardiovascular disease specifically in women. Among 93,000 young and middle-aged (29-42 years) women participating in the Nurses' Health Study II, those consuming strawberries more than once per week had a reduced risk of all-cause mortality ($p<0.05$) after covariate adjustment (14). The Iowa Women's Health Study reported a significant reduction in death from cardiovascular disease (CVD) with increasing dietary intake of strawberries in 35,000 postmenopausal women aged 55-69 (49). These findings are in contrast to the Women's Healthy Study an 11-year follow-up of almost 40,000 women enrolled in examining the influence of aspirin or vitamin E on cardiovascular outcomes. In this study, strawberry intake at baseline was not

associated with cardiovascular outcome. However, there was a decreased risk for high C-reactive protein (CRP) levels, an inflammatory biomarker associated with chronic disease (50), in women consuming higher quantities (>2 servings/week) of strawberries (13). In support of this finding the National Health and Nutrition Examination Survey (NHANES), a large-scale survey of nutritional status and health in adults and children in the U.S., noted an inverse relationship between anthocyanin intake and CRP (51). Additional cohorts also report of the relationship between strawberry anthocyanin intake a reduce risk of disease development. This includes a 14-year follow-up of over 150,000 participants from the Nurses' Health and Health Professionals Follow-Up Studies, which reported or significantly reduced risk of hypertension with higher intake (16-22 mg/day) of strawberry anthocyanins (52). Likewise, The Iowa Women's Health Study reports of a significant reduction in cardiovascular mortality with intake of strawberries and strawberry anthocyanins in a follow-up of almost 35,000 women over 16 years (49).

Randomized Controlled Trials on Strawberry Intake and Health Outcomes

Cardiometabolic Health

Cardiovascular disease is often phenotyped as a low-grade systemic inflammatory disease that promotes endothelial dysfunction (53, 54). Obesity, defined as increased visceral or central adiposity, is a major modifiable risk factor for CVD, and often characterized with increased circulating levels of pro-inflammatory cytokines, endothelial dysfunction, and metabolic dysregulation (55, 56). To date, dietary intervention trials examining the influence of strawberry intake on health have measured a number of surrogate outcomes,

known to be able to change within a relatively short period of time, and related to diet induced changes in inflammation, vascular and metabolic health.

A number of studies have assessed the impact of dietary strawberry intake on lipid profiles **(Table 2)**. A study of adults with obesity (n=20) found that consumption of FDSP equivalent to four servings of fresh strawberry per day for three weeks significantly reduced total cholesterol (TC) and small high-density lipoprotein (HDL) particles, while increasing the size of low-density lipoprotein (LDL) particles ($P<0.05$) (57). A larger size of LDL particles is associated with a reduced risk of atherosclerosis when compared to smaller LDL particle sizes (58). A study of 60 adults with obesity who were at cardiometabolic risk (abdominal adiposity and elevated serum lipids) showed significant decreases in TC, LDL-cholesterol (LDL-C), and small LDL particle concentration following intake of 50g/d of FDSP for 12-weeks ($p<0.05$), but not with 25 g/d of FDSP. No changes in HDL-C or triglycerides (TG) were observed (59). Significant decreases in total- and LDL-cholesterol, as well as small LDL particles, but not HDL-cholesterol (HDL-C) or TG, were reported participants with obesity and metabolic syndrome (n=27) following intake of 50g/d of FDSP for eight-weeks (60). Another study in women with metabolic syndrome (n=16) reported that TC and LDL-C were both significantly reduced following 4-weeks of 50 g/d of FDSP compared to their baseline values ($p<0.05$) (61). A recent study in 40 adults with moderately elevated baseline LDL-C giving either a modest amount (13 g/d) or a high amount (40 g/d) of FDSP for four weeks on serum lipids reported significant ($p<0.05$) reductions in both TC and LDL-C in

the group consuming 13g/d but not in those consuming 40 g/d or in the control group (62).

Basu et al. explored the effects of two portions (13 g/d or 32 g/d) of FDSP daily for four weeks on lipid profiles and lipoprotein particle size in 33 adults with obesity and elevated LDL-C and noted that the particle concentrations of total chylomicrons, very-low density lipoprotein (VLDL), LDL and small VLDL and LDL were significantly decreased from baseline values in the 32 g/d group, but not the 13 g/d or control groups ($p<0.0001$) (63). Interestingly, conventional measures of lipids did not change for any group. Similarly, no changes in lipid profiles were observed following intake of 50 g/d of FDSP for four weeks in a group of obese, hypercholesterolemic adults (64) or in a study of 25 overweight or obese adolescent (14-18 years) males that also supplemented with 50g/d of FDSP for one week (65). In another study that also supplemented with 50g/day FDSP for 12-weeks, no changes in lipid profiles were noted (66). In a population with type 2 diabetes mellitus (T2DM), TC and the TC:HDL ratio, but not TG or HDL-C, were significantly reduced from baseline following 6-weeks of 50g/d of FDSP (67). However, these improvements were not significantly different from the control group (67). In another cohort of 44 people who also had T2DM, intake of 200g/d of fresh strawberries for 14 days significantly reduced LDL-C compared to the control group ($P<0.001$) (68). When added to a cholesterol-lowering diet, 454 g/d (1 pound) of strawberries did not further improve serum lipids after 1-month in a hyperlipidemic population when compared to a control that followed the same diet but without additional strawberries (69). The cholesterol-lowering diet consisted of soy, viscous fiber, plant sterols, and nuts

and was followed for a period of 2.5 years prior to the strawberry intervention (69). It is important to note that the quantity of strawberry in this study is considerably higher than others mentioned equivalent to 1-pound/day of strawberries.

A two-arm parallel randomized controlled trial (RCT) supplemented 24 healthy inactive overweight females for two weeks (14 days) either with the capsules containing 25 g/d of FDSP or control capsules with similar appearance. On day 14, participants were assessed before and after a 30-minute endurance exercise session, which raised their heart rate to 75-80% of the maximum. Compared to baseline, the group supplemented with FDSP significantly reduced TC, LDL-C and TG, but increased HDL-C, while no significant change was observed in the control group (70).

A healthy population of 23 men and women supplemented daily with 500 g/d of fresh strawberries for one month showed significantly improved lipid profiles (TC, LDL-C, TG, but not HDL-C; $p<0.05$) compared to their baseline values (71). In contrast, a study in 31 healthy, non-obese adults found that the conventional lipid profile was unchanged with intake of 500 g/d of strawberry pulp following 30 days of intake (72).

The effectiveness of strawberry intake on the postprandial lipidemic response has been studied. Following an oral fat tolerance test, a single intake of 25 g of FDSP did not change TG levels in overweight adult men (n=10) over the course of four hours (73). A single intake of 40 g FDSP over the course of four hours as similarly unassociated with postprandial lipid responses following consumption of a high-fat meal in healthy

overweight and obese adults (74). A two-arm, crossover RCT investigating the impact of six-week strawberry supplementation (10 g/d FDSP; equivalent to 110 g fresh weight or 2/3 cup of sliced strawberries) on the postprandial response of 24 overweight men and women with hyperlipidemia and found supplementation of 10 g/d FDSP or six weeks showed reductions in lipid profiles following intake of a high-fat meal (75).

Taken together, the aforementioned studies suggest improvements to the lipid profile with dietary strawberry, primarily in those with elevations at baseline.

Hemostasis

Evidence pertaining to the impact of dietary strawberry on platelet function is inconclusive. A study by Djurica et al. examined the effects of FDSP on platelet reactivity in 25 adolescent overweight or obese males. After a week of supplementation at 50 g/d, adenosine diphosphate (ADP)-induced expression of P-selectin, an adhesion molecule involved in platelet aggregation, was significantly reduced from baseline values in both the FDSP and control groups (65). A study that utilized 10 g/d of FDSP for six weeks in overweight older men and women reported no significant differences in platelet aggregation between the strawberry and placebo powder beverages (76). A study in healthy men and women providing 500 g/d of fresh strawberries for one month reported a significant decrease in platelet activation ($p<0.05$) compared to baseline values (71). In overweight women with low physical activity (n=24), daily strawberry supplementation in the form of capsules containing a total of 25 g/d FDSP (consumed ~ 13g twice daily) significantly decreased circulating fibrinogen, a major coagulation factor (77).

In another two-arm parallel RCT, 24 young females (mean age 24 years) were randomly assigned to receive either 25 g/d of FDSP or a control capsule for 14 days. On the 14th day (study visit 2), the participants underwent a 30-minute endurance exercise session (a total of 50 minutes including warming up and cooling down) to reach 60-70% of their maximum heart rate. Prior to the exercise session, the levels of fibrinogen were measured and compared to the baseline levels. The results showed that there was a significant decrease in fibrinogen levels 14 days after consuming FDSP, while no significant change was observed in the control group (70).

In a two-arm crossover RCT, 20 healthy obese adults consumed either 80 g/d of FDSP or a calorie-, sugar- and flavor-control food for three weeks and assessed at two and three weeks after the start of the intervention. Fibrinogen level significantly increased in the group that consumed FDSP when compared to the control group; however, the value was within the normal range (78).

Vascular Function

Strawberries are a potentially rich source of dietary nitrate and nitrate that can be converted endogenously to vasoactive nitric oxide (NO). A study of 25 adolescent males with obesity who consumed 50 g/d FDSP for 1-week reported that fasting nitrate and nitrite levels did not significantly change within the group as a whole, but in those who demonstrated an increase, a concomitant significant increase ($p=0.014$) in the reactive

hyperemia index (RHI), whereas RHI was unaltered in those who showed no changes in their nitrate/nitrite levels (65). A second study with 60 postmenopausal women with pre- and stage-1 hypertension who consumed 25 g/d or 50 g/d of FDSP for 8-weeks showed significant reductions from baseline in arterial stiffness assessed by brachial- and femoral-ankle pulse wave velocity (PWV) ($P = 0.03$ and $P = 0.02$, respectively) in the 25 g/d group but not in the 50 g/d group (79). Curiously, an increase in nitric oxide metabolites was observed with the higher intake of strawberries, but not with the lower amount (79), suggesting that other metabolites produced from strawberry intake are involved in vascular function, possibly from microbial-derived phenolic metabolites (64). For example, when 34 adults with hypercholesterolemia drank beverages containing 50g/d FDSP for four weeks, vascular function did not differ at the end of the intervention period, but flow-mediated dilation (FMD) was significantly increased one hour after the initial strawberry intake compared to no changes in the control group ($p=0.0008$), and the short term response was significantly correlated with select circulating metabolites (64). In contrast, a study where a single intake of either 13g/d or 40g/d of FDSP (13 g FDSP=one serving; 40 g FDSP=3 servings) or the control together with a high-fat meal did not alter arterial stiffness as assessed by pulse wave analysis in adult men and women relative to consumption of a control meal (74). Similarly, FDSP did not alter endothelial function in overweight adults with elevated LDL-C following supplementation of either 13 g/d or 40 g/d for four weeks (62).

Strawberry intake may also influence vascular adhesion molecules. Two studies including 60 obese adults with metabolic syndrome found that dietary strawberry intake,

one study at 32 g/d for 4 weeks (80) and the other at 50 g/d for 8 weeks (60), both significantly decreased vascular cell adhesion molecule-1 (VCAM-1) ($p < 0.05$). In the former study, supplementation at a lower amount (13 g/d) did not yield significant changes from baseline (80).

Blood Pressure

Some studies report that strawberry consumption positively influences blood pressure, although the evidence is inconsistent. A large-scale prospective study of over 110,000 men and women from the NHS I and II and the Health Professionals Follow-Up Study explored the association between intake of flavonoids, including those found in strawberry, and incident hypertension (52). At a 14-year follow-up, individuals with the highest intakes of anthocyanins, which were predominantly from strawberries and blueberries, had an 8% reduced incidence of hypertension ($p<0.03$) when compared to those with the lowest reported intakes (52). High intakes of catechin, a flavan-3-ol present in strawberries, was also associated with a 6% reduction in hypertension ($p=0.002$) among people than 60 years old (52). In 60 postmenopausal women with pre- or stage 1-hypertension, daily strawberry consumption (25 g/d FDSP) resulted in decreased systolic, but not diastolic, blood pressure after eight weeks ($p=0.02$) (79). Interestingly, this change was not seen in participants who were consuming 50 g/d of FDSP (79). In contrast, diastolic blood pressure was significantly reduced both from baseline and control values following six weeks of intake of 50g/d FDSP in 36 females with T2DM (67). Systolic blood pressure was also significantly reduced from baseline, but this change was seen in both the strawberry and control groups (67). Another study

examined the effect of 50 g/d of FDSP in adults with obesity and hypercholesterolemia over four weeks (64). Although blood pressure did not change, systolic blood pressure was attenuated after acute (2 hour) intake ($p=0.02$) (64). The above studies are in contrast with two studies in both men and women with obesity and metabolic disturbances (abdominal adiposity and hyperlipidemia (59) or metabolic syndrome (60)) where blood pressure was not affected with provision of FDSP at 25 g/d for 12-weeks (59) or at 50 g/d for 8- (60) or 12-weeks (59).

Glucose Homeostasis/Glycemic Control

Strawberry is considered as a low glycemic fruit, even though it contains natural sugars (81). Following acute consumption, a beverage containing 500g of strawberry purée yielded a slightly lower peak glucose level when compared with a control beverage in 23 healthy women (82). When comparing strawberry jams with differing sugar content, blood glucose was maintained in the normal range after consumption of low-sugar jams, whereas jams with high sugar content resulted in significantly higher postprandial levels in both glucose and insulin (83). The addition of catechins, a form of polyphenol found in strawberries, to the low-sugar jam did not further influence postprandial glucose metabolism (83). Research suggests that dietary strawberry intake improves glycemic control, particularly by attenuating the postprandial response. A study of 21 adults with insulin resistance evaluating the response to ingestions of 0g, 10g, 20g or 40g of FDSP on postprandial glucose and insulin concentrations found that the 40g portion significantly reduced postprandial insulin concentration ($p<0.05$) when consumed with a high-carbohydrate, high-fat meal (84). Interestingly, pelargonidin-glucuronide, a

strawberry anthocyanin, was inversely associated with mean insulin concentrations in both the 20g and 40g FDSP amounts ($p<0.05$) (84). Another study examining the postprandial insulin response in overweight adults (n=24) reported that consuming a beverage containing 10g FDSP concurrently with a meal significantly reduced the postprandial insulin response ($p<0.05$) compared with a control beverage (85).

The timing of strawberry intake relative to a meal has been shown to influence the blood glucose response. Fourteen overweight adults but otherwise healthy were provided with a strawberry beverage containing 12g of FDSP to consume either two hours before, with, or two hours after a meal (86). two hours prior to or following a meal, compared to intake with a meal, Significant reductions in postprandial glucose levels were noted two hours prior to or following the meal, compared to intake with a meal, ($p=0.006$ and $p=0.03$, respectively), suggesting improved insulin sensitivity. Moreover, the strawberry-containing beverage did not result in the significant postprandial spike in glucose concentration that was seen with the control beverage ($p<0.05$), despite similar composition and sugar content (86). In contrast, an acute study supplementing with 40g of FDSP did not attenuate meal-induced metabolic alterations in adult men and women of varying body mass indices (74). Exaggerated postprandial glucose or insulin response was also not mitigated with six weeks of intake of 10g/d FDSP prior to a similar meal challenge in a group of healthy adults (76). In a short-term study, four weeks of intake of 32 g/d FDSP significantly reduced fasting insulin levels ($p=0.0002$) and homeostatic model of insulin resistance (HOMA-IR) values ($p=0.0003$), but not glucose, in adults with obesity and hyperlipidemia when compared to a lower amount (13g/d FDSP) and a

control (63). In a similar cohort (adults with hypercholesterolemia) and over the same four-week intervention period, 50 g/d of FDSP did not affect glucose or insulin levels when compared to a control (64). Another study also supplemented with 50 g/d of FDSP for six weeks and in participants with T2DM (n=23). Although supplementation resulted in decreased glycated hemoglobin (HbA1c) from baseline levels (-5.7%, $p < 0.05$), serum glucose was unchanged (87). Similar null results were reported in two different studies, both with 12-week supplementation periods in adults with obesity (n=60 and 17), one providing 25g/d and 50g/d FDSP (59) and the other providing solely 50g/d (66). Strawberry intervention did not impact measures of glycemia in either study (59, 66). These results agree with a pooled analysis of three large-scale prospective studies of approximately 187,000 participants investigating fruit consumption and incident T2DM (88). After adjustment for personal, lifestyle, and dietary risk factors, strawberry intake was not associated with reduced risk (pooled hazard ratio for every 3 servings/wk=1.03) (88).

In a three-arm, crossover RCT, a single serving of 60g homemade strawberry jam was served to participants in three different variations: sugar added (HS), non-sugar added (LS), and non-sugar added with polyphenol supplementation (LSA). The participants were provided each variation in a random order over the course of three consecutive weeks. The study monitored the participants' plasma glucose, insulin, and HOMA-IR levels at baseline and every 30 minutes until 120 minutes following a single intake. The results of the study revealed that the jam with added sugar significantly increased plasma glucose, insulin, and HOMA-IR levels compared to the natural sugar variations,

suggesting that individuals with blood sugar conditions may benefit from opting for natural sugar jam. The study highlights the potential impact of added sugar on blood sugar levels and supports the importance of making informed food choices to promote health and wellness (89).

Gut health

The impact of strawberry consumption on gut health has been studied in four clinical trials (90-93), each employing a different study design: two using an RCT crossover design (90, 93), one employing a single-arm model (91), and one with a cross-sectional study design (92). The main parameters used to gauge gut health were the gut microbiome profiles (abundance and presence) and their metabolites, such as phenolic compound conjugates (e.g., glucuronides), bile acids, SCFA, and urolithin A (90-93).

In a two-arm crossover randomized controlled trial, consumption of 50g/d of FDSP (25 g twice a day, at least six hours apart) for four weeks on gut microbiome metabolites were investigated in 34 healthy adult males and females. The results showed decreases in the plasma concentration of individual secondary bile acids, including deoxycholic acid, lithocholic acid (LCA), and their glycine conjugates, and glycoursodeoxycholic acid. Moreover, within the bile acid profiles, reductions in total glucuronide-, total oxidized-, total dehydroxyl-, total secondary, and total plasma bile acids were reported (90). A two-arm, crossover, randomized controlled trial, comparing the effects of consuming 200 g of fresh or pureed strawberries on serum metabolites and urine excretion of urolithin A and its glucuronide conjugates over a period of 92 hours postprandial in 20 healthy adults

showed no significant differences between fresh and processed strawberries, suggesting that the processing of strawberries into puree did not impact gut microbiome metabolism (93).

A single-arm study explored the effects of consuming 26 g/d (13 g twice a day, at least six hours apart) of FDSP for four weeks in 14 healthy adults. Compared to baseline values, the intake of FDSP significantly increased the abundance of specific microbial families in four operational taxonomic units (OTUs), including Christensenellaceae (Firmicutes phylum), Verrucomicrobia (Verrucomicrobia phylum), Bifidobacteriaceae (Actinobacteria phylum), and Bacteroidaceae (Bacteroidetes phylum), while simultaneously decreasing the abundance of Alcaligenaceae/Sutterella (Proteobacteria phylum). However, after a two-week washout period following the intervention, the microbial abundance trend was reversed for Christensenellaceae family. Furthermore, the plasma LCA levels were higher at the end of the study compared to baseline levels (91).

According to a cross-sectional study conducted in 124 adults aged 19-95 years, a positive relationship between strawberry consumption and the production of short-chain fatty acids was reported. The study found that an increase in strawberry intake was associated with an increase in propionate production. Moreover, the study found that anthocyanins, which are the primary phenolic compounds found in strawberries, were also positively correlated with the production of propionate, butyrate, and total SCFA (92).

Immune health

A two-arm crossover RCT assessed the effects of a three-week intake of FDSP, equivalent to four daily servings of fresh strawberries, on immune function in healthy obese males and females, compared to a flavor-matched control. The peripheral blood mononuclear cell was extracted from the blood samples and Lipopolysaccharide (LPS) from gram-negative bacteria, *Escherichia Coli* O111:B4, was used to simulate viral infection in order to assess immune function biomarkers, including CD4+ and CD8+ T cells, tumor necrosis factor alpha (TNF-α), and transcription factors related to mechanisms of oxidative stress and/or inflammation. The results of the study showed that FDSP consumption significantly decreased CD4+T cells (%), while increased CD8+ T cells (%) and TNF-α (94). These responses are all favorable, since CD4+ T cells or T helper cells support the immune response by stimulating other immune cells (e.g., macrophages and B-cells), but do not interact directly with pathogens (95). Further, CD8+ T cells or cytotoxic cells directly kill pathogens and release the pro-inflammatory cytokines TNF-α to help clear infection through necrosis or apoptosis (96-98).

Bone health

A three-arm, parallel RCT investigated the effects of consuming FDSP for eight weeks on bone health biomarkers in 60 postmenopausal women with prehypertensive conditions or stage I hypertension. Participants were assigned to one of three groups: a control group that received no FDSP, a group that received 25 g/d of FDSP, and a group that received 50 g/d of FDSP for eight weeks, with measurements collected at four and eight weeks. Consuming 25g/d FDSP, equivalent to 1.5 cups of fresh strawberries, significantly

increased insulin-like growth factor (IGF)-1 levels compared to the control group (99). The IGF-1 typically works together with growth hormone to stimulate bone resorption and formation, which support bone health (100, 101). Other indices followed in the study, including bone mineral content and density, alkaline phosphatase, osteocalcin, tartrate-resistant acid phosphatase 5b and adiponectin and leptin did not change (99).

Cognitive function

Data on the influence of strawberry consumption on cognitive function and brain health have been investigated in four prospective cohort studies (37, 41, 44, 46) and one randomized controlled trial (102). Cognitive health in these studies was assessed with biological markers and pathological burdens related to Alzheimer's disease along with general cognitive function assessments. For the Alzheimer's assessments, amyloid-β, a peptide produced during the proteolytic processing of a transmembrane protein in the brain (103, 104) is often assessed. Higher accumulation is linked to an increased risk of Alzheimer's disease pathology (103, 104). Tau is a protein in the brain that stabilizes the microtubules of neurons (105). Excessive phosphorylation of tau and its accumulation results in the neurofibrillary tangles, which impair synaptic communication and is correlated with Alzheimer's disease (106, 107).

A 24-year prospective cohort study assessed the correlation between the consumption of pelargonidin-rich foods, mainly strawberries, and the amyloid-β load and phosphorylated tau tangles in 575 people free of Alzheimer's disease at entry. The mean strawberry intake was 0.6 servings/wk and the consumption was categorized into four quartiles,

including 0.1, 0.5, 0.7, and 1.2 servings/wk. The results showed that increased pelargonidin intake was associated with reduced global Alzheimer's dementia (AD) pathology burden, amyloid-β load, and phosphorylated tau tangles among participants with no genetic risk factor for Alzheimer's disease (not APOE ε4 carriers). Additionally, strawberry intake among APOE ε4 non-carriers was also inversely associated with phosphorylated tau tangles (41). Another prospective study conducted by the same research group over a seven-year period explored the associations between strawberry consumption and AD risk in 975 dementia-free older adults. The study categorized strawberry consumption into three groups, based on frequency of intake: rarely or a few times per month, one to three times per month, or more than once per week (46). On average, participants consumed 0.64 servings of strawberries per week, with a range of 0-2 servings per week. Higher strawberry intake was associated with a reduced AD risk. Additionally, compounds found in strawberries, including vitamin C, pelargonidin, and flavonoids showed a significant trend toward reduced AD risk with increased consumption (46).

The potential link between strawberries and their main phenolic compounds, namely flavones, flavanones, and anthocyanins, and the occurrence of subjective cognitive decline (SCD) was investigated among people enrolled in the Nurse Health Study and Health Professional study. The higher consumption in each of flavone, flavanone, anthocyanin, or strawberry was significantly associated with the lower risk of SCD (37).

In a prospective cohort study, the relationship between strawberry consumption and cognitive function was evaluated through questionnaires probing categories such as verbal memory and a global composite score. Individuals who consumed at least one serving of strawberries per week demonstrated superior cognitive function, as indicated by higher scores in global and verbal memory categories, compared to those who consumed less than one serving per week (44). In a two-arm, parallel RCT, the effects of 90 days of consuming 24 g/ of FDSP (equivalent to two cups of fresh strawberries) among 37 older adults aged 60-75 years old were assessed with the California Verbal Learning Test (second edition). The study reported that the FDSP supplementation significantly improved word recognition, spatial learning, and memory compared to baseline scores, while no significant changes were observed in the control group (102).

Cancer

A four-arm, parallel, RCT involving 40 healthy young adults assessed the effects of consuming 300 g of fresh strawberries on excretion of N-nitrosodimethylamine (NDMA), a carcinogen found in some foods and drugs (108, 109). The group that consumed whole strawberries had significantly lower levels of NDMA excretion compared to the control group, who consumed foods low in NDMA, nitrate, amine, sulfur compound, ascorbic acid, and phenolic compounds (110).

A two-arm parallel RCT examined the effects of a six-month consumption of FDSP (30 or 60 g/d, twice a day; equivalent to 300 and 600 g of fresh strawberries, respectively) on outcome measures of cancerous cell growth and cancer-related biological outcomes in 75

middle-aged men and women with mild to moderate esophageal dysplastic lesions (at-risk for esophageal cancer) (111). When compared to baseline levels, the group that consumed 30 g/d of FDSP did not show a significant change in any outcomes, whereas participants who were provided with 60 g/d FDSP showed a significant reduction in the histologic grade of precancerous growth, inhibited protein expression of cancer-induced markers, including inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, nuclear factor κB (NFkB)-p65, and phospho-S6 (pS6), and delayed cell proliferation (measured by Antigen Kiel (Ki)-67) (111).

Oxidative defense

Overall, 14 clinical studies assessed the effects of strawberry intake on oxidative defense. Four were single-arm studies that compared changes in oxidative stress and related outcomes following strawberry intake to baseline values. All were short-term studies where participants consumed fresh (500 g/d) or frozen (250 g/d) or freeze-dried strawberries (50/g) for two to four weeks. Eighteen adult participants (mean age 35 years) given 500 g/d of fresh strawberries for two weeks had delayed plasma hydrogen peroxide-induced lipid oxidation, as well as decreased deoxyribonucleic acid (DNA) strand breakage, increased cell metabolic activity when exposed to hydrogen peroxide and reduced mortality of mononuclear cells (112). A second study provided 23 healthy adults (mean age 27 years) with 500 mg/d of fresh strawberries for 30 days. When compared to baseline levels, strawberry intake significantly reduced markers of oxidative stress including malondialdehyde (MDA), 8-hydroxy-2' -deoxyguanosine (8-OHdG), and Isoprostanes (113). A third single-arm study used a four-week intervention that provided

16 participants with 50g/d FDSP, which was equivalent to 500 g/d or two cups of fresh strawberries. Compared to baseline levels, the products from lipid peroxidation, MDA and 4-hydroxynonenal, were significantly decreased (114). A fourth study supplemented 21 participants with 250g/d of frozen strawberries for three weeks and noted delayed lipid oxidation compared to baseline levels (115).

A three-arm crossover RCT supplemented 33 obese, insulin resistant adults with either 13 g/d or 32 g/d of FDSP and reported decreased serum MDA, and increased serum superoxide dismutase (SOD) and total antioxidant capacity compared to fiber- or calorie-matched control groups (116). Another study by the same research group used a two-arm, crossover RCT design to assess the effect of 12 weeks of consumption of 50g/d of FDSP among 17 obese participants with knee osteoarthritis and reported significantly reduced lipid peroxidation, compared to calorie- and fiber-matched control groups (117). A three-arm, crossover RCT provided a single intake provided three forms of 60 g each of strawberry jam either with sugar added, no sugar added, or no sugar added with supplemental polyphenols. Sixteen healthy young adults (mean age 26 years) were monitored at baseline and every 30 minutes for 120 minutes and found no differences in MDA, glutathione peroxidase, or TAC between the three jams (89).

A two-arm, crossover RCT in 28 adults with hyperlipidemia (mean age 62 years) provided 454 g/d of fresh strawberries or 65 g/d of oat bran bread for four weeks, with a two-week washout period before crossing over to the other group. When the two intervention arms were compared to each other, the strawberry group showed

significantly decreased MDA in their LDL fraction compared to the oat bran bread group (118). A two-arm, crossover RCT followed responses of 23 healthy young adults (mean age 22.5 years) after a single intake of 500 g of a strawberry puree beverage at baseline, 30 minutes, and one-, two-, and four-hour time points. Compared to the calorie- and flavor-matched control beverage, the strawberry puree beverage significantly increased serum vitamin C and serum folate at all time points and delayed LDL oxidation at the one-hour measure (82).

Five parallel RCT studies assessed the effects of strawberry intake ranging from 30 to 90 days on oxidative defense when feeding either strawberry pulp (500 g/d) or FDSP (24, 25, or 50 g/d). A three-arm parallel RCT provided older adults with 24 g/d of FDSP, freeze-dried blueberry (FDBB) powder or isocaloric control for 90 days. Serum samples were collected at baseline and at 45 and 90 days and cultured in transgenic (highly aggressively proliferating immortalized (HAPI)) rat microglial cells. Compared to the controls, samples from those who consumed FDSP or FDBB for 90 days showed significantly increased NO and expression of iNOS and decreased COX-2 release following induction by LPS (119). A 12-week, four-arm parallel RCT compared the effects of FDSP (25 or 50 g/d) or isocaloric control powders (25 or 50 g/d) on outcomes related to oxidative defense. Compared to control levels, both amounts of FDSP significantly increased plasma antioxidant capacity and whole blood glutathione, while only the 25g/d FDSP group showed significantly increased catalase (120).

A two-arm parallel RCT assessed the effects of the consumption of 500g/d of frozen strawberry pulp over two 30-day interventions with a 10-day washout period. Compared to the control period devoid of strawberry intake, antioxidant capacity as measured by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) test was increased following the fruit intake (121). Another 12-week, four-arm parallel RCT provided 60 adults (mean age of 49 years) with either 25 or 50 g/d of FDSP or isocaloric control foods and reported significantly reduced levels of MDA and delayed lipid oxidation in the strawberry groups compared to the controls (122). A two-arm parallel RCT in adults with T2DM (mean age 52 years) provided either 50 g/d of FDSP (n=19) or 40 g/d of an isocalorically-matched control food (n=17) for six weeks and noted significantly increased plasma TAC along with decreased serum MDA in the strawberry group compared to the controls (123).

Inflammation

The effects of strawberry consumption on inflammatory responses or related outcomes have been investigated in eleven clinical studies. A cross-sectional study of 2,375 members from the Framingham Heart Study offspring cohort, who completed a FFQ during the 7th study examination in 1998-2001 divided participants into quartiles of intake and reported that higher strawberry intake was associated with a decrease in both the inflammation score and acute inflammation subscore, as well as an oxidative stress subscore. Additionally, the intake of both total flavonoids and anthocyanins (predominantly strawberry) were negatively associated with the inflammation score, acute inflammation subscore, and a collection of plasma and serum proinflammatory cytokines (e.g., TNF-α and interleukin (IL)-6) (43). A prospective cohort study of 38,176

participants in the Women's Health Study (mean age 54 years were followed for an average of 10.1 years reported a significant trend between higher strawberry consumption and lower levels of CRP (13). A similar outcome was reported from a two-arm parallel RCT among adults with T2DM (mean age 52 years) who received either 50 g/d of FDSP (n=19) or 40 g/d of an isocalorically-matched control (n=17) for six weeks. Those who received the FDSP had a significant decrease in serum hs-CRP levels when compared to the control group (123).

A three-arm parallel randomized controlled trial among older adults provided either 24g/day of FDSP, FDBB or an isocaloric control food for a period of 90 days. Serum samples were collected at the beginning, 45 days and 90 days after the intervention and later cultured with transgenic (HAPI) rat microglial cells. The findings of the study indicated that the serum samples from participants who consumed FDSP for 90 days had a significant decrease in TNF-α, an inflammatory cytokine (119).

Adults with moderate hypercholesterolemia (mean age 53 years) who consumed either 50g/d of FDSP or an isocaloric control for four weeks were studied in a two-arm crossover study. No significant differences in a battery of inflammatory biomarkers were noted between the FDSP and control groups (124). In contrast, a 12-week RCT of obese participants with knee osteoarthritis fed 50g/d of FDSP or an isocaloric control reported significantly reduced inflammatory biomarkers, including high sensitivity (hs)-TNF-α and soluble tumor necrosis factor receptor (sTNF-R)-268, in the strawberry group compared to the controls (117).

In a two-arm crossover RCT, 24 adult males and females with a mean age of 51 years were given either 10 g/d FDSP or a calorie-, nutrient-, and flavor-matched control for six weeks. At baseline and the six-week time point, high carbohydrate and high fat meal challenges were conducted and monitored for six hours postprandially. The results indicated that after six weeks of FDSP consumption, there was a significant decrease in plasminogen activator inhibitor (PAI)-1, while there was a trend ($p=0.05$) for decreases in IL-1β and IL-6 (125).

Seventeen obese adults with osteoarthritis participated in a two-arm crossover RCT and received 50 g/d of FDSP or a calorie-, nutrient-, and flavor-matched control in different orders for 12 weeks, with the assessment of inflammatory markers obtained at the start and completion of the intervention. Compared to the control period, FDSP intake significantly reduced IL-6, IL-1B, matrix metalloproteinase (MMP)-3, health assessment questionnaire-disability index (HAQ-DI), Intermittent and Constant Osteoarthritis Pain (ICOAP) score for constant pain, intermittent pain, and total pain (126).

A two-arm crossover RCT provided 24 overweight or obese adults with a single intake of 10 g of FDSP or a calorie-, nutrient-, and flavor- matched control and noted significantly decreased inflammatory markers, including hs-CRP and IL-6 after strawberry intake compared to the control over the course of six hours (127).

Strawberry Bioactive Pharmacokinetics

Anthocyanin glycosides are unique in that they persist in this form in the circulation shortly following consumption, suggesting that they can be absorbed directly from the stomach through a transport mechanism. The main site of anthocyanin and flavonol glycoside absorption is the small intestine following deglycosylation by epithelial □-glucosidase or lactase-phloridzin hydrolase to more lipophilic aglycones (20, 21).

Aglycones undergo phase II metabolism to form the conjugated aglycones and aglycone glycosides that are predominantly present in the circulation (20, 128). Aglycones also undergo degradation to phenolic acids and aldehydes by the colonic microbiota (20, 128). Evidence suggests that anthocyanins may have antioxidative, antiatherogenic, anticancer, antidiabetic, anti-obesity, anti-inflammatory, and neuroprotective activity (20, 22, 129).

Pharmacokinetic studies of polyphenols explore the mechanism through the absorption, digestion, metabolism and excretion following the polyphenol-rich food or extract consumption (130). Pelargonidin is the dominant anthocyanin in strawberries, which is commonly assessed in pharmacokinetic studies in its ingested form found in food (e.g., P3G) and metabolites (131, 132). The metabolites are derived from Phase I and II enzymes, resulting in conjugated products from gut microbiome metabolism, including glucuronidated and sulfated forms of pelargonidin (133). In a three-arm, crossover, randomized, controlled trial, 14 healthy young adults were given a single amount of 12 g of FDSP (containing 35 mg anthocyanins, equivalent to 132 g fresh strawberries) in three different formats: two hours before a meal, together with a meal, or two hours after a

meal. The maximum concentration (Cmax), time to Cmax (Tmax), and area under the curve (AUC) of the strawberry metabolite (plasma pelargonidin glucuronide) was significantly increased compared to the other two formats (134). A two-arm, parallel RCT assessing the pharmacokinetics of strawberry metabolites following the consumption of 24 g/d of FDSP (twice a day, at least six hours apart; equivalent to two cups of fresh strawberries) in 38 older adults showed that 90 days of FDSP supplementation increased pelargonidin metabolites, including glucuronide and rutinoside conjugates, compared to baseline levels, while a change urolithins was not observed. When compared to the calorie- and flavor-matched control, hydroxybenzaldehyde, trans-cinnamic acid, hippuric acid, and p-coumaric acid were also significantly higher (135).

Discussion

Key Compounds and Physiological Effects of Strawberry

Vitamins, Minerals, and Dietary Fiber

The health benefits of strawberry can be attributed to an array of protective nutrients and phytonutrients including folate, vitamin C, potassium, and (26, 136, 137). Vitamin C and folate were assessed in three studies that were reviewed (46, 82, 113). Vitamin C is an antioxidant that helps stabilize free radicals, and a higher concentration of this nutrient in the plasma predicts a higher antioxidant capacity in the body. Vitamin C is a potent reducing agent that protects cells against oxidative damage by scavenging free radicals and preventing formation of damaging reactive oxygen species (ROS) (138, 139). Moreover, vitamin C stabilizes free radicals by donating an electron and preventing

further damage to cells (140, 141). ROS-induced oxidative stress and inflammation play a role in the progression of several chronic diseases, including cardiovascular disease (142). Vitamin C is also required for the synthesis of collagen, a major component of connective tissue (139). Two clinical studies reported a significant increase in plasma or serum vitamin C after consuming strawberries (82, 113). A two-arm, crossover RCT assessing the effects of a single intake of 500g strawberry puree beverage on serum vitamin C levels over a four-hour time course. The study was conducted on 23 healthy young adults, with a mean age of 23 years. The results indicated a statistically significant increase in serum vitamin C levels when compared to the isocaloric control group (82).

A prospective study conducted over a period of seven years explored the relationship between strawberry consumption and the risk of AD among 975 dementia-free older adults. The average weekly consumption of strawberries was found to be 0.64 servings. The results showed that higher consumption of vitamin C was associated with a lower AD risk, indicating a possible protective effect of strawberries against AD. The findings of this study highlight the potential benefits of including strawberries in the diet of older adults to mitigate the risk of developing AD (46). In the other two clinical studies, vitamin C was assessed as the measure of *in* vitro oxidant defense, suggesting the potential ability to decrease oxidative stress. A single- arm study involving 23 healthy adults (mean age 27 years) provided 500g/d of fresh strawberries for 30 days. The results of this study showed a significant increase in the vitamin C levels of the participants when compared to their baseline (113).

Strawberries are an excellent source of folate, a crucial micronutrient involved in one-carbon metabolism, which is fundamental for DNA synthesis and the progression of chronic diseases (82, 143). Folate is also involved in the conversion to and maintenance of normal concentrations of homocysteine (144). High levels of homocysteine, or hyperhomocysteinemia, has been associated with increased risk of cardiovascular disease (145), some cancers (146), neurological conditions (147), and poor pregnancy outcomes (148). To date, only a single study assessed serum folate levels and found a significant increase in serum folate following the consumption of 500 grams of strawberry puree compared to the control group (82). While this study suggests a positive correlation between strawberry consumption and serum folate, it is important to note that folate deficiency is interrelated with other compounds, such as vitamin B6 and B12 (149). Future studies should, therefore, consider assessing these vitamins together, particularly in studies related to metabolic syndrome (149) and cognitive function (150). Overall, the assessment of macro- and micro-nutrient status in strawberry interventions or observational studies that focus on the effects of strawberries on health outcomes appears to be limited.

Dietary fiber has cholesterol-binding capacity, which enhances its excretion, thereby reducing circulating cholesterol levels (151, 152). Dietary fiber also has the capacity to hold water, which increases viscosity within the gastrointestinal tract, slowing down gastric motility and nutrient absorption, leading to increased feelings of satiety, reduced food intake and improved glucose homeostasis (152). Although fiber does not directly interact with ROS, it significantly alters the gut microbiome, resulting in changes in

metabolite profiles and modulation of oxidative stress and inflammatory responses (153, 154). Clinical studies have shown that a diet rich in fiber can suppress inflammation and oxidative stress (155, 156). Similar to vitamin C, phenolic compounds can also directly interact with ROS (157). However, the bioactivities of individual phenolic compounds can vary depending on their absorption, digestion, metabolism, and excretion (158). Large compounds such as anthocyanins and ellagitannins need to be metabolized by the gut microbiome before being absorbed in the colon (31, 159, 160). On the other hand, phenolic acids, which are smaller in size, can be easily absorbed in the gastrointestinal tract after consumption (161). These compounds and their potential physiological, protective mechanisms will be discussed in greater detail later in this review. Current interest in dietary fiber and health includes products produced by the gut microbiota after fiber fermentation including SCFA, which stimulate glucagon-like peptide-1 (GLP-1) and YY peptide (PYY), hormones involved in regulation of insulin sensitivity and satiety, respectively (152, 162). Finally, dietary fiber may have an anti-cancer effect related to increased excretion and thereby reduced concentration of circulating hormones, including steroid hormones and IGF, which are associated with cancer progression (163, 164). Anti-proliferative properties may also be mediated by SCFA (165, 166).

Strawberries are a source of dietary nitrate (167), which is associated with protective cardiovascular effects, including lowered blood pressure, improved vascular function, and reduced platelet aggregation (65, 168-177). Animal models suggest that dietary nitrate may also be associated with improved insulin sensitivity and glucose tolerance (170), but trials in humans are needed. These physiological effects are largely elicited via

the *in vivo* conversion of dietary nitrate to NO, which has bioactivity within both the cardiovascular and metabolic systems (170). Dietary nitrate is first reduced to nitrite by the oral microbiota and is further reduced to NO in the gut via pathways involving hemoglobin, myoglobin, xanthine oxidoreductase, vitamin C, and polyphenolic compounds (177). As a source of both dietary polyphenols and vitamin C in addition to nitrate, strawberries allow for enhanced generation of biologically active NO (170, 178). Nitric oxide is a potent vasodilator and is important for the regulation of vascular homeostasis, reducing blood pressure and arterial stiffness (171, 179). Further, NO exerts antithrombotic properties through the modulation of platelet reactivity, which is protective against endothelial dysfunction and atherogenesis (171, 179). The nitrate-nitrite-NO pathway is active during ischemia and hypoxia, or restricted blood flow and oxygen supply (179, 180). This is in contrast to endogenous NO formation via the conventional L-arginine-nitric oxide synthase (NOS) pathway, making dietary nitrate an alternative, effective donor of NO in times of ischemia and hypoxia, such as illness or injury (171, 180, 181). This pathway may also contribute to improved metabolic and exercise capacity (171).

Phytonutrients

Strawberries are rich in bioactive phenolics, including anthocyanins, flavan-3-ols, flavonols, EA, and ET (26). Anthocyanins are flavonoids that provide strawberries with their red pigmentation (22). Bioavailability of these compounds was previously thought to be quite low, however, recent evidence suggests bioavailability to be higher when considering anthocyanin-derived bioactive metabolites (182). Dietary anthocyanins are

present as glycosides and persist in this form in the circulation shortly following consumption, suggesting that they can be absorbed directly from the stomach. However, the main site of anthocyanin absorption is the small intestine following deglycosylation to aglycones (20, 21). Aglycones undergo phase II metabolism to form the conjugated aglycones and aglycone glycosides that are predominantly present in the circulation (20, 128). Aglycones also undergo degradation to phenolic acids and aldehydes by the colonic microbiota (20, 128). Evidence suggests that anthocyanins may have antioxidative, antiatherogenic, anticancer, antidiabetic, anti-obesity, anti-inflammatory, and neuroprotective activity (20, 22, 129). Anthocyanins present in strawberries include P3G and C3G **(Table 1)**. Data on these metabolites is limited, but evidence to date suggests that they modulate oxidative stress and inflammation by upregulating the antioxidative nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, and by downregulating the pro-inflammatory mitogen-activated protein kinase (MAPK) and NF- κB pathways (183, 184).

Flavan-3-ols are the most commonly consumed flavonoid subgroup (185). Flavan-3-ols found in strawberries include catechins and proanthocyanidins **(Table 1)**. Recently, flavan-3-ols have been proposed for an established dietary guideline (186). The U.S. Academy of Nutrition and Dietetics recommends daily flavan-3-ol intake of 400-600 mg/d to promote cardiometabolic health (7). Once ingested, flavan-3-ols undergo extensive metabolism, first conjugated in the liver, followed by microbial catabolism in the gut to a number of metabolites, which are biologically active (187, 188). Flavan-3-ol intake is associated with significant improvements in metabolic health, including glucose

metabolism and insulin sensitivity (189), thought the most recognized effect is on the vasculature (186). Flavan-3-ol intake is associated with significant improvements in FMD and lipid profiles and is inversely related to systolic and diastolic blood pressure and arterial stiffness (189-191). Supplementation with 500 mg of flavan-3-ols/day was associated with a significant 27% reduction in death from cardiovascular disease at a 3.6 year follow-up in the COcoa Supplement and Multivitamin Outcomes Study (COSMOS), a randomized clinical trial including over 20,000 participants (192). In a meta-analysis of 39 prospective cohort studies, higher intakes of catechins and proanthocyanidins were associated with a 25% and 17% lower risk of cardiovascular disease, respectively, when compared to those with lower intakes (193). The mechanisms of action by which flavan-3-ols influence vascular function include increased bioavailability of nitric oxide (186, 194) as well as modulation of inflammatory cascades (195, 196).

Flavonols are a class of flavonoids that confer white to pale-yellow pigment colors in plants (197). Similar to other flavonoids, flavonols undergo phase I and II metabolism following absorption and are mainly present in the circulation in their conjugated forms (198-200). Flavonols present in strawberries include quercetin and kaempferol **(Table 1)**, which are among the most widely distributed flavonoids in the diet (201) and exert a broad range of biological functions. Flavonol intake, particularly quercetin, is associated with favorable cardiometabolic outcomes, having antioxidative, anti-inflammatory antihypertensive, and anti-thrombotic activity as well as promoting improved lipid and glucose regulation (198, 199, 202-205). A recent meta-analysis including data from 18 randomized controlled trials reported that supplementation with flavonols significantly

increased HDL-C and reduced TC, LDL-C, triacylglycerol, fasting plasma glucose and systolic and diastolic blood pressure (206). Potential vasoprotective mechanisms include enhanced bioavailability of NO and inhibition of COX-1, resulting in improved vascular function and reduced blood pressure and reduced platelet reactivity and inflammation, respectively. (199, 206, 207). Further, flavonols suppress angiotensin-converting enzyme (ACE) activity, which is directly involved in the regulation of blood pressure (208). Flavonols may also downregulate 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A reductase, an intermediate in cholesterol synthesis, and upregulate proteins involved in reverse cholesterol transport, such as adenosine triphosphate (ATP)-binding cassettes, yielding a hypolipidemic effect (199, 209). Finally, flavonols elicit strong free-radical scavenging capacity via their modulation of the Nrf2 pathway (210, 211).

Ellagic acid and ET are phenolic acids and hydrolyzable tannins, respectively, that have gained considerable interest in recent years due to their potential association with health promotion and disease risk reduction (212). Ellagitannins are hydrolyzed in the gastrointestinal tract to EA and further metabolized by gut microbes into urolithins (212, 213). To date, 13 unique urolithins have been identified (212). Urolithin production occurs predominantly in the distal colon due to involvement of the gut microbiome (214). The ability, or lack thereof, of an individual's gut microbiota to produce certain urolithins is known as their urolithin metabotypes (UM), or metabolic phenotype. Three categories have been defined, including UM-A, which is solely urolithin-A-producing, UM-B that produces urolithin B and isourolithin A-producing in addition to urolithin A, and UM-O which does not produce urolithin (215). Current data suggests that the microbial

composition associated with UM-A may be protective of cardiometabolic health, while that associated with UM-B appears be related to gut dysbiosis and an increased risk for disease (215-218). The microbial composition associated with UM-0 has shown reduced bacterial abundance and diversity when compared with urolithin-producing metabotypes (218). Urolithins, particularly urolithin A, may confer health benefits (219-221). *In vitro* and animal studies suggest potential anti-inflammatory, -oxidative, -atherogenic, -cancer, -diabetic, -obesogenic and -dyslipidemic properties (212, 222-224). Through inhibition of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway, urolithins downregulate MAPK and NF-KB, resulting in decreased inflammation, cell adhesion, angiogenesis, and migration (224-228). Urolithins also activate the adenosine monophosphate-activated protein kinase (AMPK) pathway, which yields positive effects on lipid storage/obesity and dyslipidemia (220, 224, 229-232). Finally, urolithins defend against oxidative damage by activating the Nrf2 pathway and stimulating antioxidant response elements (AREs) (224, 233-235). Recent clinical trials in humans, although limited, report anti-aging effects through improved mitochondrial function and mitophagy (236-238).

Interindividual Variability: Understanding Towards Precision Nutrition

Precision Nutrition

Variations in metabolic responses to strawberry intake will be useful in designing dietary interventions using a precision nutrition model (239), which considers a person's unique characteristics (e.g., gut microbiome) or metabolic response to determine the most effective dietary strategies to promote health (240). This approach presents a unique

opportunity to characterize an individual by their metabolome and to inform dietary recommendations by response phenotype and can be utilized to inform personalized nutrition recommendations for optimum health.

Influence of the Gut Microbiome

Variance observed between individuals exists and is an important consideration when studying the biological effects of strawberry consumption. Strawberries contain bioactive compounds that, being largely metabolized by the gut microbiota, have the potential for considerable variability in physiological response between persons (241). Variation in responses is due, in part, to the capacity of an individual to metabolize components which in turn impacts their effect on cellular processes and health outcomes. This was initially recognized in the case of isoflavone biotransformation into equol (242) and more recently in the metabolism of ellagitannins into urolithins (212). Ellagitannins are phenolic compounds present in strawberries, as well as in other berries and in walnuts, that are metabolized by gut bacteria into a variety of urolithins (212). The production of urolithins relies on the microbes of the gut, so individual variance is due to not only to host metabolic capacity, but also to the metabolic capacity of the gut microbiome. Lower concentrations of circulating urolithins from red raspberry intake have been reported in individuals with prediabetes and insulin resistance compared to those who were metabolically healthy (243). A number of metabolites were also changed dependent on metabolic status, suggesting altered capacity to produce and potentially utilize phytochemical components or their metabolites productively (243). The relationship between these compounds and the gut is bilateral; not only does the metabolism of select

phytonutrients present in strawberries depend on the bacterial composition of the gut, but gut composition is also influenced by habitual consumption of these compounds (244, 245). For example, one study (244) found that supplementation with 1.8 g/day of ellagitannin-rich pomegranate extract for 3-weeks was able to alter the microbiota such that some participants that were originally Urolithin Metabotype-0 or non-producers became producers. Another study in a murine model reported increased urolithin-producing bacteria (Gordonibacter urolithinfaciens) in the gut following 2-weeks of daily supplementation, but not with a single intake (245), suggesting that a daily prebiotic may increase urolithin production and be cardioprotective.

Metabolic Response

Metabolic differences, presenting as "responders" and "non-responders" have been identified in strawberries. A study examining the effect of FDSP on circulating metabolites and microvascular function found individuals with either positive or negative changes in plasma nitrate and nitrite levels (65). Further, an increase in microvascular function was observed in those producing an increase is plasma nitrate/nitrite but not in non-producers (65). Differential responses have also been identified in lipid metabolism (246). One clinical study giving omega-3 fatty acids highlighted key differences in the metabolomic and transcriptomic profile of those who responded, defined by lowering of plasma triglycerides, and those who did not respond (246). Similarly, another study identified a responsive platelet aggregation phenotype as the responders and non-responders demonstrated differential effects on oxylipin (bioactive lipid-mediators

derived from polyunsaturated fatty acids) formation following consumption of extra virgin olive oil (247).

The Impact of Pesticides: Sensory Attributes and Nutrient Composition

Fruits and leaves of strawberries are reported by some to contain pesticide residues (248). Although the pesticide residues in U.S. strawberries are well below the lower tolerable limit of the reference concentration of pesticides (249), high levels have been found in strawberries sold in other countries such as China (250). Cross-sectional studies by the same research group determined the association between fruits and vegetables with low and high pesticide residue status estimated by the U.S. Department of Agriculture Pesticide Data Program and mortality and morbidities, using the baseline data of the NHS I and II and the HPFS. Fruits and vegetables such as strawberry, celery, and spinach were categorized with relatively" high" pesticide residues, while blueberry, tomatoes, and dried plums were considered "low". The two studies reported that the consumption of "low" pesticide residue fruits and vegetables of at least four servings per day was associated with lower risks of coronary heart disease and all-cause mortality. In contrast, the relationships between intakes of "high" pesticide residue fruits and vegetables and health outcomes were not observed. The authors suggested that the high pesticide residues may alter the benefits of fruit and vegetable consumption, including the protection against cardiovascular disease and mortality (251, 252).

A study compared the effects of fungicides (boscalid and difenoconazole) on nutrients and polyphenolic profiles of strawberries and showed that the concentration of most components, including total polyphenol, flavonoids, vitamin C, sucrose, fructose, and glucose, were significantly lower in strawberries treated with fungicides compared to those devoid of pesticides. The author further analyzed the potential genes that were altered by the fungicide boscalid using transcriptome and metabolome analyses and found that suppression of genes involved in sugar-acid metabolism, volatile compounds and amino acid synthesis pathways and metabolites (253). These alterations may reduce the desirable sensory characteristics of strawberries such as sweet taste and fruity and floral flavors, while increasing unwanted traits such as sour taste (254).

The challenge between demands of higher yield crops for farmers and better food quality for consumers is central to the business model of agriculture. A study assessing reduction in fungicide usage compared the sensory characteristics and polyphenolic profiles of two strawberry (*Fragaria x ananassa*) genotypes planted in fungicide, reduced-fungicide or no-fungicide conditions reported that the sensory qualities and polyphenolic compound concentrations of strawberries grown under reduced-fungicide applications were better than those grown under conventional method but not as good as in the no-fungicide group (255). This finding is consistent with another study that compared strawberries grown by conventional methods with pesticides to organically-grown strawberries and noted that organic strawberries contained higher levels of vitamin C, beta-carotene, and flavonoids such as anthocyanins, especially P3G. However, conventional strawberries contained higher concentrations of nitrate, nitrite, phosphorous, calcium, magnesium, boron, and

zinc (256). Reducing pesticide usage may also benefit farmworkers by limiting their exposure to these chemicals, which can also be mitigated by proper use of personal protective equipment (257).

White Strawberries

Rich historical usage is reported for the white Chilean strawberry (*Fragaria chiloensis*) (258). White strawberries have a pale skin due to the suppression of anthocyanin synthesis, especially P3Gs, the most abundant anthocyanins in red strawberries. Recently, white strawberries were introduced to the U.S. and Japan (10). The nutrient composition of the white strawberries in the U.S. market is currently unavailable on the USDA database. A review study compared the bioactive compound profiles of white strawberry *Fragaria chiloensis* to the commercial red strawberry *Fragaria ananassa* and reported that white strawberry contained considerably lower concentrations of anthocyanins (total anthocyanins were 2.20 and 27.9 mg/100 g fresh weight for white and red strawberries, respectively) but significantly higher levels of ellagic acid (151.38 and 36.91 mg/100 g fresh weight for white and red cultivars, respectively) (259). With the significant differences in the major polyphenol and phenolic compounds in white and red strawberries, the health benefits of these fruits are potentially different. A separate recommendation for the white strawberries may be considered as the clinical research exploring the effects of this fruit on health is currently limited to animal and *in vitro* studies (259-261).

Potential Mechanisms of Action

Chronic conditions, including CVD, T2DM, cancer, obesity, cognitive decline, and osteoporosis, are often linked to the underlying mechanisms of oxidative stress and inflammation (262). Risk factors such as older age, male and postmenopausal status, obesity, genetics, and imbalanced diet contribute to the excessive generation of reactive oxygen (ROS) and reactive nitrogen species (RNS) which can enhance oxidative stress and activate inflammatory pathways (263). Low-grade chronic inflammation can cumulatively alter metabolic processes, which can be observed through abnormal production of biological markers such as satiety hormones, bile acids, oxylipins, acute phase proteins, and vascular function following meal challenges high in carbohydrates and fats (264). Nutrient deficiencies and excessive caloric intake can induce inflammation and impair the intestinal tract permeability, which affect micronutrient absorption and metabolism (265, 266). Since the gastrointestinal tract is the largest immune organ, disruption of gut permeability and dysbiosis of microbiome abundance are associated with increased risk of progression of systemic inflammation (267, 268). Systemic inflammation can cause activated endothelium, endothelial dysfunction, and aberration in blood flow (269-271).

The presence of antioxidants in strawberries, such as vitamin C and phenolic compounds, has been shown to mitigate oxidative stress in *in vitro* experiments (272, 273). This may occur through direct interaction with ROS or the modulation of imbalanced production and elimination of free radicals via the gut microbiome (274, 275). Higher concentrations of antioxidants such as vitamin C, SOD, and GSH related to strawberry consumption has been associated with a delay in lipid/cholesterol peroxidation as observed by decreased

production of MDA in several clinical studies (71, 82, 83, 112, 114, 116, 118, 122, 123, 276, 277). Strawberry consumption was also associated with decreased productions of inflammatory cytokines (e.g., IL-6 and IL-1β), fibrinogen and acute phase proteins (e.g., CRP and hs-CRP), which typically increase in response to oxidative stress as observed in clinical and observation studies (43, 70, 78, 119, 123-125, 127, 278, 279).

Anthocyanins are phenolic compounds that possess anti-inflammatory properties and have been used to explore associations between strawberry and health outcomes, under the assumption that strawberry is the predominant source of anthocyanins. The higher consumption of total anthocyanins or pelargonidin, the predominant type in strawberries, was reported to be associated with a lower risk of hypertension, cognitive dysfunction, and reduced markers related to Alzheimer's disease, including amyloid-β and phosphorylated tau tangles (37, 44, 46). A two-arm, parallel RCT investigating the effects a 90-day supplementation of 24 g/d of FDSP (equivalent to two servings) on cognitive function in 37 healthy older adults (mean age 67 years) found improvements in short- and long-term memory compared to baseline, while responses to an isocaloric control did not change (102). The results of this study and the given strawberry potion are in agreement with observational studies that noted significant associations between higher strawberry consumption (up to two servings per week or more) with improved cognitive function and increases in favorable Alzheimer’s disease biomarkers (37, 44, 46). Anthocyanin intake may be positively associated with a higher production of SCFA, including propionate and butyrate. However, when the associations between strawberry consumption and SCFA productions were considered, only propionate was significantly

associated with a higher intake (92). The characteristics of strawberry anthocyanins may be unique from the overall anthocyanin consumption that strawberries were considered as the primary source.

A shift in SCFA production might be due to changes in microbiome profiles. A single-arm study that provided 14 healthy adults with 26 g/d of FDSP for four weeks found significant increases in the abundance of certain bacteria families, including Christensenellaceae (Firmicutes phylum), Verrucomicrobia (Verrucomicrobia phylum), Bifidobactereriaceae (Actinobacteria phylum), and Bacteroidaceae (Bacteroidetes phylum), and a decrease in Alcaligenaceae/Sutterella (Proteobacteria phylum) (280). An increase in Christensenellaceae was associated with favorable outcomes such as an inverse relationship with body mass index (BMI) and inflammatory conditions (e.g., inflammatory bowel diseases) (281). The dominant gut microbiome may result in different SCFA compounds and concentrations, since acetate and propionate are mainly produced by Bacteroidetes, while the abundance of Firmicutes favors the production of butyrate (282). In the same study, the level of LCA, a secondary bile acid, slightly decreased during the intervention, but increased two weeks later, which also occurred along with the reverse abundance of Chirstensenellaceae (280). A two-arm, crossover RCT supplementing 50 g/d of FDSP for four weeks in 34 healthy participants (mean age 53 years) also found significant decreases of secondary bile acids and their conjugated products (90). High levels of secondary bile acids were reported to be associated with increased risk of chronic conditions such as colonic inflammation and cancer (283).

Excessive consumption of common components in a typical Western diet, such as red meat and saturated fat, was related to an increase of secondary bile acid production (284).

Secondary bile acids (commonly found as LCA and deoxycholic acid) are synthesized from primary bile acids (cholic and chenodeoxy cholic acid) by colonic bacteria that contain 7-α-hydroxylase, primarily from the Firmicutes phylum (285, 286). The conversion process removes a hydroxyl group at C-7 position, resulting in more hydrophobic products, which could diffuse through cell membranes and alter their functions (285). As bile acids are synthesized from cholesterol in the liver (287), the reduction of secondary bile acids may be related to an increase in bile acid production to utilize excess cholesterol in the body. Significant changes in some of the cholesterol profiles such as decreases in TC, LDL-C, and TG and an increase in HDL-C, were observed in eight clinical studies (114, 122, 288-293).

Apart from the changes in cholesterol profiles, significant changes following strawberry intake of certain metabolites are consistent with favorable health outcomes. A single-arm study provided 21 healthy females (mean age 29 years) with 250g/d of frozen strawberries for three weeks and found a delay in LDL oxidation along with the increased concentrations of urinary pelargonidin-glucuronide, urolithin A-glucuronide, 2,5-dimethyl-4-hydroxy-3-[2H]furanone (an important flavor constituent that gives strawberries the characteristic aroma and may be in part responsible for the modest increase in antioxidant defense (276)). A two-arm, parallel RCT involving 51 participants (mean age 37 years) who were either supplemented with 500g/d of thawed strawberry

pulp for 30 days or did not consume any strawberries found a significant decrease in oxidative stress, while increases were reported in fasting plasma and urinary metabolites from strawberry polyphenols, including fasting plasma caffeic acid, plasma homovanillic acid, urinary urolithin A, and urinary 4-hydroxyhippuric acid, and an inverse relationship between a lower oxidative stress level and a higher concentration of plasma 4-hydroxyhippuric acid level (121). A two-arm, crossover RCT in 34 adults with moderate hypercholesterolemia (mean age 53 years) who were supplemented with 50 g/d FDSP (25g FDSP twice per day; equivalent to 500 g fresh strawberries) or an isocaloric control for four weeks found significant increases in 4-methoxybenzoic acid-3-sulfate, hydroxyphenylacetic acid, hydroxybenzoic acid sulfate, and urolithin A, which were consistent with a significant decrease in SBP and a significant improvement of FMD. An increase in FMD was also significantly correlated with the lower concentration of 3-(4-methoxy-phenyl)propanoic acid 3-O-glucuronide (124). A two-arm crossover RCT assessed a single intake of 10 g of FDSP (equivalent to 100 g fresh strawberries) in 24 adults compared to an isocaloric control. The results showed that plasma metabolites were associated with increases in plasma pelargonidin sulfate and P3G, whereas the levels of hs-CRP, IL-6, and insulin significantly decreased (127).

Influence of Cultivar

Seven varieties of strawberries were used in clinical studies reviewed in this article, including Honeoye (121, 294), KYSt-4 (Nohime) (295), KYSt-10, Camerosa (93, 120), Sueva (112), Alba (113), and Yumetsuzuki (82). Studies using Honeoye strawberries from Poland reported that consumption of 500 g of thawed strawberries (integrated or

organically grown) for 30 days significantly decreased plasma paraoxonase-1 (PON-1) activity and increased circulating levels of the plasma strawberry metabolites homovanillic acid and caffeic acid, and the urinary exrection of urolithin A and 4-hydroxyhippuric acid (121, 294). The enzyme that is associated with HDL, which has protective effects against coronary artery diseases (294).

A three-arm, crossover RCT from Gifu, Japan, assessed a single intake of either KYSt-4 (Nohime) or KYSt-10 (Camerosa) at 11.05 ml/kg body weight (approximately 809 ml for a 70-kg man) and found that KYSt-4 significantly increased platelet reactivity compared to baseline values, but no significant difference between anti-thrombotic activity between the two varieties was observed (295).

Two studies utilized Camerosa strawberries from different locations. The first study reported that a single intake of 200 g of fresh or pureed strawberry from Murcia, Spain and reported that serum metabolites and urinary excretion of urolithin A and its glucuronide conjugates were not significantly different between the two forms. The results suggest that the processing into puree did not affect gut microbiome metabolism under the conditions tested (93). A second study assessed the effects of 25 or 50 g/d of FDSP, predominantly Camerosa strawberry (37%) from California, U.S.A., over the course of 12 weeks in healthy adults. The results showed that both amounts significantly increased plasma antioxidant capacity and whole blood glutathione compared to an isocaloric control (120).

A single-arm study provided 500 g/d of fresh Sueva strawberries from Ancona, Italy, to adult participants (mean age 35 years) for two weeks and found that, compared to baseline levels, the supplementation significantly decreased oxidative stress as observed by lower plasma lipid oxidation and DNA strand breakage and higher cell metabolic activity when challenged with hydrogen peroxide (112). A single-arm study utilizing the Alba strawberry cultivar from Ancona, Italy provided healthy adults (mean age 27 years) with 500 g/d of fresh strawberries for 30 days. The results showed significant reductions in cholesterol (TC, LDL-C, TG) and biomarkers of oxidative stress (MDA, 8-OHdG, isoprostanes) and significant increases in *in vitro* measures of antioxidant status (fluorescence recovery after photobleaching (FRAP), oxygen radical absorbance capacity (ORAC), and Vitamin C) (113).

A two-arm, crossover RCT from Takehara, Japan provided 23 healthy young adults (mean age 22.5 years) with 500 g of Yumetsuzuki a strawberries puree beverage at baseline, and then at 30 minutes, one-, two-, and four-hour time points. Compared to a calorie-, flavor-matched control beverage, a single intake of 500 g of strawberry puree significantly increased serum vitamin C and serum folate at 30 minutes through four hours, increased the lag time to start LDL oxidation at the one-hour point, and decreased blood glucose and insulin at 30 minutes, while total cholesterol, HDL-C, LDL-C, TG, and non-esterified fatty acid were not significantly different (82).

Overall, the studies that reported the strawberry varieties of the intervention focused on the effects of strawberries on oxidative stress and plasma lipid profiles. These outcomes

are interrelated since the delayed lipid oxidation may help to reduce oxidized LDL, thereby improving the cholesterol profile, particularly LDL. Although various types of strawberries may provide similar health benefits on oxidative stress and plasma lipid profiles, further exploration is needed to determine if different cultivars have comparable effects on other health outcomes.

Sustainability: Considerations and Innovations

Strawberry processing generates non-marketable byproducts, including bruised flesh and plant waste from the sepal, calyx, and stem, which create environmental challenges for local farmers and processors (296). These wastes may be a viable source of polyphenols that could be used to fortify foods and beverages or in dietary supplements. Strawberry leaves have also been used traditionally as medicine in many countries (297). Several strawberry cultivars, including 'Festival,' 'San Andreas,' and 'Camino Real' contain considerable amounts of total polyphenols (the highest is Festival varietal at 14.97 g gallic acid equivalents (GAE)/kg) (296). Agrimoniin, a hydrolysable tannin, has also been identified as a primary polyphenol in strawberry byproduct mixtures (298). Agrimoniin is one of the main ellagitannins found in the Rosaceae family, which has been reported to have anti-inflammatory and anticancer activities *in vivo* and *in vitro* (299). One study reported Agrimoniin in three common strawberry varietals in Norway at approximately 64-83% of ellagitannins, but the content is lower as the fruits become more ripened (28). With the considerable amount of polyphenols remaining in strawberry byproducts, new potential nutraceutical ingredients may be extracted, which could help

solve environmental issues related to waste disposal and provide a new source of revenue to farmers and processors (296).

Conclusion

Recent clinical studies on the health effects of strawberries generally prefer using FDSP over fresh strawberries. The primary focus of these studies is on cardiometabolic outcomes, encompassing vascular health, lipids, metabolic health, oxidative stress, and inflammation. In the 2000s, research expanded into novel areas, including gut microbiome profiles and metabolites, as well as health outcomes beyond cardiometabolic diseases such as bone and brain function and cancer. However, evidence in these areas is limited, necessitating further research to understand potential mechanisms and effects. While some studies have identified strawberry cultivars with high antioxidant capacity or specific phenolic compounds, none have compared the effects between different cultivars, making comparison challenging. Still, outcomes between cultivars seem to be similar across studies. Higher strawberry consumption appears to be associated with positive health outcomes in observational studies. Clinical studies using whole strawberries or FDSP within the recommended intake level (~1-4 cups equivalent to whole strawberries) tend to show significant health improvements. Advanced technology offers alternative strawberry forms helping to preserve nutrients and allow for greater accessibility. Further, up-cycling strawberry by-products may help address food insecurity/increase intake, particularly in low-income populations. Implementing nutrition policies to subsidize the cost of fresh fruit is another potential strategy to address financial barriers to strawberry intake. Finally, while concerns exist regarding

pesticides and organic vs. non-organic planting methods impacting nutrient levels, conclusive evidence is lacking, with findings mostly observed in food or environmental health sciences, and limited evidence in human clinical studies.

Table 1. Summary of the Composition of Strawberry (300-302)

Essential Nutrients

Macronutrients (g/serving)				*Micronutrients (mg*/serving)*			
	Quantity per Serving (g)	**DRV (g)**	**% DV**		**Quantity per Serving (mg*)**	**DRV (mg*)**	**% DV**
Protein	1.1	50	2.2	Calcium	26.6	1,300	2.1
Fat	0.5	78	0.6	Iron	0.7	18	3.8
Carbohydrate	12.7	275	4.6	Magnesium	21.6	420	5.1
Total sugar	8.1			Phosphorus	39.8	1,250	3.2
Fiber	3.3	28	11.9	Potassium	254	4,700	5.4
				Sodium	1.7	2,300	0.1
				Zinc	0.2	11	2.1
				Copper	0.1	0.9	8.9
				Manganese	0.6	2.3	27.9
				Vitamin C	97.6	90	108.4
				Folate	39.8 µg	400 µg	10
				Vitamin A	1.7 µg	900 µg	0.2

Non-Essential Bioactives

Flavonoids (mg/serving)		*Phenolic Acids (mg/serving)*		*Other*	
Anthocyanidins		*Hydroxybenzoic acids*		*Stilbenes (mg/serving)*	
Cyanidin	4.6	4-Hydroxybenzoic acid 4-O-glucoside	2.5	Resveratrol	0.6
Delphinidin	0.9	5-O-Galloylquinic acid	0.1	*Carotenoids (µg/serving)*	
Pelargonidin	68.5	Ellagic acid	2.1	β-Carotene	11.6
Peonidin	0.1	Ellagic acid glucoside	4.7	Lutein/Zeaxanthin	43.2
Petunidin	0.2	*Hydroxycinnamic acids*			
Flavan-3-ols		5-Caffeoylquinic acid	3.2		
(-)-Epicatechin	0.7	Caffeoyl glucose	0.2		
(-)-Epicatechin 3-gallate	0.3	Cinnamic acid	0.4		
(-)-Epigallocatechin	1.3	Feruloyl glucose	0.2		
(-)-Epigallocatechin 3-gallate	0.2	p-Coumaric acid	0.4		
(+)-Catechin	5.2	p-Coumaric acid 4-O-glucoside	0.3		
(+)-Gallocatechin	0.1	p-Coumaroyl glucose	7.2		
Flavanones					
Naringenin	0.4				
Flavonols					
Kaempferol	0.8				
Myricetin	0.1				
Quercetin	1.8				

Asterisk (*) indicates unit of measure is in mg unless otherwise noted
DRV: daily reference value; DV: daily value; G: grams; mg: milligrams; µg: micrograms
1 serving (166 g) is based on one cup of sliced, raw, fresh, strawberries Values were adjusted to 1 serving (166 g)

Table 2: Strawberry Intake and Cardiovascular Health Outcomes (2000-2023)							
Citation	**Study Design**	**Study Duration**	**Participant Characteristics**	**n (M/F)**	**Intervention/ Quantity**	**Control**	**Relevant Outcomes**
Prospective Cohort Trials							
Sesso 2007 (278)	Observational cohort study Whole: Prospective Subset: Cross-sectional	10.9 year - follow-up	Healthy adult females (mean age 55 years)	Whole: 38,176 Subset: 26,966	Whole strawberry, 1-3 servings/mo, 1 servings/wk, ≥2 servings/wk	No strawberry intake	Whole: No Δ in risk of incident CVD Subset: No Δ in lipid profile
Mink 2007 (49)	Prospective cohort study	16 years	Participants from IWHS (mean age 61 years)	34,489 (0/34,489)	Frequency of strawberry consumption (times/wk), G2=1 time/wk; G3= >1 time/wk	Frequency of strawberry consumption (times/wk), G1 = <1 time/wk	1 time/week of STRS ↓RR of death from CHD when adjusted for age- and energy-adjusted and ↓RR of death from CVD when adjusted for age, energy, and others
Cassidy 2011 (52)	Prospective cohort study	14 years (NHS I and HPFS 1990-2004; NHS II 1991-2005)	Participants from NHS I and II, and HPFS (mean age 55, 36, and 56 years, respectively)	156,957 (23,043/133,914)	Anthocyanin intake (mg/d) (Strawberries and blueberries were the top two anthocyanin sources); Q5 of each study (NHS I, 16.2; NHS II, 18.0; HPFS, 21.9)	Anthocyanin intake (mg/d) (Strawberries and blueberries were the top two anthocyanin sources); Q1 of each study (NHS I, 5.7; NHS II, 6.7; HPFS, 6.8)	Participants in NHS I/II and HPFS in the highest quintile of anthocyanin intake (Q5) (predominantly from blueberries and strawberries) had an 8% ↓RR of hypertension compared to participants with the average consumption in Q1; Also, SIG for trends in all studies (the higher consumption, the lower risk)
Muraki 2013 (88)	Prospective cohort study	Study participants from 1984-2008, 1991-2009, and 1986-2008	Participants from NHS I and II and the HPFS (mean age 48-51, 36, and 50-54 years, respectively)	187,382 (36,173/151,209)	Frequency of strawberry consumption: <1 serving/ month; 1-3 servings/month; 1 serving/week; 2-4 servings/week; and ≥5 servings/week	Strawberry consumption <1 serving/ month	Hazard ratio of T2DM:↔NS in NHS I and II, but significantly ↑ in HPS in the adjusted model (age, ethnicity, smoking status, physical activity, METs, family history of diabetes, menopause status, oral contraceptive use, fruit juice consumption, HEI score, multivitamin use); RR was lower when substituting 3 servings of total or specific fruit for the same amount of fruit juice (STRS was slightly protective ~ 0.9x)
Cassidy 2015 (303)	Cross-sectional study	7th study examination (one-time,	Framingham Heart Study Offspring cohort participants	2,375 (N/A)	Frequency of strawberry intake (serving/wk); Q2:	Frequency of strawberry intake, Q: <1 serving/wk	↑ STRS intake (g/d) was associated with ↓ inflammation score, ↓acute

		some time in 1998-2001)	(mean age 60-62 years)		1-4 servings/wk; Q3: 5-6 servings/wk; ≥7 servings/wk		inflammation subscore, ↓ oxidative stress subscore (most SIG in Q3); Total flavonoids and anthocyanins are both associated with ↓inflammation score, ↓acute inflammation subscore, ↓ oxidative stress subscore and cytokines
Ivey 2017 (14)	Prospective cohort study	1991-2007	Participants from Nurses' Health Study (mean age 36 years)	93,145 (0/93,145)	Frequency of strawberry consumption, G2=1 time/week; G3= >1 time/week	Frequency of strawberry consumption, G1= <1 time/week	Strawberry consumption more than 1 per week ↓cancer, CVD, other cause mortality (only SIG with the age-adjusted model, not the multivariable adjusted)
Yang 2020 (48)	Prospective cohort study	14 years (1998-2012	Healthy adults (mean age ~57 years)	87,177 (45.7%M)	Mean strawberry intake (g/d):Q2= 3.9±4.2; Q3= 6.4±6.8; Q4 = 11.0±10.7; Q5= 21.9±27.0	Mean strawberry intake, Q1= 1.4±1.8	↑ Strawberry intake was significantly associated with ↓CHD in both males and females
Gao 2021 (47)	Prospective cohort study	14 years (1998-2012)	Healthy adults (mean age ~57 years)	47,334 (39,843/47,334)	Mean strawberry intake (g/d): Q2 = 2.4g/d; Q3= 7.7 g/d; Q4= 27 g/d	Mean strawberry intake, Q1 =0 g/d	↑ Strawberry intake was significantly associated with ↓ stroke in men (when adjusted for age, BMI, and medical history) and women (when adjusted for age, BMI, medical history, and dietary intake)
Blood Pressure and Vascular Function							
Jenkins 2008 (69)	RCT,crossover, 2-arm	4 weeks; 2-week washout	Hyperlipidemic adults who participated in the 2.5 years diet intervention (mean age 62 years)	28 (N/A)	Fresh strawberry, 454g/d	Oat bran bread, 65g/d	NS: SBP, DBP
Basu 2009 (61)	Single arm	4 weeks	Adults with MetS (mean age 51 years)	16 (0/16)	FDSP, 50 g/d	N/A	NS: SBP, DBP
Basu 2010 (60)	RCT, crossover, 2-arm	8 weeks	Adults with MetS (mean age 47 years)	27 (2/25)	FDSP, 50 g/d	Water	NS: SBP, DBP
Basu 2014 (59)	RCT, parallel, 4-arm	12 weeks	Adults with abdominal adiposity and hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	NS: SBP, DBP
Amani 2014 (67)	RCT, parallel, 2-arm	6 weeks	Adults with T2DM (mean age 52 years)	36 (13/23)	FDSP, 50 g/d	Isocaloric control powder	↓ DBP NS: SBP
Djurica 2016 (65)	RCT, crossover, 2-arm	Acute (1 hour), Short-term	Healthy adolescents (mean age 16 years)	25 (25/0)	FDSP, 50g/d	Isocaloric control powder	↑ PAT (secondary analysis) related to ↑ plasma nitrate and nitrite

		(1 week; 1-week WO)					NS: PAT (primary analysis), SBP, DBP
Richter 2017 (74)	RCT, parallel, 3-arm	Acute (0.5, 1, 2, h hours)	Overweight and obese adults (mean age 28 years)	30 (13/17)	FDSP, 40g/d	Isocaloric control powder	NS: central BP, AIx
Feresin 2017 (79)	RCT, parallel, 3-arm	8 weeks	Post-menopausal females with pre- or stage 1 hypertension (mean age 59 years)	60 (0/60)	FDSP, 25g/d or 50g/d	Isocaloric control powder	25g/d: ↓ SBP, PWV NS: DBP, endothelin-1
Schell 2017 (66)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults (mean age 58 years)	17 (4/13)	FDSP, 50 g/day	Isocaloric control powder	NS: SBP, DBP
Basu 2021 (63)	RCT, crossover, 3-arm	4 weeks; 1-week WO	Adults with one or more characteristics of MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	NS: SBP, DBP
Huang 2021 (64)	RCT, crossover, 2-arm	4 weeks; 4-week WO Acute (1, 2, hours)	Adults with moderate hypercholesterolemia (mean age 53 years)	34 (17/17)	FDSP, 50 g/d	Isocaloric control powder	↑ FMD (treatment-by-hour effect) ↓ SBP 2 hours, week 4 NS: SBP, DBP
Richter 2023 (62)	RCT, crossover, 3-arm	4 weeks; 2-week WO	Overweight and obese adults with elevated LDL-C (mean age 50 years)	40 (29/11)	FDSP, 13 g/d or 40 g/d	Control powder	NS: brachial and central BP, AIx, or PWV
Plasma Lipids							
Jenkins 2008 (69)	RCT,crossover, 2-arm	4 weeks; 2-week washout	Hyperlipidemic adults who participated in the 2.5 years diet intervention (mean age 62 years)	28 (N/A)	Fresh strawberry, 454g/d	Oat bran bread, 65g/d	NS: TC, LDL-C, HDL-C, TG
Basu 2009 (61)	Single-arm	4 weeks	Adults with MetS (mean age 51 years)	16 (0/16)	FDSP, 50 g/d	N/A	↓ TC and LDL-C NS: TG, HDL-C, VLDL-C
Basu 2010 (60)	RCT, crossover, 2-arm	8 weeks	Adults with MetS (mean age 47 years)	27 (2/25)	FDSP, 50 g/d	Water	↓ TC, LDL-C, and small LDL particles NS: TG, HDL-C, VLDL-C, HDL, IDL and VLDL particles, small and large LDL particles
Burton-Freeman 2010 (75)	RCT, crossover, 2-arm	Acute HFM (acute, 6 hours) 6 weeks	Adults with hyperlipidemia (mean age 51 years)	24 (10/14)	FDSP, 10gd	Isocaloric control powder	Acute: ↓ TG, HDL-C 6 week acute response: ↓ TC, LDL-C, TG, HDL-C

		Acute HFM (no WO)					
Zunino 2012 (57)	RCT, crossover, 2-arm	3 weeks; no WO	Obese adults (mean age 32 years)	20 (7/13)	FDSP, 50 g/d	Isocaloric control powder	↓ TC, small HDL-C particles; ↑ LDL particle size
Basu 2014 (59)	RCT, parallel, 4-arm	12 weeks	Adults with abdominal adiposity and hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	25 g/d vs 50 g/d : ↓ TC, LDL-C, and small LDL particles 50 g/d vs control: ↓ small LDL particles NS: HDL-C, TG
Amani 2014 (67)	RCT, parallel, 2-arm	6 weeks	Adults with T2DM (mean age 52 years)	36 (13/23)	FDSP, 50 g/d	Isocaloric control powder	↓ TC, TC:HDL NS: TG, HDL-C
Alvarez-Suarez 2014 (71)	Single-arm	1 month	Healthy adults (mean age 27 years)	23 (11/12)	Whole strawberry, 500 g/d	N/A	↓ TC, LDL-C, TG NS: HDL-C
Huang 2016 (86)	RCT,crossover, 3-arm	Acute (10 hours)	Healthy adults (mean age 25 years)	14 (9/5)	FDSP, 12 g (consumed before, with, or after a meal)	Isocaloric control powder, 12 g	NS: TG
Djurica 2016 (65)	RCT, crossover, 2-arm	Acute (1 hour), Short-term (1 week; 1-week WO)	Healthy adolescents (mean age 16 years)	25 (25/0)	FDSP, 50g/d	Isocaloric control powder	NS: TC, HDL-C, LDL-C, TG
Zasowska-Nowak 2016 (72)	NR, parallel, 2-arm	30 days; 10-day WO	Healthy adults (mean age 37 years)	51 (27/24)	Strawberry pulp, 500 g/d	Avoidance of strawberry	↓ PON-1 NS: TC, HDL-C, LDL-C, TG
Park 2016 (84)	RCT, crossover, 4 arm	Acute (6 hours); 3-14 day of washout period	Obese adults with insulin resistance (mean age 40 years)	21 (5/16)	FDSP, 10g, 20g, 40g (one for each visit)	0g FDSP control beverage	NS: TG, IL-6
Richter 2017 (74)	RCT, parallel, 3-arm	Acute (0.5, 1, 2, h hours)	Overweight and obese adults (mean age 28 years)	30 (13/17)	FDSP, 40g/d	Isocaloric control powder	NS: TG
O'Doherty 2017 (73)	RCT, crossover, 2-arm	OFTT 16 hours post submaximal high intensity interval cycling exercise; at least 72 h WO	Healthy overweight & obese adults (mean age 32 years)	10 (10/0)	OFTT w/ 25g of FDSP	OFTT w/ strawberry flavoring	NS compared to exercise + control: TG, HDL, LDL (all AUC), oxLDL, lipid hydroperoxides ↑ compared to Exercise + control: TG iAUC
Schell 2017 (66)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults (mean age 58 years)	17 (4/13)	FDSP, 50 g/day	Isocaloric control powder	NS: TC, LDL-C, HDL-C, TG

Etemad 2019 (77)	RCT, parallel, 2-arm	2 weeks with endurance training	Overweight adults with low physical activity (mean age 24 years)	24 (0/24)	FDSP (capsule form), 25 g (2x / day)	Control capsules (similar appearance, nutrition information was not provided)	FDSP + endurance exercise (baseline (day 0) vs day 14 of intervention (after endurance exercise): ↓TC, TG, LDL, HDL FDSP (baseline vs day14 (pre-workout): ↓TC
Yuliwati 2020 (68)	RCT, parallel, 4-arm	14 days	Adults with T2DM (age 40-55 years)	44 (34/10)	Fresh strawberries, 200 g/day	No treatment (no fruits)	↓ LDL-C
Basu 2021 (63)	RCT, crossover, 3-arm	4 weeks; 1-week WO	Adults with one or more characteristics of MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ Total VLDL and chylomicrons, small VLDL, total and small LDL particles NS: TC, LDL, HDL, TG, HDL particles, medium and large VLDL particles, large LDL particles, IDL particles
Huang 2021 (64)	RCT, crossover, 2-arm	4 weeks; 4-week WO Acute (1, 2, hours)	Adults with moderate hypercholesterolemia (mean age 53 years)	34 (17/17)	FDSP, 50 g/d	Isocaloric control powder	NS: TC, LDL-C, HDL-C, TG, ApoA and B
Richter 2023 (62)	RCT, crossover, 3-arm	4 weeks; 2-week WO	Overweight and obese adults with elevated LDL-C (mean age 50 years)	40 (29/11)	FDSP, 13 g/d or 40 g/d	Control powder	↓ 13g: TC from control, and LDL-C and TC compared to 40g/d group NS: HDL-C, TG, lipoprotein particle concentration, cholesterol efflux,
Kishimoto 2023 (82)	RCT,crossover, 2-arm	Acute (4 hours), 4-week WO	Healthy females (mean age 23 years)	23 (0/23)	Strawberry puree beverage, 500 g	Calorie-, flavor-matched control beverage, 500 g	NS: TC, HDL-C, LDL-c, Triglycerides, non-esterified fatty acid (NEFA)

Table 3: Strawberry Intake and Metabolic Health Outcomes and Strawberry Related Metabolites (2000-2023)							
Citation	**Study Design**	**Study Duration**	**Participant Characteristics**	**N (M/F)**	**Intervention/ Quantity**	**Control**	**Relevant Outcomes**
Basu 2009 (61)	Single arm	4 weeks	Adults with MetS (mean age 51 years)	16 (0/16)	FDSP, 50 g/d	N/A	NS: adiponectin, glucose
Basu 2010 (60)	RCT, crossover, 2-arm	8 weeks	Adults with MetS (mean age 47 years)	27 (2/25)	FDSP, 50 g/d	Water	NS: glucose
Henning 2010 (276)	Single-arm	3 weeks	Healthy adults (mean age 29 years)	21 (0/21)	Whole strawberry, 250 g	N/A	↑ 2,5-Dimethyl-4-hydroxy-3-[2 H]furanone, pelargonidin-glucoside, pelargonidin-glucuronide, urolithin A-glucuronide
Edirisinghe 2011 (85)	RCT, crossover, 2-arm	Acute (6hours); 3-5 d washout period	Overweight to obese adults (mean age 51 years)	14 (10/14)	FDSP, 10 g	Placebo drink with no FDSP	FDSP vs control (overall at 6 h postprandial): ↑ plasma pelargonidin sulfate, plasma pelargonidin-3-glucoside ↓ Insulin NS: glucose
Ellis 2011 (76)	RCT, parallel, 2-arm	6 weeks Week 6: Acute (6hours) with high fat/carbohydrate meal	Obese adults (mean age 51 years)	24 (10/14)	FDSP, 10 g	Isocaloric control powder	NS: glucose, insulin
Moazen 2013 (87)	RCT, parallel, 2-arm	6 weeks	Adults with T2DM (mean age 52 years)	36 (13/23)	FDSP, 50 g/d	Isocaloric control powder	↓ HbA1c
Basu 2014 (59)	RCT, parallel, 4-arm	12 weeks	Adults with abdominal adiposity and hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	50 g/d: ↑ plasma Ellagic Acid NS: glucose, insulin, HbA1c
Ibero-Baraibar 2014 (83)	RCT, crossover, 3-arm	Acute (2 hours); 1-week washout	Healthy adults (mean age 26 years)	16 (6/10)	LS: natural sugar strawberry jam , 60 g; LSA: non-added sugar strawberry jam + antioxidant, 60g; HS: Added sugar (41.8-2.6/2.7 = 39 g table sugar) strawberry jam, 60 g	N/A	HS vs LS, LSA: ↑ glucose, insulin, HOMA-IR at 30 mins (60 mins for insulin and HOMA-IR) ↓ free fatty acids (at 30, 60, 90, 120 mins)
Alvarez-Suarez 2014 (71)	Single arm	1 month	Healthy adults (mean age 27 years)	23 (11/12)	Whole strawberry, 500 g/d	N/A	NS: glucose

Djurica 2016 (65)	RCT, crossover, 2-arm	Acute (1 hour), Short-term (1 week; 1-week WO)	Healthy adolescents (mean age 16 years)	25 (25/0)	FDSP, 50g/d	Isocaloric control powder	↑ plasma nitrate and nitrite, 1 hr post intake
Sandhu 2016 (304)	RCT, crossover, 3-arm	A single intake (2h before, with, or 2h after breakfast)	Overweight and obese adults (mean age 25 years)	14 (9/5)	FDSP, 12 g	Isocaloric control powder	Pharmacokinetics of: pelargonidin-3-O-glucoside, pelargonidin-3-O-rutinoside, pelargonidin glucuronide
Park 2016 (84)	RCT, crossover, 4 arm	Acute (6 hours); 3-14 day of washout period	Obese adults with insulin resistance (mean age 40 years)	21 (5/16)	FDSP, 10g, 20g, 40g (one for each visit)	0g FDSP control beverage	40 g FDSP: ↓Insulin AUC/iAUC, glucose (iAUC), insulin: glucose ratio For all intake levels: ↑ pelargonidin-3-O-glucoside, pelargonidin-glucuronide, cyanidin-3-O-glucoside
Huang 2016 (86)	RCT,crossover, 3-arm	Acute (10 hours)	Healthy adults (mean age 25 years)	14 (9/5)	FDSP, 12 g (consumed before, with, or after a meal)	Isocaloric control powder, 12 g	NS: glucose, insulin
Richter 2017 (74)	RCT, crossover, 2-arm	Acute (0.5, 1, 2, h hours)	Overweight to obese adults (mean age 28 years)	30 13/17)	FDSP, 40g/d	Isocaloric control powder	NS: glucose, insulin Men trend for lower glucose response with FDSP intake compared to control
Feresin 2017 (79)	RCT, parallel, 3-arm	8 weeks	Post-menopausal females with pre- or stage 1 hypertension (mean age 59 years)	60 (0/60)	FDSP, 25g/d or 50g/d	Isocaloric control powder	50g/d: ↑ nitrate and nitrite
Schell 2017 (66)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults (mean age 58 years)	17 (4/13)	FDSP, 50 g/day	Isocaloric control powder	NS: glucose, HbA1c, Nitrite
Sandhu 2018 (305)	RCT, parallel, 2-arm	90 days	Adults with normal to obese BMI (mean age of control and strawberry group, 69 and 67, respectively)	38 (N/A)	FDSP, 24 g/day	Isocaloric control powder	↑ pelargonidin glucuronide, pelargonidin-3-O-rutinoside, at 2 h from day 45 to day 90 of FDSP intake ↑ Urolithin A, B glucuronide, isourolithin A glucuronide, (day 45 to day 90 of FDSP intake; NS between 2 h after FDSP) ↑4-hydroxybenzaldehyde, trans-cinnamic acid, hippuric acid, and p-coumaric acid (FDSP vs control at 0 and 2 h after)
Basu 2018 (277)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults with knee OA (mean age 57 years)	17 (4/13)	FDSP, 50g/d	Isocaloric control powder	NS: C-peptide, ghrelin, GIP, glucagon, insulin, leptin, resistin, visfatin

Huang 2021 (64)	RCT, crossover, 2-arm	4 weeks; 4-week WO Acute (1, 2, hours)	Adults with moderate hypercholesterolemia (mean age 53 years)	34 (17/17)	FDSP, 50 g/d	Isocaloric control powder	↑3-Methoxyphenylacetic acid, 3-Hydroxyphenyl-γ -valerolactone-4-sulfate, 4-Hydroxyphenylacetic acid, Hydroxybenzoic acid–sulfate, Urolithin A NS: glucose, insulin
Basu 2021 (63)	RCT, crossover, 3-arm	4 weeks; 1-week WO	Adults with one or more characteristics of MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ insulin, HOMA-IR ↑ glucagon NS: glucose, HbA1c, adiponectin, C-peptide, GIP, GLP-1, ghrelin, leptin, resistin, visfatin
Richter 2023 (62)	RCT, crossover, 3-arm	4 weeks; 2-week washout	Adults with with overweight and obesity and elevated LDL-C (mean age 50 years)	40 (29/11)	FDSP, 13 g/d or 40 g/d	Control powder	NS: glucose, insulin
Kishimoto 2023 (82)	RCT,crossover, 2-arm	Acute (4 hours), 4-week WO	Healthy females (mean age 23 years)	23 (0/23)	Strawberry puree beverage, 500 g	Calorie-, flavor-matched control beverage, 500 g	↑ glucose, insulin (0.5 hours)

Table 4: Strawberry Intake and Platelet Reactivity and Markers of Inflammation (2000-2023)							
Citation	**Study Design**	**Study Duration**	**Participant Characteristics**	**N (M/F)**	**Intervention/ Quantity**	**Control**	**Relevant Outcomes**
Naemura 2006 (306)	RCT, crossover, 3-arm	A single intake	Healthy adults (female and male mean age 22 years and 21 years, respectively)	30 (10/20)	Strawberry filtrates (KYSt-4 or KYSt-10); consumed for first intake at 7.7 ml/kg body followed by a second intake at 3.85 ml/kg body weight, 30 min post first intake	Water	KYSt-4 significantly ↑ occlusion time (OT) as % change from baseline NS: Lysis time
Sesso 2007 (278)	Observational cohort study Whole: Prospective Subset: Cross-sectional	10.9 year follow-up	Healthy adults (mean age 55 years)	Whole: 38,176 Subset: 26,966	Whole strawberry, 1-3 servings/mo, 1 servings/wk, ≥2 servings/wk	No strawberry intake	Subset: NS: CRP
Jenkins 2008 (69)	RCT,crossover, 2-arm	4 weeks; 2-week washout	Hyperlipidemic adults who participated in the 2.5 years diet intervention (mean age 62 years)	28 (N/A)	Fresh strawberry, 454g/d	Oat bran bread, 65g/d	NS: CRP
Basu 2010 (60)	RCT, crossover, 2-arm	8 weeks	Adults with MetS (mean age 47 years)	27 (2/25)	FDSP, 50 g/d	Water	↓ VCAM-1 NS: ICAM-1
Edirisinghe 2011 (85)	RCT, crossover, 2-arm	Acute (6hours); 3-5 d washout period	Overweight to obese adults (mean age 51 years)	14 (10/14)	FDSP, 10 g	Placebo drink with no FDSP	FDSP vs control (overall at 6 h postprandial): ↑plasma pelargonidin sulfate, plasma pelargonidin-3-glucoside ↓ hs-CRP, IL-6, Insulin NS: PAI-1, glucose, IL-1beta, TNF-a
Ellis 2011 (76)	RCT, parallel, 2-arm	6 weeks Week 6: Acute (6hours) with high fat/carbohydrate meal	Obese adults (mean age 51 years)	24 (10/14)	FDSP, 10 g	Isocaloric control powder	NS short term intake: hsCRP, IL-6, PAI-1, IL-1B, TNF-alpha, glucose, insulin; 6h postprandial: ↓PAI-1
Zunino 2012 (57)	RCT, crossover, 2-arm	3 weeks; no WO	Obese adults (mean age 32 years)	20 (7/13)	FDSP, 50 g/d	Isocaloric control powder	NS: Il-1beta, -8, TNF-a, Complement 3c, sICAM-1, sVCAM-1, CRP, ORAC, TAS, 8-Isoprostane ↑ Fibrinogen

Moazen 2013 (87)	RCT, parallel, 2-arm	6 weeks	Adults with T2DM (mean age 52 years)	36 (13/23)	FDSP, 50 g/d	Isocaloric control powder	↓ hs-CRP
Bialasiewicz 2014 (307)	NR, parallel, 2-arm	30 days	Healthy adults (mean age 37 years)	51 (24/27)	Strawberry pulp, 500 g/d	Avoidance of strawberry	↑ Uro-A and 4-hydroxyhippuric acid
Basu 2014 (59)	RCT, parallel, 4-arm	12 weeks	Adults with abdominal adiposity and hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	NS: hsCRP, sICAM-1, sVCAM-1
Alvarez-Suarez 2014 (71)	Single-arm	1 month	Healthy adults (mean age 27 years)	23 (11/12)	Whole strawberry, 500 g/d	N/A	↓ platelet activation
Djurica 2016 (65)	RCT, crossover, 2-arm	Acute (1 hour), Short-term (1 week; 1-week WO)	Healthy adolescents (mean age 16 years)	25 (25/0)	FDSP, 50g/d	Isocaloric control powder	NS: platelet reactivity
Huang 2016 (86)	RCT,crossover, 3-arm	Acute (10 hours)	Healthy adults (mean age 25 years)	14 (9/5)	FDSP, 12 g (consumed before, with, or after a meal)	Isocaloric control powder, 12 g	Consuming FDSP before a meal vs baseline: ↓ IL-6 (while no significant change when consuming FDSP with or after a meal)
Park 2016 (84)	RCT, crossover, 4 arm	Acute (6 hours); 3-14 day of washout period	Obese adults (mean age 40 years)	21 (5/16)	FDSP, 10g, 20g, 40g (one for each visit)	0g FDSP control beverage	NS: IL-6
Schell 2017 (66)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults (mean age 58 years)	17 (4/13)	FDSP, 50 g/day	Isocaloric control powder	FDSP vs control (at Week 12): ↓ IL-6, IL-1B, MMP-3, disability index (HAQ-DI), ICOAP-constant pain, ICOAP-intermittent pain, ICOAP-total pain NS: MMP-8, hsCRP
Basu 2018 (277)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults with knee OA (mean age 57 years)	17 (4/13)	FDSP, 50g/d	Isocaloric control powder	↓ hsTNF-α and sTNF-R2
Etemad 2019 (77)	RCT, parallel, 2-arm	2 weeks with endurance training	Overweight adults with low physical activity (mean age 24 years)	24 (0/24)	FDSP (capsule form), 25 g (2x / day)	Control capsules (similar appearance, nutrition information was not provided)	FDSP + endurance exercise (baseline (day 0) vs day 14 of intervention (after endurance exercise): ↓ fibrinogen FDSP (baseline vs day14 (pre-workout): ↓ fibrinogen
Rutledge 2019 (308)	RCT, parallel, 2-arm, double-blinded & in vitro study in	90 days	Healthy adults (age 60-75 years); HAPI rat microglial cells	N/A	FDSP, 24 g/d	Isocaloric control powder	Serum from FDSP supplemented older adults ↑ LPS-induced NO production, ↓ LPS induced expression of

	HAPI rat microglial cells						iNOS, ↓ LPS-induced release of TNF-α, ↓ LPS-induced expression of COX-2 in HAPI rat microglial cells *ex vivo*
Basu 2021 (63)	RCT, crossover, 3-arm	4 weeks; 1-week WO	Adults with one or more characteristics of MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ PAI-1 NS: hsCRP
Basu 2021 (80)	RCT, crossover, 3-arm	4 weeks; 1-week washout	Adults with one or more characteristics of MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ sVCAM-1, TNF-a NS: nitrate and nitrite, sICAM-1, sP-, E-selectin, IL-6, IL-1beta
Huang 2021 (64)	RCT, crossover, 2-arm	4 weeks; 4-week WO Acute (1, 2, hours)	Adults with moderate hypercholesterolemia (mean age 53 years)	34 (17/17)	FDSP, 50 g/d	Isocaloric control powder	NS: hsCRP
Richter 2023 (62)	RCT, crossover, 3-arm	4 weeks; 2-week WO	Overweight and obese adults with elevated LDL-C (mean age 50 years)	40 (29/11)	FDSP, 13 g/d or 40 g/d	Control powder	NS: CRP, 8-isoprostanes, TNF-a, IL-6

Table 5: Strawberry Intake and Gut Health Outcomes (2000-2023)							
Citation	**Study Design**	**Study Duration**	**Participant Characteristics**	**N (M/F)**	**Intervention/ Quantity**	**Control**	**Relevant Outcomes**
Truchado 2012 (309)	RCT, crossover, 2-arm	A single intake; 2-week washout	Healthy adults with normal BMI (age 25-30 years)	20 (8/12)	Fresh strawberry, 200g Strawberry Puree, 200g	N/A	NS for processing in urinary excretion of urolithin A glucuronide and urolithin A
Fernández-Navarro 2016 (310)	Cross-sectional study	N/A	Healthy adults (age 19-95 years)	124 (38/86)	FFQ	N/A	Strawberry consumption from diet (+) association w/ propionate production (SCFA)
Sandhu 2018 (305)	RCT, parallel, 2-arm	90 days	Adults with normal to obese BMI (mean age of control and strawberry group, 69 and 67, respectively)	38 (N/A)	FDSP, 24 g/day	Isocaloric control powder	↑ Urolithin A, B glucuronide, isourolithin A glucuronide, (day 45 to day 90 of FDSP intake; NS between 2 h after FDSP)
Zhao 2021 (311)	RCT,crossover, 2-arm	4 weeks	Healthy adults (mean age 53 years)	34 (N/A)	FDSP, 50g/d	Isocaloric control powder	↓ Secondary BA and glycine conjugates
Ezzat-Zadeh 2021 (280)	Single-arm	4 weeks	Healthy adults (mean age 30 years)	14 (6/8)	FDSP, 26g/d	N/A (placebo group omitted due to prebiotic content)	↑ *20 OTUs* ↓ *4 OTUs* No Δ in fecal microbial metabolites or SCFA
Basu 2023 (312)	RCT, crossover, 3-arm	4 weeks; 1-week washout	Adults with MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ Serum valine and leucine ↑ Serum phosphate, hydroxyphenyl propionic acid, and propionic acid

Table 6: Strawberry Intake and Cognitive or Brain Health Outcomes (2000-2023)

Citation	Study Design	Study Duration	Participant Characteristics	N (M/F)	Intervention/ Quantity	Control	Relevant Outcomes
Agarwal 2022 (313)	Prospective cohort study	24 years (1997-2021; dietary assessment started in 2004)	Deceased adult participants of Memory and Aging Project (mean death age 90 years)	575 (70%F)	Strawberry consumption in quartiles (overall mean intake 0.61±0.52 servings/week); Q1= 0.10±0.17; Q2= 0.48±0.25; Q3= 0.67±0.32; Q4= 1.19±0.53)	Q1= 0.10±0.17 serving/week	↑ Pelargonidin intake (Q3 and Q4 vs Q1) is associated with ↓amyloid-β load; Q3 vs Q1 is associated with ↓ Phosphorylated tau tangles; ↑ strawberry intake among APOE ε4 non-carriers is associated with ↓ Phosphorylated tau tangles; ↑Pelargonidin intake among APOE ε4 non-carriers is associated with ↓ global Alzheimer's dementia (AD) pathology burden in Q4, ↓ amyloid-β load in Q3, and ↓ Phosphorylated tau tangles in Q4
Yeh 2021 (314)	Prospective cohort study	FFQ-NHS (started 1984-2006); FFQ-HPFS (started 1986-2002) ; subjective cognitive decline (SCD) was assessed in 2012-2014 for NHS and 2008-2012 for HPFS	Healthy adults (female mean age 48 years; male mean age 51)	77,335 (27,842/49,493)	strawberry was considered as the main source of anthocyanins and provided the total anthocyanins in mg/d); Q2= 13.2±7.2; Q3= 16.8±9.9; Q4= 19.2±13.2; Q5=18.6±15.7	Q1= 8.1±5.1 mg/d	↑ Intake of flavones, flavanones, anthocyanins, and strawberry intake is associated with ↓ ORs of 3-unit increments in Subjective cognitive decline (SCD)
Agarwal 2019 (315)	Prospective cohort study	Mean follow-up 6.7 years	Healthy adults (mean age 81 years)	925 (75%F)	≥1 serving/wk strawberry intake	None or <1 serving/mo strawberry intake	↓ risk AD
Devore 2012 (316)	Prospective cohort study	21 years (1980-2001; recruitment during 1995-2001)	Participants from NHS (mean age 74 years)	16,010 (0/16,010)	Frequency of strawberry consumption,G2 = 1 serving/week; G3 = ≥2 servings/week	Frequency of strawberry intake, Q1 = < 1 serving/ week	↑ strawberry intake is associated with ↑ mean difference of global score and verbal memory score of cognitive decline (the higher difference compared to Q1 is considered as better cognitive function)
Miller 2021 (317)	RCT, parallel, 2-arm	90 days	Healthy adults (mean age 68 years)	37 (22/15)	FDSP, 24 g/d	Isocaloric control powder	↑ Word recognition and spatial learning and memory No Δ in gait or balance

Table 7: Strawberry Intake and Oxidant Defense (2000-2023)							
Citation	**Study Design**	**Study Duration**	**Participant Characteristics**	**N (M/F)**	**Intervention/Quantity**	**Control**	**Relevant Outcomes**
Naemura 2006 (306)	RCT, crossover, 3-arm	A single intake	Healthy adults (female and male mean age 22 years and 21 years, respectively)	30 (10/20)	Strawberry filtrates (KYSt-4 or KYSt-10); consumed for first intake at 7.7 ml/kg body followed by a second intake at 3.85 ml/kg body weight, 30 min post first intake	water	KYSt-4 significantly ↑ occlusion time (OT) as % change from baseline No Δ: Lysis time, antioxidant activity
Jenkins 2008 (69)	RCT,crossover, 2-arm	4 weeks; 2-week washout	Hyperlipidemic adults who participated in the 2.5 years diet intervention (mean age 62 years)	28 (N/A)	Fresh strawberry, 454g/d	Oat bran bread, 65g/d	Compared to baseline: ↑ protein thiols ↓ MDA-equivalent (TBARS) in LDL fraction
Basu 2009 (61)	Single-arm	4 weeks	Adults with MetS (mean age 51 years)	16 (0/16)	FDSP, 50 g/d	N/A	↓ MDA and HNE NS: hsCRP, oxLDL
Burton-Freeman 2010 (75)	RCT, crossover, 2-arm	Acute HFM (acute, 6 hours) 6 weeks Acute HFM (no WO)	Adults with hyperlipidemia (mean age 51 years)	24 (10/14)	FDSP, 10gd	Isocaloric control powder	Acute: ↓ oxLDL
Henning 2010 (276)	Single-arm	3 weeks	Healthy adults (mean age 29 years)	21 (0/21)	Whole strawberry, 250 g	N/A	↑ oxLDL ↑ 2,5-Dimethyl-4-hydroxy-3-[2 H]furanone, pelargonidin-glucoside, pelargonidin-glucuronide, urolithin A-glucuronide NS: GST, comet assay, 8-OH2dG
Zunino 2012 (57)	RCT, crossover, 2-arm	3 weeks; no WO	Obese adults (mean age 32 years)	20 (7/13)	FDSP, 50 g/d	Isocaloric control powder	NS: ORAC, TAS, 8-Isoprostane
Moazen 2013 (87)	RCT, parallel, 2-arm	6 weeks	Adults with T2DM (mean age 52 years)	36 (13/23)	FDSP, 50 g/d	Isocaloric control powder	↓ MDA ↑TAC
Alvarez-Suarez 2014 (71)	Single-arm	1 month	Healthy adults (mean age 27 years)	23 (11/12)	Whole strawberry, 500 g/d	N/A	↓ MDA, 8-OHdg, isoprostanes, AAPH-RBC hemolysis ↑ ORAC, FRAP, vitamin C NS: uric acid
Bialasiewicz 2014 (307)	NR, parallel, 2-arm	30 days	Healthy adults (mean age 37 years)	51 (24/27)	Strawberry pulp, 500 g/d	Avoidance of strawberry	↓ Resting LBCL ↑ Uro-A and 4-hydroxyhippuric acid

							No Δ in agonist (fMLP)-induced LBCL, plasma antioxidant activity or circulating phagocytes
Basu 2014 (59)	RCT, parallel, 4-arm	12 weeks	Adults with abdominal adiposity and hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	50 g/d vs control: ↓ MDA, HNE
Ibero-Baraibar 2014 (83)	RCT, crossover, 3-arm	Acute (2 hours); 1-week washout	Healthy adults (mean age 26 years)	16 (6/10)	LS: natural sugar strawberry jam , 60 g; LSA: non-added sugar strawberry jam + antioxidant, 60g; HS: Added sugar (41.8-2.6/2.7 = 39 g table sugar) strawberry jam, 60 g	N/A	NS: MDA, GPx, uric acid, TAC
Tulipani 2014 (318)	Single-arm	2 weeks	Healthy adults (mean age 35 years)	18 (8/10)	Whole strawberry, 500 g/d	N/A	↑ Lipid peroxidation lag time ↓ Oxidative RBC hemolysis and mononuclear cell mortality No Δ in plasma total antioxidant capacity or RBC membrane lipid peroxidation
Basu 2014 (59)	RCT, parallel, 4-arm	12 weeks	Adults with abdominal adiposity and hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	↓ Serum MDA
Alvarez-Suarez 2014 (71)	Single-arm	30 days	Healthy adults (mean age 27 years)	23 (11/12)	Whole strawberry, 500 g/d	N/A	↓ MDA, 8-OHdG, Isoprostanes, and oxidative RBC hemolysis ↑ ORAC and FRAP
Basu 2016 (319)	RCT, parallel, 4-arm	12 weeks	Adults with hyperlipidemia (mean age 49 years)	60 (5/55)	FDSP, 25g/d or 50g/d	Isocaloric control powder	↑ Plasma antioxidant capacity, whole blood GSH, and serum catalase activity No Δ in trace elements or GSH enzyme activity
Park 2016 (84)	RCT, crossover, 4 arm	Acute (6 hours); 3-14 day of washout period	Obese adults (mean age 40 years)	21 (5/16)	FDSP, 10g, 20g, 40g (one for each visit)	0g FDSP control beverage	NS: oxLDL, IL-6
Huang 2016 (86)	RCT,crossover, 3-arm	Acute (10 hours)	Healthy adults (mean age 25 years)	14 (9/5)	FDSP, 12 g (consumed before, with, or after a meal)	Isocaloric control powder, 12 g	NS: oxLDL

O'Doherty 2017 (73)	RCT, crossover, 2-arm	OFTT 16 hours post submaximal high intensity interval cycling exercise; at least 72 h washout period	Healthy overweight & obese adults (mean age 32 years)	10 (10/0)	OFTT w/ 25g of FDSP	OFTT w/ strawberry flavoring	NS compared to exercise + control: oxLDL, FOX1
Richter 2017 (74)	RCT, crossover, 2-arm	Acute (0.5, 1, 2, h hours)	Overweight to obese adults (mean age 28 years)	30 13/17)	FDSP, 40g/d	Isocaloric control powder	NS: MDA/TBARs, oxLDL
Feresin 2017 (79)	RCT, parallel, 3-arm	8 weeks	Post-menopausal females with pre- or stage 1 hypertension (mean age 59 years)	60 (0/60)	FDSP, 25g/d or 50g/d	Isocaloric control powder	25g/d: ↑ SOD
Basu 2018 (277)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults with knee OA (mean age 57 years)	17 (4/13)	FDSP, 50g/d	Isocaloric control powder	↓ HNE
Basu 2021 (80)	RCT, crossover, 3-arm	4 weeks; 1-week washout	Adults with one or more characteristics of MetS (mean age 53 years)	33 (2/31)	FDSP, 13g/d or 32g/d	Isocaloric control powder	↓ MDA, ↑ SOD, TAS NS: catalase, GSH, GR, GPX,
Richter 2023 (62)	RCT, crossover, 3-arm	4 weeks; 2-week WO	Overweight and obese adults with elevated LDL-C (mean age 50 years)	40 (29/11)	FDSP, 13 g/d or 40 g/d	Control powder	NS: 8-isoprostanes
Kishimoto 2023 (82)	RCT,crossover, 2-arm	Acute (4 hours), 4-week WO	Healthy females (mean age 23 years)	23 (0/23)	Strawberry puree beverage, 500 g	Calorie-, flavor-matched control beverage, 500 g	↑ vitamin C, folate (all time points), oxLDL lag time (1 hour)

Table 8: Strawberry Intake and Other Health Outcomes (2000-2023)							
Bone Health							
Citation	**Study Design**	**Study Duration**	**Participant Characteristics**	**N (M/F)**	**Intervention/Quantity**	**Control**	**Relevant Outcomes**
Feresin 2021 (320)	RCT, parallel, 3-arm	8 weeks	Postmenopausal females with pre- and stage 1-hypertension (mean age 59 years)	60 (0/60)	FDSP, 25g/d or 50g/d	Isocaloric control powder	↑ IGF-1 ↑ osteocalcin (NS) No Δ in bone-specific alkaline phosphatase or tartrate-resistant acid phosphatase-5b
Immune Health							
Zunino 2013 (321)	RCT, crossover, 2-arm	3 weeks; no washout	Obese adults (mean age 32 years)	20 (7/13)	FDSP, 50 g/d	Isocaloric control powder	No Δ in production of T-cell cytokines ↑ proliferation of activated $CD8^+$ T-lymphocytes and production of TNF-α
Obesity							
Basu 2018 (277)	RCT, crossover, 2-arm	12 weeks; 2-week washout	Obese adults with knee OA (mean age 57 years)	17 (4/13)	FDSP, 50g/d	Isocaloric control powder	No Δ in obesity-related hormones
Cancer							
Chung 2002 (322)	RCT, parallel, 4-arm	A single intake	Healthy adults (mean age 24 years)	40 (24/13)	Whole strawberries, 300 g	Control group refrained from consumption of high NDMA, nitrate, amine, sulfur compound, ascorbic acid and phenolic compound-containing foods	↓ mean N-nitrosodimethylamine (NDMA) excretion after intake of whole strawberries, garlic juice, or kale juice compared to the control group
Chen 2012 (323)	RCT, parallel, 2- arm	6 months	Adults diagnosed with mild/moderate esophageal dysplasia (mean age 57 years)	75 (37/38)	FDSP, 60 g/d or 30 g/d	N/A	60g/d FDSP ↓precancerous growth, Inhibit protein expression of iNOS, COX-2, pNFkB-p65, and pS6 Inhibit cell proliferation

Includes human clinical trials that focus solely on strawberry as a functional food with outcomes of known physiological relevance. Excludes interventions using strawberry-containing mixtures and strawberry extracts. Also excludes interventions where strawberry intake was in combination with other potentially confounding factors (ie. diet or lifestyle modifications including physical activity and dietary counseling).

AD: Alzheimer's dementia; AIx: augmentation index; ApoA: apolipoprotein A; ApoB: apolipoprotein B; BA: bile acids; BMI: body mass index; BP: blood pressure; CHD: coronary heart disease; C_{max} : maximum concentrations; COX-2: cyclooxygenase-2; CVD: cardiovascular disease; DiaBP: diastolic blood pressure; FDSP: freeze-dried strawberry powder; FMD: flow-mediated dilation; fMLP: N-Formylmethionine-leucyl-phenylalanine; FRF: flavonoid-rich fruits; g/d: grams per day; GSH: glutathione; HCFM: high-carbohydrate, moderate-fat meal; HFM: high fat meal; HNE: hydroxynonenal; HPFS: Health Professional Follow-up Study; HS: high-sugar; Hs-CRP: high sensitivity C-reactive protein; hs-TNF-α: high-sensitivity tumor necrosis factor-α; IGF-1: insulin-like growth factor-1; IL-6: interleukin-6; iNOS: inducible nitric oxide synthase; IWHS: Iowa Women's Health Study; Knee OA: Knee osteoarthritis; LBCL: luminol enhanced whole blood chemiluminescence; LDL-C: low-density lipoprotein cholesterol; LS: low-sugar; LSA: low-sugar including antioxidant; MAP: Rush Memory and Aging Project; MDA: malondialdehyde; MetS: metabolic syndrome; mo: month; NDMA: N-nitrosodimethylamine; NHS: Nurses' Health Study; NO: nitric oxide; NR: non-randomized; NS: non-significant; OFTT: oral fat tolerance test; OTUs: operational taxonomic units; PAI-1: Plasminogen activator inhibitor-1; PAT: peripheral arterial tonometry; pNFκB-p65: phospho-NFκB-p65, PON-1: paraoxonase-1; PP: postprandial; pS6: phospho-S6; PWV: pulse wave velocity; RBC: red blood cell; SCFA: short-chain fatty acids; SFFQ: semiquantitative food frequency questionnaire; SOD: superoxide dismutase; sTNF-R2: soluble tumor necrosis factor receptor-2; sVCAM-1: soluble vascular adhesion molecule-1; SysBP: systolic blood pressure; TAC: total antioxidant capacity; TG: triglyceride; T_{max} : time when C_{max} was achieved; TNF-α: tumor necrosis factor-α; Uro-A: Urolithin A; VLDL-C: very low-density lipoprotein cholesterol; wk: week; 8-OHdG: 8-hydroxy-2-deoxyguanosine

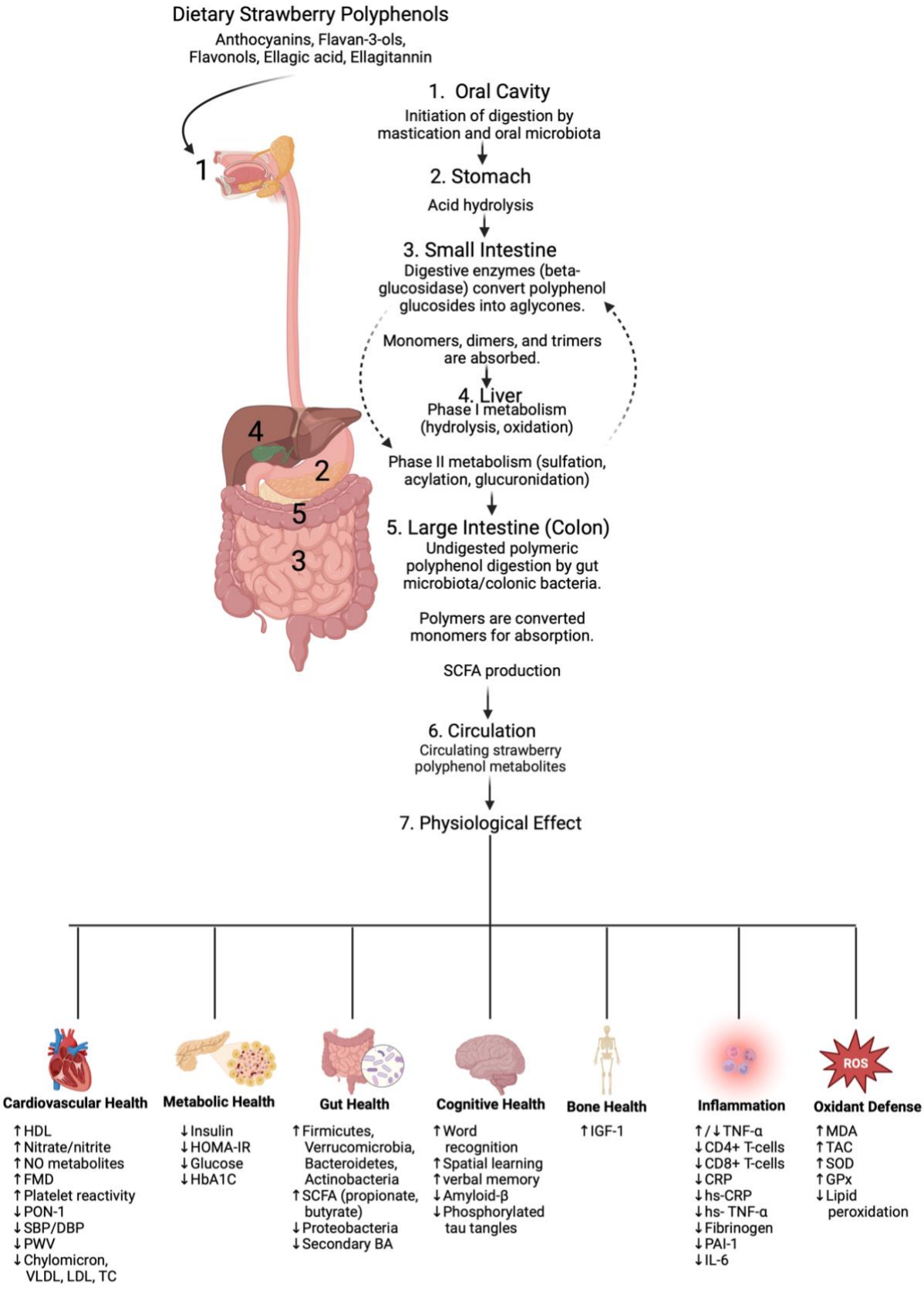

Figure 1. Metabolism and Physiological Effects of Strawberry Polyphenols

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CHAPTER IV

The Effects of Daily Walnut Intake on Vascular Function and Lipid Metabolism in Postmenopausal Women

Abstract

Background: Evidence suggests that walnut intake is associated with improved cardiovascular health. Walnuts provide essential polyunsaturated fatty acids (PUFA), linoleic and alpha linolenic acids, that may mediate the observed benefits. Metabolites of these fatty acids, called oxylipins, are known for their biological effects including regulation of inflammation, vasodilation, and platelet reactivity.

Methods: Twenty overweight and obese, postmenopausal women, aged 45 to 65 years, were enrolled in an 18-week dietary intervention trial. Participants followed their habitual diet for a 6-week baseline period, followed by a 12-week intervention period in which they supplemented their habitual diet with 40g/day of walnuts. The primary endpoint was microvascular function as measured by reactive hyperemia index (RHI). Secondary endpoints included plasma lipids and plasma and lipoprotein-esterified oxylipins assessed by liquid chromatography–mass spectrometry (LC-MS). Repeated Measures Analysis of Variance (RM-ANOVA) was used to assess within-subject change from habitual diet to six and 12 weeks of daily walnut intake.

Results: Daily consumption of 40g/d walnuts for 12 weeks did not yield significant changes in the RHI ($p=0.30$). Total cholesterol and low-density lipoprotein (LDL)-cholesterol did not significantly change ($p=0.23$ and $p=0.06$, respectively), over the course of the trial, although the overall trend was towards reduced levels. Participants with elevated total cholesterol above 200 mg/dL at baseline experienced more pronounced reductions than those in the optimal range, which did reach statistical significance ($p=0.02$ for both). No significant changes were found for blood pressure, high-density lipoprotein (HDL)-cholesterol, triglycerides, augmentation index (AIx), or platelet reactivity.

Conclusion: Daily addition of 40g walnuts to the habitual diet did not improve vascular function in overweight and obese, postmenopausal women. However, lipid profile was improved, particularly in those at higher cardiovascular risk at baseline.

Introduction

A substantial body of research has demonstrated that the acute and short-term intake of walnuts improves a number of cardiometabolic outcomes including total- and LDL-cholesterol, blood pressure and vascular function, with the latter predominately measured using flow-mediated dilation (FMD), a measure of endothelial-dependent macrovascular function that is predominately nitric oxide (NO) dependent (1-8). Notably, measures of microvascular function, such as the reactive hyperemia index (RHI) are less dependent on NO (9). We have previously reported improved RHI with the intake of 40g/day of walnuts for four weeks (24) by hypercholesterolemic, postmenopausal women, with the improvement in RHI associated with the change in sum of plasma epoxides and was most strongly associated with 14(15)-epoxyeicosatrienoic acid (EET), known for its vasodilatory properties (24). Epoxides are a subgroup of oxylipins derived from omega-6 and -3 fatty acids, more specifically, cytochrome P450 (CYP450)-derived metabolites of arachidonic acid (AA) (10). The purpose of the current study is to extend these previous findings and evaluate the effect of six and 12 weeks of daily walnut intake on microvascular function. Additional secondary outcomes of cardiometabolic health such as plasma lipids, and platelet function were also measured.

Methods

Participants

Twenty overweight and obese, postmenopausal women, aged 45 to 65 years, were recruited from the greater Sacramento, CA area through public and online advertisements. Criteria for inclusion were an age between 45 – 65 years, a body mass index (BMI) between 25.0 – 35.0 kg/m2, and postmenopausal status (defined as absence of menses for at least two years). Exclusion criteria included dislike or allergy to walnuts, use of prescription medications and supplements (except for a standard multivitamin/mineral formula), self-reported daily use of non-steroidal anti-inflammatory drugs or other over the counter medications that can affect the study outcomes, habitual diet high in foods known to influence vascular health (defined as ≥ three cups/day fruit, coffee, tea, or dark chocolate; ≥ four cups/day vegetables), regular consumption of nuts (defined as ≥ three servings/week), routine high-intensity exercise, cancer or chronic disease (diabetes, renal, liver, heart, or peripheral artery disease), malabsorption, drug or alcohol use, abnormal complete metabolic panel (CMP) or complete blood count (CBC) deemed clinically relevant by the study physician, and a baseline reactive hyperemia index (RHI) ≥ 2.7. This trial was registered (NCT03900403) at ClinicalTrials.gov and protocol approved by the Institutional Review Board of the University of California, Davis.

Study Design

Twenty participants completed the 18-week dietary intervention trial where 40 g/day of walnuts were added to the habitual diet. The trial consisted of four study visits scheduled six weeks apart. This study design allowed for data collection from an individual's habitual diet for six weeks followed by 12 weeks of daily walnut intake. Each participant's 12-week supplemental walnut period was compared to their initial six weeks of habitual diet.

Potential participants were screened via telephone interview followed by an in-person clinical screening visit at the Ragle Human Nutrition Research Center (RHNRC) on the University of California Davis (UCD) campus. This clinical screening included a fasting blood collection, blood pressure, height, and weight were measured. Participants also completed a screening questionnaire that provided additional detail on their diet and lifestyle, to include medication use, and past medical history. Fasted blood samples were collected for a CMP and CBC for analysis at the Department of Pathology at the UCD Medical Center (UCDMC) in Sacramento, CA. Preliminarily eligible participants were asked to come to the RHNRC for a pre-visit, where baseline RHI was evaluated.

Those eligible for enrollment were asked to return for four study visits scheduled before and after six weeks on their habitual diet, and six and 12 weeks after incorporating 40 g/day of walnuts into their diet (**Figure 1**). One week prior to and during the intervention period, participants were instructed to refrain from consuming any type of nuts, except for the walnuts provided and the use of products that had the potential to influence study procedures (e.g., aspirin, non-steroidal anti-inflammatory drugs (NSAIDS), antihistamines, and mouthwash). A 40-gram quantity of walnuts was chosen based on the recommended intake level by the 2004 Food and Drug Administration qualified health claim for walnuts and cardiovascular health (11) and the quantity previously shown to impart vascular benefit in a preceding exploratory study (12). Participants were asked to incorporate a 40-gram serving of walnuts per day into their diet as a snack or part of a meal and instructed not to cook or heat the walnuts. They were also instructed to consume their walnuts in the morning the day prior to their study visit in order to avoid a potential acute response.

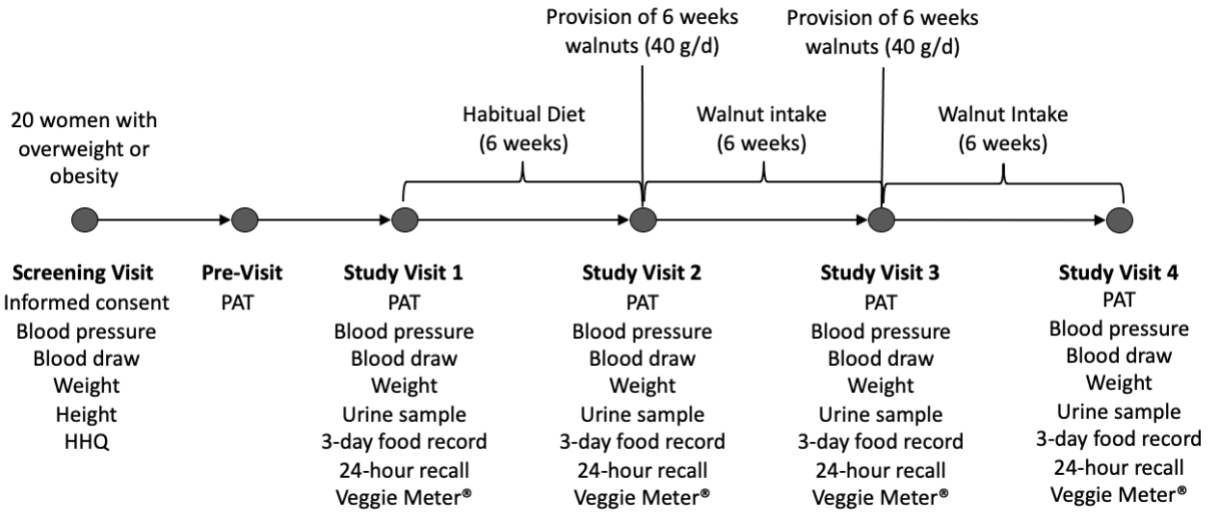

Figure 1: Study Design
HHQ: health and habits questionnaire; PAT: peripheral arterial tonometry

For each study visit, participants were arrived at the RHNRC following an overnight (12 hour) fast. Participants were asked to keep a 3-day food record (two weekdays and one weekend day) in the week prior to their study visit. On the morning of the study, they also collected a first morning urine, and were instructed to bring these items (food records and urine sample) with them to the study visit. Once at the facility, weight and waist circumference was recorded, and three seated blood pressure measurements were collected five minutes apart after a 15-minute rest using an automated oscillometric blood pressure unit (Masimo Root; Masimo Corporation, Irvine, CA). After a 10-minute rest period in the supine position, RHI was assessed by peripheral arterial tonometry (PAT; EndoPAT 2000; Itamar Medical, Caesaria, Israel). In addition to their 3-day food record, the prior day's diet was assessed using the Automated Self-Administered 24-Hour (ASA24) Dietary Assessment Tool. Moreover, reflection spectroscopy (Veggie Meter®; Longevity Link Corporation, Salt Lake City, UT) was measured, which is considered a proxy measure of fruit and vegetable intake via skin carotenoids (13, 14). Finally, blood was collected

for metabolomics, nitrate/nitrite and platelet reactivity assays and for comprehensive metabolic and lipid panels and complete blood counts, which were done by the University of California Davis Medical Center (UCDMC) Pathology Lab.

Compliance was monitored by a self-administered log. The primary endpoint was microvascular function as measured by the reactive hyperemia index (RHI). Secondary outcome measures included blood pressure, plasma lipids and plasma and lipoprotein-esterified oxylipins assessed by liquid chromatography–mass spectrometry (LC-MS). Plasma and urinary urolithins, quantified via LC-MS, were also assessed.

Microvascular Function and Augmentation Index (AIx)

Microvascular function, assessed by the RHI, was measured using PAT. All measurements were conducted by a trained researcher and occlusion validity was set at $\geq$70%. Participants were asked to relax quietly in a supine position for a minimum of ten minutes prior to the procedure. Room temperature was kept at a constant, comfortable temperature of about 72-74 degrees Fahrenheit. Speaking and movement in the room was minimized throughout the entirety of the protocol. A single use, plethysmographic probe was placed on the pointer finger of each hand, and a blood pressure cuff was placed on the proximal forearm of the left arm. A 6-minute baseline period of peripheral blood flow was recorded, after which the forearm cuff was inflated to approximately 60 mmHg above resting systolic blood pressure, or just enough to reduce blood flow. This period, called the occlusion period, had a duration of five minutes, after which time the forearm cuff was deflated, pressure released, and the resulting reactive hyperemic response recorded for the final 5-minute period. Reactive hyperemia index, Framingham reactive

hyperemia index (fRHI), and augmentation index (AIx) were calculated by the EndoPAT software. Reactive hyperemia index is defined as the ratio of hyperemic pressure in the occluded arm to baseline pressure in the non-occluded arm (15). The fRHI is an alternative reactive hyperemia score proposed by researchers from the Framingham Heart Study that uses the natural logarithmic transformation of the RHI ratio, does not include a baseline correction factor, and only utilizes post-occlusion readings from 90 to 120 seconds (16). Augmentation index is a measure of arterial stiffness defined as the difference between the early and late systolic peak pressures as a percentage of pulse pressure (17).

Platelet Aggregation

Optical platelet aggregometry was measured in citrated blood using a Chrono-Log 700 whole blood/optical 2-channel lumi-aggregometer (Havertown, PA). Twenty minutes after blood was collected, platelet-rich plasma (PRP) was separated from whole blood by centrifugation at 200 × g for ten minutes at 25°C and transferred into a separate tube. Platelet-poor plasma (PPP) was obtained via subsequent centrifugation of whole blood at 1500 × g for ten minutes at 25°C and also separated. Prior to testing, PRP was incubated at 37°C for a minimum of three minutes. Platelet aggregation was stimulated with agonists using one and three µg/mL collagen and ten µg/mL adenosine diphosphate (ADP) in duplicate at a stirring speed of 1200 rpm.

Plasma and Lipoprotein-Esterified Oxylipins

Lipids were extracted from plasma and isolated lipoprotein fractions in the presence of isotopically labeled surrogates, using 8:10:11 (v/v/v) isopropanol/cyclohexane/0.1M ammonium acetate. Organic phase sub-fractions were either methylated for fatty acid methyl ester analysis

on a 6890/5973N GC-MS (Agilent Technologies), or trans-methylated and hydrolyzed to yield oxylipin free acids, which are trapped and re-concentrated by hydrophobic/lipophilic solid phase extraction and quantified on a API-6500 QTRAP LC-MS/MS system using modifications of previously reported procedures (18).

Lipoprotein Fractionation

Lipoproteins in 35 µL of plasma were fractionated by size exclusion chromatography using modifications of reported procedures (19). Specifically, resolution was increased by using reconfigured modern materials including a 10/300cm Superose Increase column in-line with a 10/100cm Superdex 200 Increase column and carefully modified buffering systems. Very-low density lipoprotein (VLDL), LDL and HDL fractions were collected. It has been documented that a single freeze thaw event does not significantly disrupt lipoprotein structure or composition (18).

Plasma Ellagitannin (ET) and ET Metabolites

Conjugated and unconjugated urolithins and other ET-derived metabolites were quantified in plasma as their aglycone on an API-4000 QTRAP UPLC-MS/MS system on a 50 x 2.1 mm, 2.5 µm HSS T3 using adaptations of published methods (20). Metabolites were extracted in the presence of appropriately labeled surrogates by protein precipitation with acidified acetonitrile. Total urolithin A and B were quantified after glucuronidase and sulfatase treatment in the presence of appropriately labeled surrogates, then quenched with cold acetonitrile. Deconjugation efficiency was confirmed by evaluating the removal of conjugated species and

quantifying the clearance of introduced nitrophenyl-glucuronide and nitrophenyl-sulfate as previously described (21).

Statistical Analysis

Based on a mean between-group difference in RHI response of 0.4 and a standard deviation of 0.375 from our pilot study, (12) a sample size of 18 was required to achieve an $\alpha = 0.05$ at 80% power. Twenty participants were enrolled in order to account for potential attrition, which has typically been less than 10% in our clinical studies among postmenopausal women.

Data was checked for normality and transformed as needed (JMP Pro 17; SAS Institute Inc., Cary, NC). Each participant's 12-week supplemental walnut period was compared to their initial six weeks of habitual diet. Repeated Measures Analysis of Variance (RM-ANOVA) was used to assess the potential six and 12 week differences between habitual diet and walnut intake in vascular and platelet function, and oxylipins, and assess changes over time in response to each treatment. Tukey's test was used for post-hoc comparisons, with a P value <0.05 as the level of significance. Multivariate analysis was conducted to assess the relationship between the changes in physiologic responses and metabolic mediators.

Metabolomics data was assessed by partial least squares-discriminant analysis (PLS-DA), with group classification based on change in vascular or platelet function. These multivariate analyses were performed using JMP Pro 17 (SAS Institute Inc., Cary NC). Prior to PLS-DA, data was curated such that analytes with <70% completeness of data were removed from consideration. Curated data was screened for outliers and missing data was imputed by a two-component

probabilistic principle components analysis with univariate scaling (22). Following transformation to a normal distribution, tested using the Shapiro-Wilk W-test, the PLS-DA was conducted using the orthogonal scores algorithm with univariate scaling and leave-one-out cross-validation. Variables were clustered by Spearman correlation coefficients using the Minkowski distance and Ward agglomeration. Multiple comparison adjustments followed the False Discovery Rate correction recommendations of Benjamini and Hochberg (23).

Results

Study enrollment occurred from October 2019 to June 2023. Of 22 enrolled, 20 postmenopausal women completed the study and were included in the analysis (**Figure 2**).

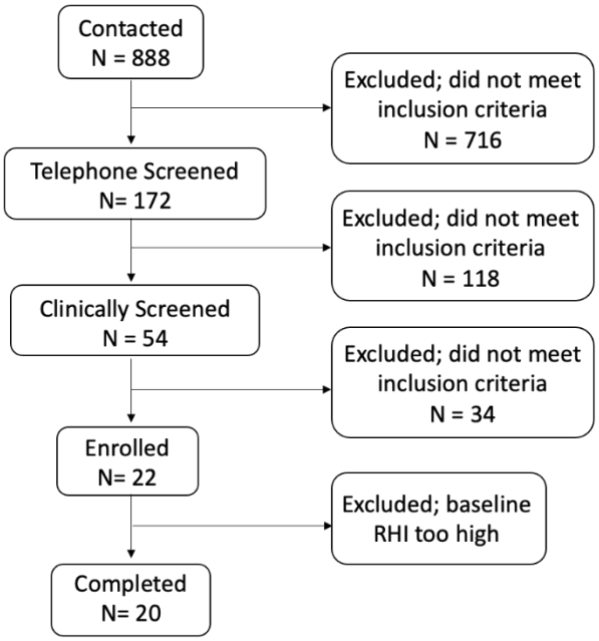

Figure 2: Consort Diagram

Their mean age at baseline was 57 ± 3.9 years of age. Participants were overweight or obese (mean BMI 30.4 ± 3.1) with mild hypercholesterolemia (mean total cholesterol 216.6 ± 37.5 mg/dL; mean LDL-cholesterol 135.4 ± 34.8 mg/dL). Generally, participants had normal blood pressure and normal vascular function at baseline (**Table 1**). The majority (70%) of participants were Caucasian, with 20% identifying as Mexican American/Chicano and the remaining 10% identifying as a mixture of races.

Table 1: Participant Characteristics at Baseline

Variable	Mean ± SD
Demographic Characteristics	
Age, years	57 ± 3.9
BMI, kg/m^2	30.4 ± 3.1
Lipid Profile	
Total Cholesterol, mg/dL	216.6 ± 37.5
HDL-C, mg/dL	61.3 ± 14.9
LDL-C, mg/dL	135.4 ± 34.8
Triglycerides, mg/dL	99.5 ± 40.0
Blood Pressure	
Systolic, mmHg	123.8 ± 13.1
Diastolic, mmHg	78.5 ± 7.5
Vascular Parameters	
RHI	2.2 ± 0.6
fRHI	0.8 ± 0.3
AIx@75, %	14.6 ± 12.7

Values are mean ± SD. BMI: body mass index; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; RHI: reactive hyperemia index; AIx@75: augmentation index corrected for heart rate of 75 beats per minute

Based on self-reported 3-day food records and compliance logs, compliance with the dietary intervention was 83%. Participants' habitual diet prior to supplementation with walnuts as well as their walnut-supplemented intake is detailed in **Table 2**. Total energy intake was similar between diets (habitual diet 1974.6 ± 467.2 total calories; walnut-supplemented diet 1971.3 ±

445.0 total calories; p=0.980; **Supplemental Table 1**), despite the addition of energy-dense walnuts to the diet, suggesting potential nutrient displacement. Intakes of macronutrients were similar before and after walnut supplementation, with an overall trend for increased fat intake with the addition of walnuts to the diet. This included a significant increase in the intake of polyunsaturated fats (PUFA) with walnut intake beyond the habitual diet for both omega-3 and -6 fatty acids ($p < 0.001$ for all).

Table 2: Self-Reported Dietary Intake

	Habitual Diet		**Walnut-Supplemented Diet**		
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	
	Mean ± SD				**P-Value**
Total Calories (kcal)	1834.0 ± 626.6	1542.7 ± 554.6	2003.6 ± 601.0	1962.7 ± 810.1	0.05
Protein (g)	75.5 ± 26.7	62.9 ± 26.3	84.0 ± 27.9	69.8 ± 31.9	0.10
Carbohydrate (g)	165.5 ± 60.8	153.5 ± 64.0	170.3 ± 67.8	188.1 ± 78.3	0.35
Fiber (g)	15.0 ± 6.6	15.7 ± 7.3	16.4 ± 8.1	20.4 ± 9.4	0.12
Fat (g)	83.9 ± 38.6	75.6 ± 31.5[a]	105.2 ± 35.1[b]	91.9 ± 39.7	0.03
Saturated Fat (g)	27.9 ± 11.0	23.7 ± 11.2	29.1 ± 13.2	26.3 ± 13.1	0.44
Monounsaturated Fat (g)	29.9 ± 17.6	27.6 ± 12.0	31.9 ± 11.9	26.2 ± 11.6	0.46
Polyunsaturated Fat (g)	19.0 ± 12.6[a]	18.0 ± 8.7[a]	36.4 ± 16.5[b]	27.2 ± 14.9[b]	<.0001*
Omega-3 Fatty Acids (g)	2.0 ± 1.6[a]	1.6 ± 1.1[a]	4.5 ± 2.3[b]	3.5 ± 1.9[b]	<.0001*
Omega-6 Fatty Acids (g)	16.6 ± 10.8[a]	16.0 ± 7.5[a]	30.7 ± 13.8[b]	22.8 ± 12.3[ab]	<.0001*

Values are the mean ± SD obtained from 3-day food records completed the week prior to each study visit. Records from visits 1 and 2 were used to assess habitual intake and those from visits 3 and 4 were used to assess walnut-supplemented intake. P-values <0.05 were considered statistically significant. Asterisk (*) denotes statistical significance p<0.0001; Different letters showcase statistically significant differences between means; SD: standard deviation; kcal: kilocalories; g: grams

The BMI significantly increased over the course of the trial (mean BMI Visit One: 30.4 kg/m^2 vs. mean BMI Visit Four: 30.9 kg/m^2; p<0.0001; **Table 1**). However, note that BMI had begun to increase between visits one and two, prior to the addition of walnuts to the diet (mean BMI Visit One: 30.4 kg/m^2 vs. mean BMI Visit Two: 30.5 kg/m^2).

Microvascular Function

At baseline, microvascular function was at a level associated with low cardiovascular risk (24). Daily consumption of 40g/d walnuts for 12 weeks did not yield significant changes in the primary outcome of RHI (p=0.30) or the Framingham Reactive Hyperemia Index (p=0.31; **Figure 3**), although RHI was significantly lower during study visit one for those starting the study in the Fall (p=0.0045). No significant changes were found for systolic or diastolic blood pressure or augmentation index (AIx) **(Table 3)**.

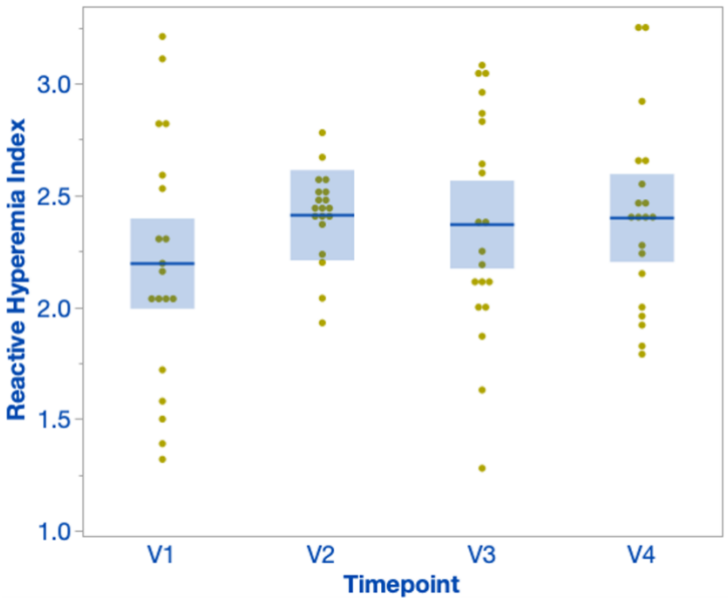

Figure 3. RHI with daily consumption of 40g/d walnuts for 12 weeks

Platelet Reactivity

An increasing trend in platelet aggregation was observed (Mean AUC Visit One: 484.4 ± 66.7 vs. Visit 4: 525.3 ± 48.6; p=0.5; Mean MaxA Visit One 82.1 ± 5.6 vs. Visit Four: 85.7 ± 5.0; p=0.11), when three µg/mL collagen was used as the clotting agonist. This change was not observed for the other agonists, including a lower quantity of collagen (one µg/mL collagen) and ADP **(Table 3)**.

Table 3: Pre- and Post- Intervention Differences with 6 and 12 Weeks of Daily Walnut Intake

	Habitual Diet		**Walnut-Supplemented Diet**		
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	
	Mean ± SD				**P-Value**
Anthropometric Measures					
BMI, kg/m^2	30.4 ± 3.1[a]	30.5 ± 3.3[ab]	30.7 ± 3.2A[bc]	30.9 ± 3.2[c]	<0.0001*
Lipid Profile					
Total Cholesterol, mg/dL	216.6 ± 37.5	214.6 ± 36.2	208.1 ± 34.3	208.8 ± 29.1	0.23
HDL-C, mg/dL	61.3 ± 14.9	63.5 ± 17.5	61.8 ± 17.1	62.4 ± 18.7	0.68
LDL-C, mg/dL	135.4 ± 34.8	131.7 ± 33.7	127.2 ± 31.8	126.8 ± 29.4	0.06
Triglycerides, mg/dL	99.5 ± 40.0	97.1 ± 38.3	95.9 ± 39.4	97.8 ± 40.2	0.87
Blood Pressure					
Systolic, mmHg	123.8 ± 13.1	122.3 ± 10.7	121.2 ± 8.9	123.9 ± 10.9	0.47
Diastolic, mmHg	78.5 ± 7.5	77.4 ± 8.0	76.8 ± 6.5	77.1 ± 6.6	0.65
Vascular Parameters					
RHI	2.2 ± 0.6	2.4 ± 0.2	2.4 ± 0.5	2.4 ± 0.4	0.30
fRHI	0.8 ± 0.3	0.9 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.31
AIx@75, %	14.6 ± 12.7	12.0 ± 17.0	12.3 ± 14.2	11.8 ± 13.8	0.83
Platelet Aggregation					
Collagen (3 µg/mL), AUC	484.4 ± 66.7	500.9 ± 42.8	490.5 ± 64.5	525.3 ± 48.6	0.05

Collagen (1 µg/mL), AUC	429.3 ± 84.8	444.0 ± 74.7	397.7 ± 140.8	446.0 ± 81.6	0.44
ADP (10 µg/mL), AUC	536.2 ± 74.5	533.7 ± 62.8	531.3 ± 67.4	551.6 ± 58.6	0.63
					0.11
Collagen (3 µg/mL), MaxA	82.1 ± 5.6	82.8 ± 5.6	83.3 ± 6.9	85.7 ± 5.0	0.48
Collagen (1 µg/mL), MaxA	73.6 ± 11.5	76.6 ± 10.1	69 ± 21.0	76.5 ± 11.2	0.54
ADP (10 µg/mL), MaxA	80.1 ± 10.3	81.0 ± 9.4	80.2 ± 9.9	83.5 ± 8.3	0.71
Skin Carotenoids	206.3 ± 106.9	187.0 ± 92.6	216.0 ± 139.0	203.2 ± 108.7	
Carotenoid Score					

Values are the mean ± SD; P-values <0.05 were considered statistically significant. Asterisk (*) denotes statistical significance p<0.0001; Different letters showcase statistically significant differences between means; SD: standard deviation; RHI: reactive hyperemia index; fRHI: Framingham reactive hyperemia index; AIx@75: augmentation index corrected for heart rate of 75 beats per minute; ADP: Adenosine diphosphate; AUC: area under the curve; MaxA: maximum aggregation

Lipid Profile

Over the course of the trial, total cholesterol and LDL-cholesterol did not change significantly (p=0.23 and p=0.06, respectively), although the overall trend was towards reduced levels. For those with hypercholesterolemia (TC>200 mg/dL) at baseline, 12 weeks of walnut intake significantly reduced total- and LDL-cholesterol by 8% and 11%, respectively (p=0.02 for both; **Figure 4**). No significant changes were found for HDL-cholesterol or triglycerides.

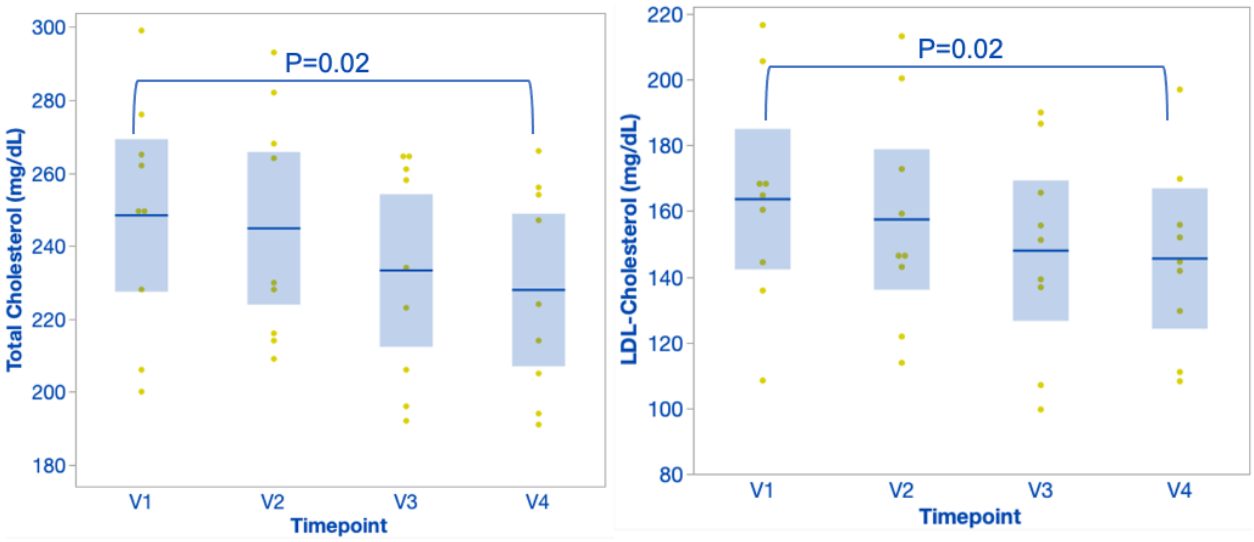

Figure 4. Changes in total- and LDL-cholesterol in participants who were hyperlipidemic (TC>200 mg/DL) at baseline

Discussion

The consumption of nuts, specifically walnuts, is associated with improved cardiovascular outcomes (25, 26). Reductions in total cholesterol, LDL-cholesterol, and blood pressure, along with improvements in vascular function have been reported (1-7). A pilot study from our group found that short-term (six week) walnut intake (40g/d) improved microvascular function in postmenopausal women (12). The results of our current study were not consistent with these previous findings. This is also inconsistent with previous reports where vascular function was improved following intake of walnuts (5, 27). A study of 40 overweight adults with visceral adiposity and symptoms of metabolic syndrome found that the addition of 56 g/d of walnuts for eight weeks yielded significant improvements in endothelial function as assessed by FMD (5). Another study evaluating the acute effects of walnut intake in overweight adults observed postprandial improvements in FMD in addition to decreased vascular adhesion molecules with the addition of 60 g of walnuts to a high-fat meal (27).

Although they are both measures of vascular function, FMD and PAT have distinct differences that may, at least in part, explain the discrepancy in our results from studies described above. Flow-mediated dilation is predominantly mediated by NO, whereas dilation induced during PAT can be attributed to a broader range of mechanisms, including endothelial derived hyperpolarizing factor (EDHF) thought to be present in walnuts, that are less dependent on NO. Also, FMD is a measure of vascular function primarily of the macrovasculature while PAT is representative of the microvasculature or smaller, peripheral vessels (28).

A reduced vascular function for those starting the study during the fall is particularly noteworthy given that exposure to poor air quality from local wildfires is associated with negative impacts on cardiovascular health (29). Another plausible possibility is that participants had relatively healthy baseline values **(Tables 1 and 3)**, leaving little room for improvement.

A 40-gram serving of walnuts provides approximately 15 grams of linoleic acid (LA), an omega-6 polyunsaturated fatty acid (PUFA), and four grams of alpha-linolenic acid (ALA), an omega-3 PUFA (30). Dietary ALA is converted to eicosapentaenoic acid (EPA)and docosahexaenoic acid while dietary LA is converted to arachidonic acid (AA). Polyunsaturated fatty acids can undergo bioactivation by cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes and produce a variety of lipid products collectively referred to as oxylipins. A sub-class of oxylipins, eicosanoids, which includes the EPA- and AA-derived metabolites, exert an array of biological effects. Eicosanoids, particularly EET, have been shown to exert anti-inflammatory and vasodilatory effects (31-33), while prostacyclin (PGI2) has both vasodilatory and anti-aggregatory properties through activation of the cyclic adenosine monophosphate (cAMP)

pathway (34). Epoxyeicosatrienoic acid has vasodilatory and anti-aggregatory properties through its' capacity as an EDHF (35, 36). Through hyperpolarization, EET stimulates vasodilation of vascular smooth muscle cells (VSMC). In addition to VSMC, EET hyperpolarizes platelets, which inhibits their adhesion to endothelial cells (37).

In agreement with numerous studies on walnut supplementation, the current study reported improvements in lipid profiles, particularly in those with elevated baseline cholesterol values (38-44). In 84 adults with metabolic syndrome, supplementing with 45 g/d of walnuts for 16 weeks increased HDL-C levels such that 51.2% of participants reversing their metabolic syndrome status (38). Further, replacement of saturated fatty acids with unsaturated fats from walnuts (57-99 g/d) for six weeks yielded significant reductions in total cholesterol, LDL-C, and non-HDL-C among 34 individuals who were overweight and at-risk for cardiovascular disease (43, 44). Improvements in the lipid profile attributed to walnut intake are pronounced, but not limited to those with dyslipidemia at baseline. A study by Bamberger et al. evaluating the effect of an isocaloric replacement of macronutrients with walnuts on plasma lipids in healthy adults (n=194) found that replacement of carbohydrate or fat with 43 g/d walnuts resulted in significant reductions in total cholesterol, LDL-cholesterol, non-HDL-cholesterol, triglycerides, and apolipoprotein B (42). Also in healthy men and women (n=18), three weeks of 42 g/d of walnut intake significantly reduced LDL-C concentration by 7% (41). In another study of participants (n=50) with a normal lipid profile, adding 40 g/d of walnuts to the diet for a four week period resulted in significant decreases in LDL-C and triglycerides compared to controls (40). Finally, a two-year intervention with 628 heathy, elderly adults reported decreased total, LDL-, and

intermediate-density lipoprotein- cholesterol with the addition of 30-60 g/d of walnuts (~15% energy) to the habitual diet (39).

The improvements in lipid response reported in the present study may be attributed, in part, to the ellagic acid (EA) and ellagitannin (ET) content of walnuts. A 40 gram serving of walnuts contains approximately 11 grams of ellagic acid (45). Ellagitannins are hydrolyzed in the gastrointestinal tract to EA and further metabolized by gut microbes into urolithins (46, 47). The ability, or lack thereof, of an individual's gut microbiota to produce certain urolithins is known as their urolithin metabotypes (UM), or metabolic phenotype: UM-A is distinctly urolithin-A-producing, UM-B is urolithin B- and isourolithin A-producing in addition to urolithin A, and UM-O is non-urolithin-producing (48). Urolithin metabotype-A is associated with levels of apolipoprotein A and HDL-cholesterol, whereas UM-B is associated with apolipoprotein B, total cholesterol, LDL, VLDL, and oxidized LDL-cholesterol (49). Urolithins, particularly urolithin A, may confer health benefits (50-52). In vitro and animal studies support potential dyslipidemic properties (46, 53, 54). Urolithins have been shown to activate the adenosine monophosphate-activated protein kinase (AMPK) pathway, which yields positive effects on lipid metabolism (51, 54-58).

There are several analyses pending as part of the present study. Future urolithin analysis will allow for differentiation of participants by metabotype, which will help to explore the potential contribution of urolithins to changes in lipid response. We will also assess whether urolithin metabotype is influenced by increasing intake of ET-rich foods, such as walnut, as preclinical and clinical studies have suggested (59, 60). We previously reported a strong association between PUFA-derived epoxides, specifically 14(15)-EET, and microvascular function (12).

Metabolomics data will demonstrate the presence, or absence, of specific oxylipins that are known to be vasoactive, such as EET and PGI2, and will determine whether or not these metabolites are associated with vascular response, or lack thereof, in the current study. Metabolomics will also help to identify phenotypic differences associated with habitual diet that may influence individual metabolic response. Taken together, the aforementioned analyses will help elucidate the mechanisms driving physiological responses seen with walnut supplementation, both in this study and beyond.

The current study recruited postmenopausal women with overweight and obesity. Indeed, this population is at increased cardiovascular risk related to their age, weight, and menopausal status. Future studies intending to measure vascular response related to vasoactive epoxides, specifically, should consider recruiting those at a lower baseline level to allow for room for improvement. Finally, seeing as select results were trending towards significance, a larger sample may be of benefit to fully extrapolate these differences.

Conclusion

In conclusion, the addition of 40g walnuts per day to the habitual diet for 12 weeks did not significantly improve RHI in postmenopausal women with overweight or obesity. However, the study confirms previous findings that short-term walnut intake improves the lipid profile. Significant reductions in total- and LDL-cholesterol in participants with hyperlipidemia at baseline suggest improvements in those at higher cardiovascular risk.

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Supplemental Material

Supplemental Table 1: Habitual Diet vs. Walnut-Supplemented Diet

	Habitual Diet	**Walnut-Supplemented Diet**	
	Mean ± SD	**Mean ± SD**	**P-Value**

Total Calories (kcal)	1974.6 ± 467.2	1971.3 ± 445.0	0.98
Calories from Fat (kcal)	743.5 ± 202.3	863.0 ± 192.3	0.10
Protein (g)	74.6 ± 19.0	70.8 ± 19.2	0.57
Carbohydrate (g)	227.4 ± 70.3	218.9 ± 100.1	0.78
Fiber (g)	23.2 ± 15.2	18.3 ± 9.1	0.28
Fat (g)	82.7 ± 22.5	96.0 ± 21.4	0.10
Saturated Fat (g)	27.6 ± 9.1	26.6 ± 8.8	0.75
Polyunsaturated Fat (g)	8.4 ± 2.9	24.7 ± 5.1	<0.0001*
Omega-3 Fatty Acids (g)	0.7 ± 0.3	1.2 ± 1.2	0.07
Omega-6 Fatty Acids (g)	5.9 ± 2.0	8.1 ± 5.8	0.16

Values are the mean ± SD obtained from 3-day food records (n=16) completed the week prior to each study visit. Records from visits one and two were used to assess habitual intake and those from visits three and four were used to assess walnut-supplemented intake. P-values <0.05 were considered statistically significant. Asterisk (*) denotes statistical significance p<0.0001. SD: standard deviation; kcal: kilocalories; g: grams

Supplementary Table 2: Self-Reported Dietary Intake (24-hour Recall)

	Habitual Diet		**Walnut-Supplemented Diet**		
	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	
	Mean ± SD				**P-Value**
Total Calories (kcal)	2104.7 ± 742.9	1844.5 ± 467.4	2111.4 ± 531.2	1831.2 ± 488.4	0.15
Protein (g)	77.1 ± 29.9	72.2 ± 18.0	72.0 ± 22.3	69.6 ± 20.8	0.68
Carbohydrate (g)	234.1 ± 99.0	220.6 ± 70.9	229.3 ± 82.7	208.5 ± 166.6	0.86
Fiber (g)	20.0 ± 12.8	26.6 ± 25.6	19.3 ± 9.9	17.2 ± 9.9	0.26
Fat (g)	93.1 ± 38.9	72.3 ± 20.3	96.4 ± 23.3	95.6 ± 31.3	0.05
Saturated Fat (g)	32.3 ± 16.7	22.9 ± 7.9	26.5 ± 8.6	26.6 ± 12.0	0.11
Monounsaturated Fat (g)	16.1 ± 9.8	15.1 ± 5.9	15.9 ± 7.2	18.2 ± 7.0	0.69
Polyunsaturated Fat (g)	8.0 ± 5.1[a]	8.7 ± 3.3[a]	23.7 ± 5.6[b]	25.6 ± 8.2[b]	<0.0001*
Omega-3 Fatty Acids (g)	0.7 ± 0.5	0.7 ± 0.4	1.3 ± 1.6	1.2 ± 1.2	0.16
Omega-6 Fatty Acids (g)	6.0 ± 4.0	5.7 ± 2.8	7.8 ± 8.0	8.4 ± 6.3	0.43

Values are the mean ± SD obtained from 24-hour recalls completed the day of each study visit. Recalls from visits 1 and 2 were used to assess habitual intake and those from visits 3 and 4 were used to assess walnut-supplemented intake. P-values <0.05 were considered statistically significant. Asterisk (*) denotes statistical significance p<0.0001; Different letters showcase statistically significant differences between means; SD: standard deviation; kcal: kilocalories; g: grams

CHAPTER V

Effects of Short-Term Consumption of Strawberry Powder on Select Parameters of Vascular Health in Adolescent Males

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Cardiovascular disease and childhood obesity

A dramatic increase in the prevalence of childhood (ages 2–19) obesity, defined as age and sex adjusted body mass index at or above the 95th percentile, has been noted in the US.[1] Similar to adults, obesity in children impacts all major organ systems and increases the risk of morbidity and mortality.[2,3] Compared to normal weight children, those who are obese are more likely to endure adverse health effects, including an increased prevalence of cardiovascular risk factors such as insulin resistance, hypertension, hyperlipidemia, and endothelial dysfunction[4–11] that persist into adulthood.[3,12,13] In response to the above, the American Heart Association has proposed targeting children, adolescents, and young adults in pursuit of their 2020 Impact Goal of reducing deaths attributable to vascular disease and stroke by 20%.[14]

Cardiovascular disease (CVD) is the number one cause of death in the US.[14] By 2030, an estimated 44% of the US population is projected to suffer from CVD, resulting in annual costs in excess of $350 billion.[14,15] Risk factors for CVD are divided into lifestyle factors, including smoking, lack of physical activity, poor diet and excess body fat[14] and clinical factors, including high cholesterol, high blood pressure, and poor glucose control.[14] Atherosclerotic CVD is characterized as a chronic inflammatory disease and disordered lipid metabolism initiated by endothelial dysfunction and promoted by a number of cell types such as platelets.[16–18] Vascular homeostasis is maintained in part by the vasodilators nitric oxide (NO), prostacyclin, endothelial derived hyperpolarizing factors (EDHF), and vasoconstrictors such as thromboxane and endothelin.[19,20] These mediators also help regulate both smooth muscle cell proliferation, inflammation and platelet activation.[21–23] Endothelial dysfunction describes the partial or total loss of balance and regulatory function between vasoconstrictors and vasodilators, growth

promoting and inhibiting factors, and pro- and anti-atherogenic factors.[21]Atherosclerotic CVD is generally characterized as a disease of adulthood, however, fatty streak lesions that are in part secondary to maternal hypercholesterolemia can be detected in fetuses and young children.[21–23]

Apart from maternal factors, poor diet, low physical activity and obesity can increase the development of cardiometabolic disease throughout the life span.[24–26] In addition, micronutrient deficiencies that are associated with under- and over-nutrition can perturb growth, and promote inflammation and infection that can increase chronic disease risk.[27,28] For example, in overweight and obese children, increased inflammation is associated with reduced iron status,[29–32] with hypoferremia potentially mediated through the induction of iron regulators such as hepcidin.[33] Lower socioeconomic status is also associated with increased childhood obesity, increased infection and lower vascular function in adulthood.[28] Therefore, weight control coupled with improvements in dietary patterns at an early age are key to reducing the risk for chronic disease development. Currently, dietary patterns that are high in the intake of plant foods, similar to a Mediterranean dietary pattern, are recommended for the prevention of CVD.[34] These dietary patterns are essential nutrient rich, providing vitamins, minerals, fiber, fats and polyphenols that either alone or through their interactive effects can be of benefit towards cardiovascular health in both children and adults. Therefore, understanding how specific foods may be of benefit can provide further insight for future refinements of dietary recommendations. The following review will focus on strawberries as a potential “vascular healthy” food in overweight adolescents.

Methodologies to assess vascular health in children

As an early step in the atherosclerotic process, assessing vascular function in children is useful to identify those who may be at increased risk.[7,35,36] These measures are considered physiologically relevant towards future disease development, and are appropriate for use towards dietary health claims.[37] Endothelial dysfunction is commonly assessed using the non-invasive ultrasound technique, flow-mediated dilation (FMD).[9,38] This technique measures endothelium-dependent dilation of the brachial artery, a large conduit vessel, in response to shear stress induction of NO after reactive hyperemia,[38,39] and serves as an indicator of endothelial function.[38,39]Nitric oxide-evoked vasodilation plays a critical role in the control of vascular function through the initiation of vascular smooth muscle relaxation by a cascade of steps that leads to reductions in intracellular calcium.[40] Peripheral arterial tonometry (PAT) measures digital arterial pulse wave amplitude in the microvasculature.[41,42] A reactive hyperemia index (RHI) can be calculated after endothelium-mediated changes in microvessel tone is elicited with hyperemia.[42] Given the substantial cross-talk between the endothelium and smooth muscle, measurements of arterial stiffness using either pulse wave velocity (PWV) or the augmentation index (AIx) are also useful assessments of vascular function in children.[36,43,44] The above techniques are associated with cardiovascular risk factor burden,[36,43,45–47] but are mechanistically distinct. For example, under standard conditions, FMD is predominately NO-dependent,[48] while PAT is only partially NO mediated, due to the additional influence of circulating metabolites that are potential EDHFs.[49,50]

When assessing endothelial function in children, variability in time to peak response, vessel size, and pubertal maturation needs to be considered.[7,35] The peak vascular response to reactive hyperemia can occur later in children compared to adults.[7,35] For FMD, the brachial artery diameter may need to be measured several times over a 120 seconds reactive hyperemia interval

or calculated as the area under the curve in order to account for the later peak response time.[7,51] Whether similar calculations are needed for RHI are subject to debate.[51] Pubertal and hormonal changes that occur in childhood and adolescence also impact measures of vascular function.[52] Pubertal status significantly correlates with RHI and lower RHI values in younger or prepubertal groups may reflect immature vascular function rather than dysfunction.[51] The RHI increases during puberty in both sexes as reproductive hormones upregulate NO synthase and activity.[52] The phase of the menstrual cycle also needs to be considered.[35] Sex differences in measures of vascular function are well-recognized.[53–55] Such variability can be partially attributed to the smaller size of the heart and major blood vessels of females compared to males of the same age and race, as well as body height.[56,57]

Potential benefits of strawberry intake for cardiovascular health

Current dietary guidelines stress the importance of a healthful dietary pattern abundant in fruits, vegetables, whole grains, low-fat or nonfat dairy, seafood, legumes, and nuts, with only modest intakes of red meat, refined grains and added sugars.[58] The vascular benefits of plant-based whole foods such as fruits and vegetables can be attributed, in part, to their high content of vitamins, minerals, fiber, and a diversity of bioactive compounds such as polyphenols.[59] An increased intake of polyphenols has been associated with decreased risk for CVD,[60–62] improved endothelial function and reduced platelet reactivity.[60,63–65]

Strawberries are rich in polyphenolic compounds, including anthocyanins, flavanols, flavonols, ellagic acid (EA), and ellagitannin (ET),[63,66] and can provide a source of dietary nitrate.[67,68] Dietary nitrate has been shown to induce positive changes to vascular

health[69] through its conversion to nitrite and NO, which in turn can induce vasodilation and inhibit platelet aggregation.[69]Strawberries also provide an array of vitamins, minerals and fiber, whose health benefits are well-recognized.[59] Epidemiological studies suggest that high anthocyanin intakes (16–22 mg day^{-1}) provided from strawberries and blueberries are associated with an eight percent lowered risk of hypertension and reduced CVD mortality.[70] Several dietary interventions support the concept that strawberry consumption has favorable effects on vascular outcomes attributed to improved endothelial function and plasma lipid profiles, and inhibition of platelet aggregation, lipid peroxidation, and inflammatory responses (Table 1). Other studies report no apparent effects.[72] This may be due to differences in study design and population, or inherent variability in metabolic response. It is also important to note that the outlined trials were mostly conducted in adults, while only limited information is available for children and adolescents.

Table 1 Dietary strawberry intake and cardiovascular surrogate outcomes[a]

Type	Quantity	Study duration	Subject characteristics (mean age)	*n*	Response
WS	454 g per 2000 kcal per day	4 weeks	Hyperlipidemic adults (62 years)	28	↓ TBARS, ↑ protein thiols No effect on plasma lipids[72]
FDSP	25 g	4 weeks	Females with MetS (51 years)	16	↓ TC, LDL, MDA[73]
FDSP	50 g	8 weeks	Obese adults with MetS (47 years)	27	↓ TC, LDL, small LDL particles, VCAM-1 No effect on TG, HDL[65]
FDSP	10 g	6 weeks + PP with HFM	Hyperlipidemic adults (51 years)	24	Acute: ↓ TG, LDL, HDL, OxLDL Chronic: ↓ TC, LDL, HDL, TG[74]
FDSP	10 g	PP with HFM	Overweight adults (51 years)	24	↓ IL-6, hsCRP, insulin[75]
FDSP	10 g	6 weeks + PP with HFM	Overweight adults (51 years)	24	↓ PAI-I, IL-1b[76]
FDSP	320 g	3 weeks	Obese adults (30 years)	20	↓ Total cholesterol and small HDL particles ↑ LDL particle size[77]
FDSP	50 g	6 weeks	Adults with type 2 diabetes mellitus (52 years)	36	↓ CRP, MDA, HbA1C[78]
WS	500 g	4 weeks	Healthy adults (27 years)	23	↓ TC, LDL, TG, MDA, 8-OHdG, isoprostanes[79]
FDSP	25 g 50 g	12 weeks	Adults with abdominal adiposity hyperlipidemia (49 years)	60	50 g: ↓ TC, LDL, small LDL particles 25 & 50 g: ↓ MDA, no effect on HDL, TG, CRP or adhesion molecules[80]
FDSP	50 g	1 week	Overweight and obese adolescent males (16 years)	25	↑ Microvascular function related to plasma nitrate/nitrite[71]
FDSP	40 g	4 weeks + PP	Overweight and obese adults (28 years)	30	No effect on arterial stiffness or plasma lipids[81]
FDSP	25 g 50 g	8 weeks	Stage 1 hypertensive, postmenopausal females (60 years)	60	Low dose: ↓ SBP, PWV High dose: ↑ nitrate/nitrite[82]

WS: whole strawberries; FDSP: freeze-dried strawberry powder; MetS: metabolic syndrome; PP: postprandial; HFM: high-fat meal; g: grams; MDA: malondialdehyde; TBARS: thiobarbituric acid reactive substances; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein, OxLDL: oxidized low-density lipoprotein; TG: triglycerides; CRP: C-reactive protein; hs-CRP: high-sensitivity C-reactive protein; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; VCAM-1: vascular adhesion molecule-1; HbA1C: hemoglobin A1C (glycated hemoglobin); SBP: systolic blood pressure; PWV: pulse wave velocity; NOX2: NADPH oxidase 2; IKK: inhibitory κB kinase; IL-6: interleukin 6; IL-1B: interleukin 1 beta; PAI-1: plasminogen activator inhibitor-1. [a] Includes human clinical trials of known physiologically relevant measures related to cardiovascular function. Therefore, excludes studies of antioxidant capacity that are not direct measures of known oxidant products where the relationship to physiology and disease development has been established. Also, excludes studies where either the amount or fresh strawberries or FDSP intake was not clearly defined.

Effects of a freeze-dried strawberry powder on parameters of vascular health in adolescent males: a randomized trial

Given the dearth of information on strawberries and vascular health in children, we conducted a randomized, controlled, double-blind, crossover trial to assess whether the acute (one hour) or short-term (one week) consumption of a freeze-dried strawberry powder (FDSP) can influence vascular health in adolescents. The children were at increased cardiovascular risk due to their elevated adiposity (>75th percentile for age and sex). We hypothesized that vascular function would increase following the intake of FDSP compared to a control polyphenol-free power. Microvascular function as measured by PAT, platelet reactivity, and plasma nitrate and nitrite (nitrate/nitrite) concentrations were assessed before and after the one-week daily consumption of 50 grams of FDSP or an isocaloric, macronutrient-matched control powder that was devoid of polyphenols (Table 2; Fig. 1). The powders were divided into two 25 g servings. The children were instructed to mix the powder in water, consuming one packet at breakfast and the other at dinner.

Twenty-five adolescent (aged 14–18 years) males were enrolled into the trial. The mean age was 16 years, and the participants on average were healthy, with normal blood pressures, fasting lipid profiles and fasting blood glucose levels.

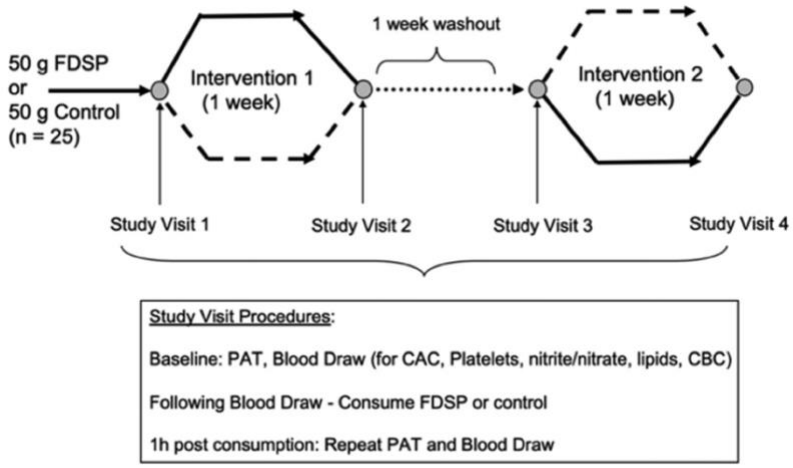

Fig. 1 Study design and visit procedures.[71]

The composition of both FDSP and control powder is described in Table 2, with the polyphenolic content outlined in Table 3. Fifty grams of FDSP is equivalent to approximately three cups (450 g) of whole strawberries. The control powder also contained dietary fiber and potassium that may provide some benefit towards vascular health and therefore should not be considered as a true "placebo". The isoenergetically matched powders were produced and provided by the California Strawberry Commission.

Table 2 FDSP composition per 50 grams

	FDSP	Control powder
Calories (kcal)	180	180
Fructose (g)	11	12
Glucose (g)	10.3	9
Sucrose (g)	7.2	10
Total sugar (g)	28.4	30.5
Carbohydrate (g)	39	42
Protein (g)	3.2	0
Total dietary fiber (g)	8.1	4.3
Potassium (mg)	839	350

Table 3 FDSP polyphenol content per 50 grams

Polyphenol	Content (mg)
Pelargonidin-3-glucoside	198.5
Procyanidin B1	15.31
(+)Catechin	12.52
Ellagic acid	6.30
Cyanidin-3-glucoside	5.82
Isoquercetin	3.31
Rutin	1.68
Quercetin	0.73
Tiliroside	0.37
Gallic acid	0.2
Sinapic acid	0.2
Kaemferol	0.18
p-Coumaric acid	0.13
2-Hydroxycinnamic acid	0.1
3,4-Dihycrobenzoic acid	0.08
Syringic acid	0.01

Peripheral arterial tonometry was assessed after an overnight fast and one hour after the intake of the assigned powder. The measurement provided the data for the parameters: (1) RHI, (2) Framingham RHI (fRHI), an index associated with cardiovascular risk factors,[45] (3) AIx, (4) platelet reactivity assessed by flow cytometry, and (5) total plasma nitrate/nitrite. An initial analysis demonstrated that neither short-term (seven-day) nor acute FDSP intake improved microvascular function (Fig. 2) when assessed as a group. Likewise, no significant differences were observed in blood pressure, plasma lipids, or platelet reactivity.[71] Compliance with daily powder intake was difficult to assess as very few children returned their packaging as requested.

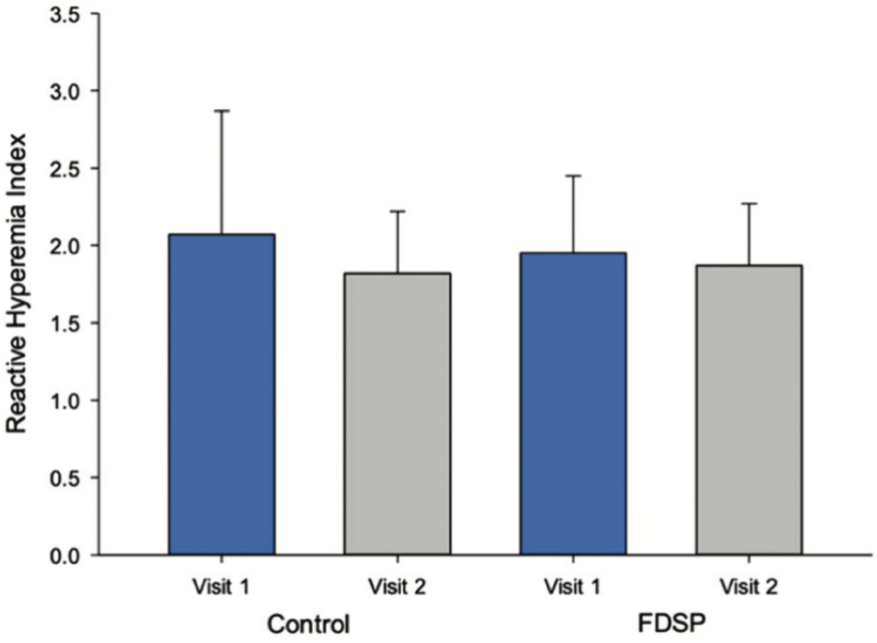

Fig. 2 Reactive hyperemia index with short-term consumption of FDSP *vs.* control powder.

A significant increase in total plasma nitrate/nitrite levels was observed one hour after the intake of FDSP compared to control powder intake.[71] However, total fasting plasma nitrate/nitrite was not significantly changed with FDSP compared to control powder after one week of intake. Based on the results of the acute intake, and the observation that strawberries are considered a source of dietary nitrate[67] with a half-life of five to eight hours,[83] a subset analysis was conducted between those who had an increase in the one week change in total plasma nitrate/nitrite compared to control powder intake ("Responder"), and those who did not ("Non-Responder"; Fig. 3, left panel). Among children who had a significant increase in circulating nitrate/nitrite levels, an increase in both RHI (Fig. 3, right panel) and fRHI was observed, while those showing no detectable increases in plasma nitrate/nitrite had no improvement in vascular function.[71]

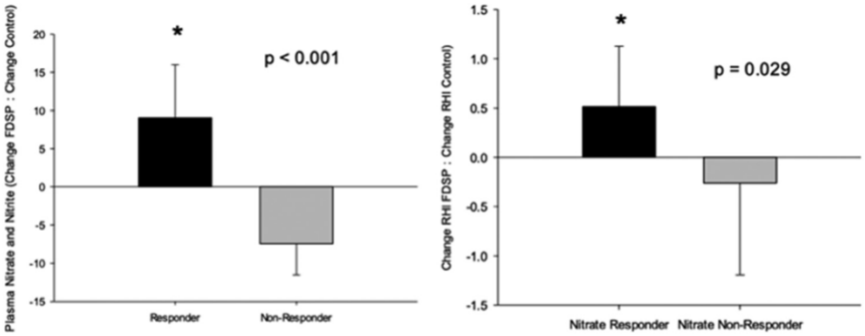

Fig. 3 Individuals who had an increase in fasting plasma nitrate and nitrite levels with one week of FDSP intake relative to control powder intake (left panel) also had an improved vascular function response as measured by the reactive hyperemia index (RHI; right panel).

Potential mechanisms

Nitrate/nitrate response

The above results demonstrate an improvement in vascular function after FDSP intake by overweight adolescents that is associated with plasma nitrate levels. Our findings are in agreement with Feresin *et al.* who reported increased plasma levels of nitrate/nitrite after short-term (four and eight weeks) FDSP intake (50 g day^{-1}).[82] Dietary nitrate has gained interest as a potentially bioavailable supply of NO, with green leafy vegetables as a predominate source.[83] Nitrate is reduced to nitrite by commensal bacteria in the oral cavity, and further reduced to NO *via* numerous pathways that involve polyphenols, vitamin C, deoxygenated myoglobin, xanthine oxidoreductase, and deoxygenated haemoglobin (Fig. 4).[84] The potential production of NO through oxygen-independent means is of particular importance during tissue ischemia and exercise, which are situations of reduced blood flow and increased tissue oxygen demand.[85] Indeed, circulating nitrate/nitrite levels have been positively associated with FMD response,[86,87] and the intake of nitrate from beetroot juice has been observed to reduce blood pressure and improve vascular function in healthy adults.[83,87,88]

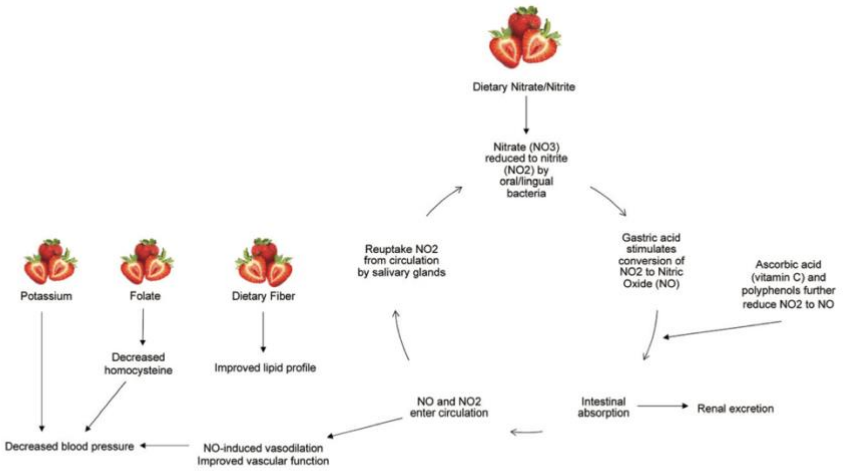

Fig. 4 Potential mechanisms modulating the vascular effects of strawberry consumption. NO3: nitrate; NO2: nitrite; NO: nitric oxide.

Rodriguez-Mateos and colleagues demonstrated the potential interactive effects of nitrate-containing foods with polyphenol-rich foods.[89] Nitrate intake enhanced the FMD response to cocoa flavanol intake.[89] Importantly, the amount of nitrate provided was similar to the potential intake from typical amounts of leafy greens.[89] Interestingly, the intake of both flavanols and nitrate together, but not separately, increased the stomach production of NO,[89] which is in agreement with other trials.[90] As will be discussed in the following sections, strawberries can provide a substantial amount of both vitamin C and polyphenols, both with the potential to reduce nitrate to bioactive NO. The potential interactive effects these components may elicit on the vascular response should be explored further.

Strawberry polyphenols

The FDSP products for the forementioned trial were particularly rich in the anthocyanin pelargonidin-3-glucoside (P3G), with a single daily intake providing approximately 200 mg, which is 13 to 15-fold higher than that of the flavan-3-ols procyanidin B1 and catechin,

respectively. The FDSP intake also provided 6 mg of the anthocyanin cyanidin-3-glucoside. In addition to flavonoids, the FDSP provided phenolic and hydroxycinnamic acids (Table 3). Anthocyanins are glycosylated flavonoids that, depending on the pH, provide red, purple, and blue pigmentation to berries, grapes and flowers.[91] Following dietary intake, anthocyanins are present in the circulation as conjugated aglycones and aglycone glycosides or degraded to phenolic acids and aldehydes.[92,93] Within an hour of FDSP intake, P3G is predominately converted to its glucuronide form (pelargonidin-*O*-glucuronide), with considerably lower circulating amounts of P3G and pelargonidin-3-rutinoside, along with several phenolic acids and aldehydes, including hippuric acid, 3,4-dihydroxybenzaldehyde, 4-hydroxybenzaldehyde, *p*-coumaric, and 3-hydroxybenzoic acid.[93–95]

Numerous investigators have utilized *in vitro* systems to investigate the role of strawberry polyphenols on a number of parameters. However, it should be noted that positive effects have been predominately observed with the anthocyanidin pelargonidin and not the specific anthocyanins that are found within strawberry or its' physiologically relevant metabolites. Amini *et al.* observed that *in vitro* administration of 0.08 μmol L^{-1} of P3G extract to stimulated human whole blood significantly increased the concentration of interleukin-10, an anti-inflammatory cytokine.[96] Pelargonidin, but not P3G, increased clotting time (prothrombin time) and reduced the ratio of plasminogen activator inhibitor (PAI-1): tissue plasminogen activator (t-PA) in human umbilical vein endothelial cells.[97] In isolated rat aorta, pelargonidin inhibited thromboxane induced vasoconstriction in an endothelium-independent fashion,[98] while cyanidin-3-glycoside enhanced endothelial nitric oxide synthase expression in bovine artery endothelial cells in a dose-and time-dependent manner, with the highest response observed at 0.1 μmol

L^{-1}.[99] While the above *in vitro* work is promising, data from dietary interventions that specifically examine the association between circulating strawberry polyphenol or phenolic metabolites and physiological effects have yet to be established. However, in a diabetic mouse model, the addition of serum providing strawberry metabolites attenuated endothelial dysfunction and markers of inflammation.[100] In addition, other anthocyanin-rich foods such as blueberries, have demonstrated improvements in vascular function that are associated with the presence of a number of circulating phenolic and aromatic acids.[101]

Fifty grams of the FDSP used in the above trial provided 6.3 micrograms of EA (Table 3). Ellagitannins are another potential source of bioactive polyphenols[102] that are hydrolyzed in the gastrointestinal tract to EA, and further metabolized by the gut microbiota to urolithins.[103] In animal models, the addition of polyphenolic-rich strawberry extracts to the diet beneficially affects colonic enzymes and improves gut dysbiosis, while increasing short chain fatty acid production.[104–106] The influence of strawberry polyphenols on microbial derived metabolites are of considerable interest as they can influence cellular signalling and physiological response both locally in the gastrointestinal tract and systemically after absorption. Short chain fatty acids have received considerable interest as potential blood pressure regulators.[107] Urolithins have been observed to reduce oxidative damage[108,109] and inflammation.[102,108]Finally, dietary nitrate can be reduced by the gut microbiota to bioactive NO and as we have demonstrated above, can be associated with improved vascular response.[71]

Other cardioprotective nutrients

Beyond polyphenols and nitrate, strawberries contain additional vasculoprotective nutrients, such as folate, vitamin C, potassium, and dietary fiber (Table 4). When folate levels are low, the conversion of homocysteine to methionine is decreased and homocysteine levels rise.[110] Elevated serum homocysteine has been associated with increased vascular disease risk.[111,112]

Low levels of vitamin C are associated with vascular disease.[113] Thirty-nine grams of FDSP provides 171% of the Recommended Dietary Allowance for vitamin C. Plasma levels of vitamin C are significantly increased two to four hours after the intake of 300 g of fresh strawberries.[114] Vitamin C scavenges free radicals, and protects lipoproteins from oxidative damage.[115] In addition, vitamin C has been shown to improve both arterial stiffness and endothelial function.[116,117]

Potassium influences vascular disease risk through its critical role in blood pressure regulation.[118,119] Dietary fiber has the ability to bind cholesterol and increase its' excretion, lowering circulating cholesterol levels.[120,121] This allows for less lipoprotein to be susceptible to oxidation and atherogenesis.[122]

Table 4 Vitamins, minerals and fiber provided in 39 g of FDSP (2018 formulation provided by the California Strawberry Commission)

	Content	% RDA
Folic acid (mcg)	108	27
Pantothenic acid (mcg)	0.35	7
Thiamin (mg)	0.02	5
Niacin (mg)	1.83	11
Vitamin C (mg)	154	171
Potassium (mg)	659	19
Dietary fiber (g)	5.1	20

Research considerations when evaluating the potential health effects of strawberries

A number of variables exist that could contribute to differential findings with products such as FDSP. Examples of these follow.

Metabotype

Recent studies have demonstrated a relationship between urolithin metabotype (UM) and cardiovascular risk factors.[123,124] Three UMs have been identified: Metabotype A (urolithin-A producing), Metabotype B (urolithin-A and/or -B producing), and Metabotype 0 (not urolithin-producing).[125] Studies suggest that UM-A is cardioprotective, while UM-B may be associated with gut dysbiosis and disease.[123,125] Correlations between UM and cardiometabolic risk factors in individuals have been reported in overweight or obese adults;[89] information about adolescents and children is currently lacking. UM-A has been correlated with levels of apolipoprotein A and high-density lipoprotein-cholesterol (HDL), while UM-B has been associated with apolipoprotein B, total cholesterol, very low density lipoprotein (VLDL)-cholesterol, low density lipoprotein (LDL)-cholesterol, and oxidized LDL-cholesterol.[89] These patterns may be significant in that apolipoprotein A and HDL are associated with decreased cardiovascular risk,

while apolipoprotein B and its' lipoproteins (LDL and VLDL) are associated with increased risk.[126] Similar results of enhanced endothelial function have been reported following acute (three-day) consumption of ET and production of their microbial metabolites.[124] Taken together, these results suggest that individuals who produce UM-B are at increased risk of CVD, whereas production of UM-A may confer vasculoprotective effects.[89,124] Ideally, metabotypes should be considered in future studies that assess the potential benefits of ET-rich foods and cardiovascular health.

Food matrix

An important influence of the food matrix on the potential health benefits of strawberries is suggested by studies demonstrating a change in anthocyanin pharmacokinetics with the addition of food.[95] While FDSP allows for a consistent and convenient supplementation across studies, it is important to note that the amount of P3G provided in 50 g of FDSP is about 12 times higher than the amount found in whole strawberries.[127] Food processing, preservation, and storage conditions affect the stability and bioavailability of the active compounds in strawberries. For example, the freeze-drying process may denature anthocyanin content and reduce bioactivity.[128] Juicing and preserving strawberry may increase the level of EA relative to fresh strawberry *via* hydrolysis.[129] The conversion of ET to EA can be reduced by using low processing temperatures. Prolonged storage at low temperature can also enhance hydrolysis, although at a slower rate.[129] Additional data are needed on the potential interactive effects between strawberry bioactives and other nutrients within a diet. The above highlights the need for more information on the influence of a complex diet on the potential health effects of strawberries. Such interactions can be positive as well as negative. The identification of positive

interactions is particularly important as it may provide insight for the development of new vascular-health foods. Conversely, the identification of negative interactions may be of great value in the design of new food processing and handling techniques that can amplify the health effects of strawberries.

Appropriate controls for strawberry investigative trials

An inherent difficulty with most nutrition studies is the identification of appropriate controls or placebos. This is particularly difficult when examining the potential health effects of whole foods that contain a number of components that either on their own or through their interaction with each other are bioactive. With respect to strawberries, an attempt has been made to develop dietary powders that can be used in dietary intervention trials that are matched with respect to several nutrients excluding polyphenols and are thought to drive many of the health effects reported with strawberry intake. It is important to stress that the "control" powders used in such studies will likely underestimate the positive health effects that these foods can provide. Thus, the positive health effects observed with powders used in strawberry research are underestimates of the positive health effects of strawberry intake. Complimenting this caveat with respect to control food or diet, it is important to note that placebos can elicit biological effects, despite containing no pharmaceutical compounds or known bioactive components.[130] Placebo treatments in randomized controlled clinical trials have demonstrated significant improvement in symptoms.[131] These effects have been seen both with discrete and disclosed provision of placebos. Interestingly, positive effects seen with the provision of an "open-label placebo", where participants are knowingly prescribed placebo treatment, still have been shown to evoke some level of response.[130,132–134] The mechanisms behind these phenomena are not well

understood, but are thought to include neurobiological and psychological mechanisms, classical conditioning, and simple hope for change.[130,135]

Summary

The literature to date strongly supports the concept that the regular consumption of strawberries can be associated with improvements in cardiovascular health. Dietary interventions that examine the influence of strawberry intake on measures of vascular function are currently limited, especially for longer periods of intake. Moreover, it is reasonable to further examine the health effects of strawberries in additional at-risk populations that include children and adults. Trial designs that capture the relationship between circulating strawberry-derived metabolites and physiologic response are desired. This would include studies that assess the effects of strawberry intake on vascular health in a variety of populations, as hormonal status, sex, age, genetic polymorphisms and microbial metabolism can affect polyphenol metabolism and ultimately cardiometabolic response.[136] Current recommendations stress a dietary pattern that is high in plant foods. Therefore, a better understanding of the synergy between the diverse constituents of strawberries within the diet and their relationship to vascular health is desired, including a better appreciation of individual metabotypes in response to strawberry intake. The above information will better enable practical recommendations about the amount and frequency of strawberry intake to consume on a regular basis as part of a healthy vascular dietary pattern over the lifespan.

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CHAPTER VI

Concluding Remarks

Cardiovascular disease, often beginning in childhood, is a significant contributor to morbidity and mortality in the U.S. (1, 2). Dietary intervention is a common and effective treatment strategy (3, 4). Evidence demonstrates that certain diets, such as the Mediterranean and Vegetarian diets, promote cardiovascular health (5-7). These plant-based diets contain biologically active polyphenolic compounds and polyunsaturated fatty acids that may elicit vasculoprotective effects through a variety of mechanisms. Nuts and berries are rich in the aforementioned compounds of interest (8).

Chapter I provides an in-depth discussion of both the fundamental challenges and promising future directions in research with nuts and berries. With unique nutritional profiles, including an array of bioactive compounds and phytonutrients, nuts and berries are considered part of a healthy eating pattern and are associated with improved cardiovascular outcomes (9). Despite a large body of literature, heterogeneity in study design, test material, control foods, and feasible portion sizes limits its' clinical application. New understanding of the bioactive compounds found in both nuts and berries has reinforced their role for use in personalized nutrition efforts. Future research utilizing advanced multi-omics and personal biomonitoring technologies will undoubtedly yield new insights (8).

Chapter II is a review of recent literature on the effects of strawberry intake on human health. The impact of strawberry on cardiometabolic outcomes, including vascular function, glycemic control, lipid profile, inflammation, and oxidant defense, is well-documented (10). Although limited, research efforts have expanded into novel areas, such as gut health, cognitive function, bone health, and cancer. Recent advances in research related to the gut microbiome and

microbial metabolism have established a new understanding of the phytonutrients in strawberries and the potential mechanisms by which strawberry intake may promote health (11).

Chapter III details an 18-week dietary intervention trial supplementing with walnuts. Previous work from our laboratory found improved microvascular function as assessed by reactive hyperemia index (RHI) in hypercholesteremic, postmenopausal women with the intake of 40g/day of walnuts for four weeks, which was associated with increased plasma epoxides (12). The present work sought to confirm and extend these findings by lengthening the intervention period to 12-weeks but did not yield the same level of improvement. Twelve weeks of 40g/day walnut consumption did, however, improve lipid profile, particularly in those with hyperlipidemia at baseline. Pending metabolomic analysis will help elucidate the mechanisms underlying the described physiological changes related to walnut intake.

Chapter IV reviews clinically relevant studies on the effects of freeze-dried strawberry powder (FDSP) on vascular health, with focus on a dietary intervention trial conducted in overweight or obese adolescent males. Fifty grams of FDSP daily for one week influenced fasting nitrate/nitrite levels differentially in participants, yielding ‘responder’ and ‘non-responder’ phenotypes. In responders, in whom fasting nitrate levels increased with FDSP intake, RHI was significantly increased compared to controls, an improvement that was not seen in non-responder phenotypes (13). These results support the role of strawberry in vascular health and emphasize the importance of inter-individual variability, metabolomics, and personalized nutrition.

Appendix A describes a cross-sectional study evaluating vascular function in individuals following either a vegetarian or omnivorous diet containing red meat (14). Vegetarians tend to have reduced cardiovascular disease risk compared with omnivores (15). While mechanisms are not completely clear, it is thought following a vegetarian diet may be protective of the vascular endothelium (16). In this study, however, participants had similar indices of vascular function and arterial stiffness regardless of dietary pattern, although those following a vegetarian diet did have lower central pulse pressure and forward pressure wave amplitude which may reduce cardiovascular risk.

Appendix B discusses diet quality, psychosocial health, and cardiometabolic risk factors within the context of adolescent obesity (17). The prevalence of obesity in youth has increased dramatically in recent years and is coupled with increased prevalence of chronic cardiometabolic diseases that are usually seen in adults, such as hypertension, hyperglycemia, and dyslipidemia. To address this issue, it's recommended to implement theory-driven, multifaceted interventions in schools and communities, focusing on promoting knowledge and self-efficacy for sustained behavior change. Such strategies should encompass a comprehensive approach targeting both stress reduction and cardiometabolic symptoms, while also empowering adolescents to make informed dietary choices (17).

This dissertation focuses on the role of plant-based diets and associated nutrients and phytonutrients in promoting vascular health. Plant-based diets provide an array of vitamins, minerals, fibers, polyphenolic compounds and essential fatty acids, which elicit cardioprotective effects (18). The research described demonstrates the benefits and proposed mechanisms of plant

foods, particularly nuts and berries, in mitigating cardiovascular risk. Findings reviewed herein generally align with similar research on the benefits of plant foods and vascular health, particularly on endothelial function, blood pressure, and lipid profiles (5, 19-21). Appreciating the synergy between the diverse constituents of dietary plants and their relationship to vascular health is critical. Future research should utilize a personalized approach with an emphasis on multi-omic approaches and inter-individual variability in physiological response. This information can be used to inform personalized nutrition recommendations and promote vascular health over the lifespan.

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APPENDIX A

Arterial Stiffness and Endothelial Function are Comparable in Young Healthy Vegetarians and Omnivores

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Introduction

Roughly half of the US population aged 20 years and older suffers from at least 1 form of cardiovascular disease (CVD) [1]. Traditional CVD risk factors are well established, and comprise smoking, physical inactivity, overweight or obesity, cholesterol, diabetes, high blood pressure (BP), and poor nutrition [1]. Beyond these, important nontraditional risk factors for the development of CVD include endothelial dysfunction and arterial stiffness [2,3]. The endothelium, or inner lining of blood vessels, plays a critical role in the synthesis and release of different vasodilatory molecules. Among those, nitric oxide, a critical vasodilator, maintains homeostasis within blood vessels by regulating cell growth, platelet and leukocyte interactions, thrombogenicity, and cell proliferation and angiogenesis [4]. Of clinical relevance, endothelial dysfunction has been shown to present before any clinical evidence of CVD and is an independent predictor of cardiovascular risk and events [5,6]. Arterial stiffness and in particular stiffening of the central arteries is also an independent predictor of cardiovascular events [7]. Both endothelial dysfunction and arterial stiffness have been shown to be influenced by diet. Proper nutrition plays an important role in minimizing CVD risk. Specifically, diets high in saturated fat and sodium, and low in whole grains, fruits, and vegetables are associated with increased CVD risk [8]. This dietary pattern represents the typical diet for most Americans [9]. Red meat consumption, especially processed meat, has been related to increased mortality and the occurrence of adverse cardiovascular events [10], although this evidence is not consistent [11,12]. In contrast, plant-based diets are associated with a reduced risk for CVD [13–16]. These diets are characterized by a partial or total elimination of animal product consumption and are often rich in grains, legumes, vegetables, fruits, and nuts, and low in saturated fat, cholesterol, salt, and added sugars [13,17]. Typical benefits of following a plant-based diet include a lower

body mass index (BMI) [18], lower BP [15], lowered cholesterol levels [19,20], and reduced CVD incidence [21] compared with an omnivorous diet. Furthermore, an analysis of 5 prospective cohorts found that following a plant-based diet for at least 5 years resulted in the lowest mortality among vegetarians compared with nonvegetarians with similar lifestyle habits [22], whereas consuming 2 servings per week of red meat com- pared with 0 servings per week has been shown to be associated with incident CVD [23].

Plant-based diets may be beneficial in reducing CVD risk but the mechanism(s) responsible have not been elucidated. Increased consumption of antioxidant compounds, polyphenols, and fiber, components abundant in this diet, have been suggested to contribute to this benefit [24–26]. Indeed, consumption of these nutrients and bioactive components may provide a more favorable environment for the endothelium and subsequently lower CVD risk. Diets high in sodium [27,28] and/or fat [29], a characteristic of the typical American diet, can result in endothelial dysfunction and arterial stiffness. To the best of our knowledge, limited work has assessed differences in vascular function between omnivores and vegetarians. Middle-aged adult vegetarians were shown to exhibit a greater vascular function [30] and less arterial stiffness [31] than age-matched omnivores. Whether or not these differences present earlier in a younger population has yet to be examined. This is important because as adults age, differences occur at the level of the vasculature [32] but whether vegetarian diets play a role in younger adults is unknown. Therefore, our goal was to determine whether differences in vascular function exist in a younger group of vegetarians and omnivores. We hypothesized that vegetarians would exhibit greater vascular function compared to their omnivore counterparts. Endothelial function was assessed by brachial artery flow-mediated dilation (FMD) and passive leg movement (PLM),

arterial stiffness was assessed by carotid-to- femoral pulse wave velocity (PWV), and wave reflection was assessed by augmentation index (AIx).

Methods and Materials

Participants

This protocol was approved by the institutional review board at the University of Delaware (#841654-14) and conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent. Participants were men and women between the ages of 18 and 45 years old; free of CVD, hypertension, diabetes, cancer, kidney disease; not using medications for the mentioned conditions; not obese (BMI ≥ 30 kg/m2), smokers; pregnant or lactating; or highly trained endurance athletes. Vegetarians were defined as those following a plant-based diet for at least 5 years with no occasional consumption of meat, poultry, or fish. They could be ovo-lacto-vegetarians (consumption of eggs, milk, and dairy), lacto-vegetarians (consumption of milk and dairy), or vegans (no consumption of eggs, milk, nor dairy) [33]. Omnivores were defined as consuming red meat at least 2 times per week.

Experimental protocol

All screening and study visits were conducted at the Cardio- vascular Nutrition laboratory located on the Science, Technology and Research campus at the University of Delaware. Potential participants were asked to come in for an initial screening visit, where height (Health O Meter Professional 500KL-BT, Pelstar, McCook, IL), weight (Tanita TBF 300a), and BMI (kg/cm2), were assessed. Height and weight values are shown rounded to the nearest centimeter and kilogram. Resting seated BP were assessed in triplicate (GE DinaMap Pro- Care 100

Monitor) and the average is reported. Participants also completed a medical history questionnaire, Global Physical Activity Questionnaire [34] and a food frequency questionnaire (Diet History Questionnaire II (DHQ-II); version 2.0. National Institutes of Health, Epidemiology and Genomics Re- search Program, National Cancer Institute, 2010). The DHQ-II consisted of 134 food items and 8 dietary supplement questions and asked about intake over the previous year. It was used to verify the self-reported vegetarian or omnivore status as well as to collect data on usual intake. Participants self- reported the number of years they were following a vegetarian diet. To investigate how well the consumed foods aligned with the Dietary Guidelines for Americans (DGA), we quantified the Healthy Eating Index (HEI, 2015), an index of diet quality, from the DHQ-II responses [35]. Women were asked to fill out a menstrual cycle form for the purposes of scheduling their experimental visit during the early follicular phase of their menstrual cycle. Last, PLM was demonstrated on the subject to eliminate a potential learning effect. For the experimental visit, participants were asked to report to the laboratory following a 6-hour fast, to avoid caffeine and alcohol consumption for 12 hours, and to abstain from exercise for 24 hours before the study visit. A venous blood sample was taken to measure hemoglobin (HemoCue Hb 201p model, Lake Forest, CA), hematocrit (Clay Adams Brand, Readacrit Centrifuge, BD Diagnostics, Sparks, MD), and lipid profile (LabCorp, Burling- ton, NC). Participants rested in a supine position for 20 minutes before vascular measurements. Assessment of vascular function included pulse wave analysis (PWA), PWV, FMD, and PLM.

Pulse wave analysis

Central aortic pressure was synthesized using the SphygomoCor XCEL system (SphygmoCor, ATCOR Medical, Sydney, Australia) from a measured brachial artery pressure waveform using a

generalized transfer function. Central pressures and AIx were derived from this method. AIx was calculated as (Ps – Pi)/(Ps – Pd), where Ps = systolic pressure, Pd = diastolic pressure, and Pi = pressure at the inflection point. Forward and reflected wave components were determined by wave separation analysis using a modified triangular flow wave [36]. AIx was normalized to heart rate (HR) at 75 beats/min. Mean arterial pressure (MAP), HR, and augmentation pressure were also measured.

Pulse wave velocity

Carotid-to-femoral PWV was measured by recording both a carotid and femoral artery waveform simultaneously while the subject remained in the supine position. A high-fidelity strain gauge transducer (Millar Instruments) was placed on the carotid artery and a blood pressure cuff was placed over the femoral artery and pulse transit time was determined by the time delay between the feet of the carotid and femoral waveforms. External distances were measured using a tape measure. The distance between the carotid measurement site to the sternal notch and from the sternal notch to the femoral site was measured. The distance from the carotid measurement point to the sternal notch was subtracted from the distance from the sternal notch to the femoral measurement point and used as propagation distance. PWV was calculated by dividing the 2 recording sites by the pulse transit time (PWV = propagation distance/transit time).

Assessment of endothelial function

Macrovascular function

We assessed endothelial function at the macrovascular and microvascular level. At the macrovascular level, endothelial function was assessed by FMD in accordance with established

guidelines [37]. The subject rested in the supine position with their right arm extended at a 90° angle from the torso and at heart level, palm facing up and remained in this position during the test. A pneumatic rapid inflation/deflation cuff (D.E. Hokanson, Bellevue, WA) was placed distal to the olecranon process to provide a stimulus to forearm ischemia. A 10-MHz linear phased array probe attached to a high-resolution ultrasound machine (Terason, uSmart 3300, Burlington, MA) was used to image the brachial artery and placed approximately midway between the antecubital and axillary regions [38]. On optimal identification of the vessel, the probe was held stable with a probe holder, and the ultrasound parameters were set to optimize the longitudinal, B-mode images of the lumen-arterial wall interface. Ultrasound images, containing diameter and blood flow measures, were recorded during the entire test with an image capturing system (Camtasia Studio Version 8). After a 1-minute baseline period, the cuff was inflated to 200 mm Hg for 5 minutes, and then was deflated for 2 minutes, in which reactive hyperemia occurred. Brachial artery diameter was determined from the 1-minute baseline average and peak diameter was determined during the reactive hyperemia period after applying a 3-second-wide median filter to each data point using the Cardiovascular Suite software package (QUIPU, Pisa, Italy). FMD was expressed as percent change from baseline [(peak diameter – baseline diameter)/baseline diameter] × 100. Doppler data were used to calculate flow and shear rate as: Flow (mL · min – 1) = p(1/2 · vessel diameter)^2 · (1/2 · Vpeak); Shear rate (s – 1) = 4 · Vpeak · vessel diameter –1; where Vpeak = centerline velocity. It has been shown that the shear rate area under the curve (AUC) from cuff release to peak diameter best represents the reactive hyperemia shear stimulus for FMD [37].

Microvascular function

At the microvascular level, endothelial function was assessed via PLM as described by Gifford and Richardson [39]. Participants were tested in the up- right/seated posture. Measurements of femoral arterial blood velocity and vessel diameter were performed in the passively moved leg distal to the inguinal ligament and proximal to the deep and superficial femoral bifurcation using the NextGen LOGIQ e Ultrasound (GE Healthcare, Milwaukee, WI). The protocol consisted of 1 minute of resting baseline measurement followed by two minutes of continuous passive leg flexion- extension through a 90° to 180° range of motion at a frequency of 1 Hertz. A metronome was used to maintain the cadence. Passive movement of the leg was performed by a member of the research team, while the contralateral leg remained fully extended and supported at the same height. Before the start of and throughout the protocol, participants were encouraged to remain passive and resist the urge to assist with leg movement. To avoid a startle reflex and active resistance to the passive movement, participants were made aware that passive movement would take place in approximately 1 minute. To minimize the chance of an anticipatory response, participants were not informed when passive leg movement would begin. For those participants who were unable to remain passive during the measurement, additional trials were performed. If after several attempts, the participant could not remain passive throughout the trial, their data were excluded. Vessel diameter was determined at a perpendicular angle along the central axis of the scanned area. Blood velocity was measured using the same transducer with a frequency of 5 MHz. Arterial diameter was measured and mean velocity (angle corrected, and intensity-weighted area under the curve) was calculated. Leg blood flow was calculated using arterial diameter and mean velocity. Peak change in leg blood flow was calculated as peak minus baseline leg blood flow and leg blood flow area AUC was calculated for the first 60 seconds of PLM.

Statistical analyses

The dietary data collected in the DHQ-II was analyzed using the Diet∗Calc Analysis Program (version 1.5.0, National Cancer Institute, Epidemiology and Genomics Research Program, October 2012). The primary outcome was FMD. Secondary outcomes were percent change and AUC during PLM, PWV, and AIx. An a priori power analysis with an alpha of 0.05, 95% power, and assuming a difference in FMD of at least 2% between the 2 groups, we needed 27 participants in each group in an unpaired samples *t* test (G∗ Power). This difference in FMD is based off the Lin et al. [30] study in which they saw a 10% difference in their older vegetarian and omnivore groups. Given our younger group, we anticipated a smaller difference and conservatively used a 2% change. Although not powered to find sex differences on diet and main outcomes, we per- formed an exploratory analysis using a 2 × 2 analysis of variance with diet and sex as factors. A Tukey honestly significant difference post hoc analysis was used to assess for multiple comparisons. JMP Pro 16 was used to carry out the analysis. Data are presented as mean ± SD. $P < .05$ was considered statistically significant.

Results

Participant characteristics

One hundred and four subjects were screened. Forty-six were excluded because of high BP, high BMI, lack of interest, smoking status, diabetes, not consuming enough red meat, or not being a vegetarian long enough. Participants' characteristics from the screening including demographics, blood chemistries, hemodynamics, and physical activity levels are presented in Table 1. Twenty-eight omnivores (15 women/13 men) and 30 vegetarians (25 women/5 men) participated in the

study. The average duration of vegetarianism was 13 years and ranged from 5 to 44 years. The only difference observed in demographics was weight, which was higher in the omnivore group. Blood chemistry and hemodynamic values were similar between the 2 groups, with the exclusion of pulse pressure, which was higher in the omnivore group. Physical activity assessed by Global Physical Activity Questionnaire did not differ between groups.

Table 1 – Group characteristics of healthy omnivores and vegetarians.

Variable	Omnivores	Vegetarians	P
Demographics			
N, women/men	15 W/13 M	25 W/5 M	
Race, Asian/Black/White	7/1/20	6/1/23	
Age, yr	26 ± 6	26 ± 8	0.853
Weight, kg	70 ± 12	63 ± 10	**0.023**
Height, cm	172 ± 9.3	168 ± 6.9	0.050
BMI, kg/m^2	23.3 ± 2.8	22.3 ± 3	0.175
Total physical activity, min/week	487 ± 384	574 ± 590	0.51
Blood chemistry			
Hemoglobin, mg/dL	13.9 ± 1.6	13.4 ± 2	0.306
Hematocrit, %	43.8 ± 4.1	42.5 ± 5.2	0.361
Total cholesterol, mg/dL	173 ± 36	164 ± 34	0.404
High-density lipoprotein, mg/dL	60 ± 12	58 ± 17	0.718
Low-density lipoprotein, mg/dL	96 ± 30	87 ± 28	0.275
Cholesterol: HDL	2.9 ± 0.8	2.9 ± 0.9	0.980
Non-HDL, mg/dL	113 ± 34	106 ± 33	0.469
Triglycerides, mg/dL	79 ± 42	90 ± 56	0.469
Hemodynamics			
Systolic BP, mm Hg	114 ± 12	110 ± 10	0.129
Diastolic BP, mm Hg	66 ± 8	68 ± 9	0.455
Pulse pressure, mm Hg	48 ± 10	42 ± 8	**0.009**
Mean arterial pressure, mm Hg	71 ± 7	70 ± 7	0.623

Values are means ± SD. BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein; M: men; W: women. Blood markers were collected for 21 omnivores and 27 vegetarians.

Diet History Questionnaire II

Results from the DHQ-II are displayed in Table 2. All nutrient data were normalized to 1000 kcal except for food groups. Omnivores consumed more energy, protein, saturated fat, cholesterol, magnesium, vitamin B12, and choline than the vegetarians. In contrast, vegetarians consumed more carbohydrates and fiber. When dietary intake was broken down by food groups, omnivores consumed meat, fish, and poultry regularly. Furthermore, the DHQ-II confirmed that the omnivore group consumed 2 servings of red meat per week. Vegetarians ate more fruits and

omnivores ate more eggs. No other differences were observed regarding food groups.

Supplementary Table 1 presents the results from the DHQ-II separated by diet and sex.

We also quantified the HEI score based on the DHQ-II responses**. Supplementary Table 2** shows HEI results separated by diet and sex. No interaction or main effect of diet were found. Only a main effect of sex (P = .041) was found, which showed that women had higher HEI scores regardless of dietary pattern.

Table 2 – Dietary intake calculated by the DHQ-II in healthy omnivores and vegetarians.

Variable	Omnivores	Vegetarians	P
Energy, kcal/d	2040 ± 734	1651 ± 673	.040
Macronutrients			
Total carbohydrate, g	104 ± 25	132 ± 23	<.001
Total protein, g	45 ± 8	33 ± 7	<.001
Total fat, g	43 ± 7	40 ± 9	.131
Saturated fat, g	13 ± 2	11 ± 3	.002
Monounsaturated fat, g	17 ± 4	16 ± 5	.207
Polyunsaturated fat, g	8 ± 2	9 ± 4	.110
Nutrient			
Fiber, g	9 ± 3	16 ± 6	<.001
Cholesterol, mg	197 ± 84	86 ± 84	<.001
Na^{+}, mg	1651 ± 190	1606 ± 371	.562
K^{+}, mg	1578 ± 387	1915 ± 718	.029
Mg^{+}, mg	176 ± 47	240 ± 91	.002
Ca^{+2}, mg	556 ± 259	557 ± 215	.976
Vitamin B12, mcg	3 ± 1	1 ± 1	<.001
Choline, mcg	207 ± 53	153 ± 43	<.001
Food groups			
Grains, oz	5.4 ± 2.8	4.6 ± 2.8	.282
Whole grains, oz	0.8 ± 0.5	0.9 ± 0.6	.337
Refined grains, oz	4.6 ± 2.4	3.6 ± 2.3	.142
Meat, poultry, and fish, oz	5 ± 2.6	0 ± 0	<.001
Red meat, oz	2 ±1.5	0 ± 0	<.001
Eggs, oz	1 ± 0.8	0.5 ± 0.6	.004
Dairy, cup	1.8 ± 1.6	1.2 ± 1.2	.112
Fruit, cup	1.1 ± 0.6	1.8 ± 1.2	.009
Vegetables, cup	2.2 ± 1.2	2.9 ± 2.7	.211

Values are means ± SD. Nutrient data has been normalized to energy intake per 1000 kcal except food groups, which are expressed according to U.S. Department of Agriculture MyPyramid Equivalents Database.
Abbreviations: Ca^{+2}, calcium; DHQ-II, Diet History Questionnaire II; K^{+}, potassium; Mg^{+}, magnesium; Na^{+}, sodium.

Pulse wave analysis, pulse wave velocity, and wave reflection

Hemodynamic measures from the PWA are presented in Table 3. Central systolic and diastolic pressures as well as central MAP showed no differences between groups. However, central pulse pressure was higher in the omnivore group. Al- though AIx showed no differences between the groups, forward pressure wave amplitude was higher in the omnivore group. There were no

differences in the backward pressure wave amplitude nor reflection magnitude. PWV was not different between groups. **Supplementary Table 3** shows PWA, PWV, and wave reflection separated by diet and sex.

Table 3 – Blood pressure and hemodynamic measures in healthy omnivore and vegetarian participants.

Variable	Omnivores	Vegetarians	*P*
Heart rate, beats/min	60 ± 10	63 ± 7	.104
Central SBP, mm Hg	101 ± 10	100 ± 9	.698
Central DBP, mm Hg	70 ± 7	71 ± 7	.825
Central MAP, mm Hg	81 ± 9	82 ± 7	.611
Central PP, mm Hg	32 ± 5	29 ± 5	.048
AP, mm Hg	2 ± 4	3 ± 4	.550
AIx, %	6.9 ± 12.3	8.8 ± 13.5	.569
AIx75, %	-1.4 ± 16	3.3 ± 14	.238
Forward wave amplitude, mm Hg	26 ± 3	24 ± 3	.048
Backward wave amplitude, mm Hg	12 ± 2	11 ± 2	.641
Reflection magnitude, %	46 ± 6	48 ± 9	.378
Pulse wave velocity, m/s	5.6 ± 0.8	5.3 ± 0.8	.171

Values are means ± SD.
Abbreviations: AIx, augmentation index; AIx75, augmentation index normalized to 75 beats/min; AP, augmentation pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure.

Measures of endothelial function

Endothelial function was assessed by brachial artery FMD and PLM, as shown in Table 4 and Figure 1. Baseline diameter, peak diameter, absolute FMD response and area under the curve shear to peak diameter did not differ between groups. Furthermore, % FMD (omnivores = 6 ± 2.9%; vegetarians = 6.9 ± 3.3%; *P* = .290) was not different between groups (Fig. 1A). Baseline LBF during PLM was higher in omnivores and so was peak flow, although this difference was trending. Peak change as well as percent change in LBF were similar between groups. LBF AUC for the first 60 seconds of PLM (omnivores = 132.7 ± 96.9 mL; vegetarians = 125.6 ± 76.6 mL; *P* = .784) was also similar between groups (Fig. 1B). **Supplementary Table 4** presents endothelial function measures separated by diet and sex.

Table 4 – Endothelial function measures in healthy omnivore and vegetarian participants.

Variable	Omnivores	Vegetarians	P
Brachial artery FMD			
Baseline diameter, mm	3.73 ± 0.73	3.58 ± 0.73	.334
Peak diameter, mm	3.95 ± 0.72	3.83 ± 0.81	.426
Absolute FMD response, mm	0.21 ± 0.09	0.25 ± 0.15	.450
AUC shear rate to peak, A.U.	20585 ± 8004	22113 ± 7417	.484
Passive leg movement			
Baseline flow, mL/min	220 ± 111	158 ± 68	.041
Peak flow, mL/min	674 ± 377	504 ± 230	.081
ΔLBFpeak, mL/min	454 ± 298	346 ± 191	.163
Percent change, mL/min	203 ± 88	252 ± 192	.502

Values are means ± SD.
Abbreviations: ΔLBFpeak, peak change in leg blood flow; A.U., arbitrary unit; AUC, area under the curve; FMD, flow-mediated dilation.

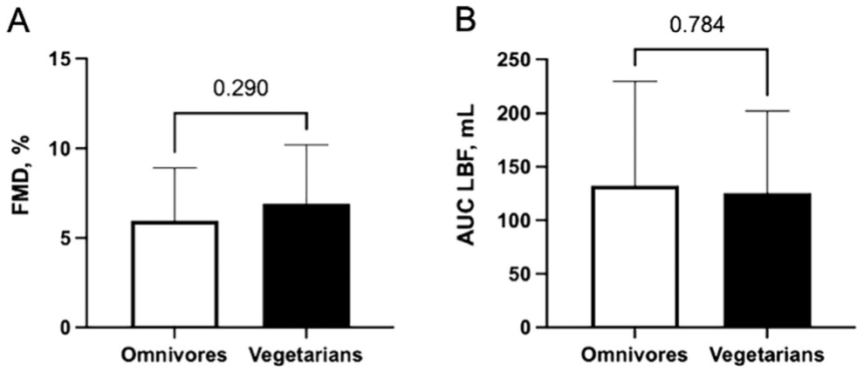

Figure 1 – (A) Brachial artery flow-mediated dilation (FMD) in omnivores (n = 22, white bar) and vegetarians (n = 29, black bar). (B) Area under the curve (AUC) leg blood flow for the first 60 seconds of passive leg movement (PLM) in omnivores (n = 21, white bar) and vegetarians (n = 26, black bar). Values are means ± SD.

Discussion

The present study sought to determine whether differences in vascular function exist between healthy, normotensive vegetarians and their omnivorous counterparts. Contrary to our hypothesis, there were no differences in arterial stiffness, augmentation index, or endothelial function between young healthy normotensive adults who have been consuming a vegetarian diet for at least 5 years and those who have been consuming 2 servings of red meat per week. We observed a lower central pulse pressure and a lower forward wave amplitude in vegetarians; however, PWV, although greater in omnivores, was not different between our 2 groups. Our data suggest that young healthy adults have comparable vascular function independent of dietary pattern. Although the dietary data confirmed that our vegetarians consumed a plant-based diet

with greater intake of fiber and potassium, this was not associated with an additional health benefit beyond central pulse pressure and forward wave reflection with regard to the vasculature at this time. Omnivores and vegetarians did not differ either in diet quality, which was evaluated with the HEI, 2015. It may be that this plant-based dietary pattern becomes more critical during later stages of the lifespan.

The literature has widely reported that those who follow plant-based diets have a lower BP [15,31,40,41,42,43]. We did not find peripheral BP differences between our vegetarian and omnivore groups which may be attributed to their younger age, nonsmoking status, nonobese BMI, and overall healthy lifestyle. Those studies demonstrating a lower BP in their vegetarian groups have studied middle-age to older adults (mean age was 50 and 55 years) who appear to gain a benefit from following a plant-based diet for much of their lives [21,44,45]. Furthermore, physical activity levels were similar between our 2 groups, and regular activity is an important lifestyle factor that may offset poor dietary choices. Beyond measuring peripheral BP, we also evaluated central pressures, which have not been previously reported. Omnivores in our study exhibited a higher central pulse pressure and forward wave amplitude which may over time lead to arterial stiffness [46]. This is important as elevations in central pressures can occur in response to an increased forward pressure wave amplitude that leads to increased left ventricular workload, left ventricular hypertrophy, and decreased diastolic perfusion pressure. Ultimately, this can lead to decreased coronary blood flow [47], suggesting that over time, an in- creased risk for CVD may occur in those consuming red meat regularly.

We investigated several measures of arterial stiffness including carotid-to-femoral PWV and AIx in our study. Both parameters did not differ between groups. Acosta-Navarro et al. [48] showed decreases in PWV in healthy middle-aged (mean age was 46 years) vegetarian men compared with their omnivore counterparts. They concluded that a vegetarian diet (of at least 4 years) was independently and negatively associated with PWV [31]. AIx, a measure of wave reflection, has not previously been evaluated between vegetarians and omnivores. Although we did not see any differences in our young healthy cohort, we did see a greater forward pressure wave amplitude in the omnivore group. As part of our exploratory analysis, we observed that men had increased central pressures and forward pressure wave amplitude as well as PWV than women independent of dietary pattern, as shown in other studies [49,50]. Furthermore, there is some evidence to suggest that a healthy diet is associated with lower arterial stiffness and wave re- flection. In a study by Sauder et al. [51], diet quality was evaluated using the 2010 DGA Index (DGAI-2010) and correlated with cardiovascular outcomes in approximately 6000 healthy middle-age to older adults (mean age was 48 years) from the Framingham heart study who are part of the Offspring and Third Generation cohorts. A higher DGAI-2010 score, reflecting greater adherence to the DGA guidelines, was associated with lower PWV and AIx, even after adjusting for CVD risk fac- tors. Although the DGAI scored meat consumption and did not separate out vegetarians from omnivores, it is interesting to note that a healthier diet is associated with less arterial stiffness. In our study, we saw some differences in dietary intake of specific nutrients between the 2 groups although the vegetarian dietary pattern did not provide an overall benefit in this study. It is important to consider that our omnivore group was relatively healthy and younger than the subjects in these studies [48]; therefore, this may have reduced the likelihood of seeing

differences. Finally, the HEI score was not different between our omnivores and vegetarians. Last, women overall had a higher HEI score than men independent of dietary pat- tern.

Our study sought to determine whether differences in endothelial health are present in younger vegetarians compared with their meat-eating counterparts as endothelial dysfunction may precede the development of CVD. We did not find any differences between the 2 groups in regards to brachial artery FMD, a measure of macrovascular function. To date, only 1 study has compared FMD between omnivores and vegetarians. They found that vegetarians (mean age, ~59 years; mean duration of vegetarianism, 8 years) had a greater FMD compared with omnivores. Furthermore, those vegetarians with the highest FMD values had followed a vegetarian diet for the longest [30]. This suggests that vegetarians in our study may experience more benefits with time if they continue to follow a vegetarian diet pattern as they age. In contrast, a study in Chinese individuals found that vegetarians (mean age, 45 years; average duration of vegetarianism, 16 years) exhibited increased carotid intima media thickening, decreased endothelial function as assessed by brachial artery FMD, and increased MAP compared with an age-matched omnivore group [52]. They attributed their negative findings to a deficiency in vitamin B12, a nutrient that naturally occurs in foods of animal origin only. In a subsequent study, those vegetarians supplemented with vitamin B12 for 12 weeks had improved FMD [53]. We also found that vitamin B12 intake was lower in our vegetarian group, which is not surprising given that B12 is found in animal-based foods, or those products fortified with B12. However, this does not appear to have impacted endothelial function as we found no differences between groups. Although we did not measure plasma levels of vitamin B12 to confirm the presence of a deficiency, this is an important area to consider in future research.

Finally, our FMD results coincide with Page et al. [54], who observed no differences between healthy, young (mean age was 20-25 years), vegan (for at least 6 months), and omnivorous men. We also measured endothelial function at the microvascular level evaluating blood flow responses to PLM . PLM is considered a robust measure of vascular function using the femoral artery and has been studied in a variety of populations [55,56,57]. Although brachial artery FMD is thought to reflect the nitric oxide-mediated contribution to dilation, PLM may reflect a greater percentage of the nitric oxide- mediated contribution to dilation as 60% to 80% of the hyperemic response can be attributed to nitric oxide [58,59]. To date, no studies have looked at the relation between a vegetarian diet and nitric oxide-mediated contributions to dilation using PLM. One study reported that after four weeks of following a Mediterranean diet (including white meat), healthy young volunteers (mean age, 25-26 years) experienced an increased microvascular blood flow measured via Laser Doppler Flowmetry compared with those following a vegan diet (without animal products) [60]. In this study, the PLM parameters were not different between groups suggesting that younger, healthy vegetarians did not have a greater overall vasodilatory response. We did observe a higher base- line leg blood flow in omnivores however, this is likely be- cause of a greater number of men in this group. In line with other studies [61], men in our study also had a higher base- line and peak brachial artery diameter than women independent of the diet followed. In summary, our measures of endothelial function at both the macrovascular and microvascular level showed no differences between vegetarians and omnivores.

The results of this study should be interpreted in the con- text of its strengths and limitations. Strengths include all women being tested in the early follicular phase to account for hormonal influences on endothelial function as well as using several indices of endothelial function and

arterial stiffness. Regarding limitations, we relied on self-report and the use of a food frequency questionnaire that asked about food consumption over the past year to confirm vegetarian and omnivore status. We also had an uneven distribution of men and women in each group because of difficulty recruiting vegetarian men and omnivore women who ate red meat regularly. Last, we did not assess sleep, which has been shown to impact vascular function [62].

Conclusion

In this study, we aimed to address potential differences in vascular function in a group of young healthy normotensive adults following either a vegetarian diet or an omnivorous diet containing red meat. Our participants, regardless of dietary pattern, had similar endothelial function and arterial stiffness. However, we did observe that our vegetarians had a lower central pulse pressure and forward pressure wave amplitude which may lower the risk of future CVD. Future re- search is needed to elucidate the cellular mechanisms regulating the relation between vegetarian and omnivores diets and cardiovascular health as well as examining these measures in older vegetarians.

Supplementary Tables

Table S1. Dietary Intake separated by Diet and Sex in Healthy Omnivore and Vegetarian Participants

Variable	Omnivores		Vegetarians		*P*		
	Men	Women	Men	Women	Diet	Sex	Diet*Sex
Energy, kcal/day	2222 ± 784	1882 ± 674	2151 ± 687	1551 ± 638	0.347	**0.030**	0.544
Macronutrients							
Total Carbohydrate, g	106 ± 29	102 ± 22	118 ± 33	134 ± 20	**0.004**	0.414	0.160
Total Protein, g	46 ± 11	44 ± 4	34 ± 5	33 ± 7	**<0.001**	0.483	0.769
Total Fat, g	43 ± 9	43 ± 5	44 ± 14	39 ± 7	0.596	0.321	0.207
Saturated fat, g	13 ± 3	14 ± 2	14 ± 2	10 ± 3	0.136	0.168	**0.021**
Monounsaturated fat, g	17 ± 6	17 ± 3	17 ± 8	15 ± 4	0.426	0.505	0.865
Polyunsaturated fat, g	8 ± 2	8 ± 2	10 ± 7	9 ± 3	0.155	0.938	0.718
Nutrient							
Fiber, g	9 ± 3	9 ± 2	10 ± 2	17 ± 6	**0.002**	**0.027**	**0.012**
Cholesterol, mg	186 ± 89	206 ± 84	160 ± 110	72 ± 71	**0.002**	0.185	**0.037**
Na^{+}, mg	1658 ± 223	1644 ± 163	1514 ± 226	1625 ± 395	0.383	0.607	0.507
K^{+}, mg	1648 ± 425	1517 ± 354	1479 ± 344	2003 ± 746	0.376	0.273	0.070
Mg^{+}, mg	185 ± 58	168 ± 35	185 ± 65	251 ± 92	0.072	0.278	0.068
Ca^{+2}, mg	576 ± 198	538 ± 307	520 ± 178	565 ± 224	0.847	0.963	0.581
Vitamin B12, mcg	3 ± 1	3 ± 1	2 ± 1	1 ± 1	**<0.001**	0.713	0.337
Choline, mcg	203 ± 55	210 ± 52	168 ± 54	151 ± 41	**0.002**	0.761	0.411
Food Groups							
Grains, oz	2.6 ± 0.7	2.6 ± 0.9	3.4 ± 1.7	2.7 ± 1.2	0.140	0.343	0.333
Whole grains, oz	0.4 ± 0.2	0.4 ± 0.3	0.5 ± 0.2	0.6 ± 0.4	0.072	0.697	0.604
Refined grains, oz	2.2 ± 0.6	2.2 ± 0.8	2.9 ± 1.5	2.1 ± 1.0	0.245	0.210	0.185
Meat, poultry, and fish, oz	2.7 ± 1.6	2.4 ± 0.9	0 ± 0	0 ± 0	**<0.001**	0.529	0.624
Red meat, oz	0.9 ± 0.4	1.0 ± 0.6	0 ± 0	0 ± 0	**<0.001**	0.672	0.567
Eggs, oz	0.3 ± 0.3	0.4 ± 0.6	0.6 ± 0.2	0.4 ± 0.3	0.183	0.684	0.265
Dairy, cups	0.9 ± 0.5	0.8 ± 0.6	1.2 ± 0.6	0.6 ± 0.6	0.862	0.120	0.305
Fruit, cups	0.6 ± 0.4	0.5 ± 0.3	0.6 ± 0.4	1.3 ± 0.8	**0.046**	0.134	0.071
Vegetables, cups	1.2 ± 0.9	1.1 ± 0.5	0.8 ± 0.3	2.1 ± 1.7	0.457	0.129	0.072

Values are means ± SD. Nutrient data has been normalized to energy intake per 1,000 kcal except food groups which are expressed according to U.S. Department of Agriculture MyPyramid Equivalents Database. Ca^{+2}, calcium; K^{+}, potassium; Mg^{+}, magnesium; Na^{+}, sodium

Table S2. HEI-2015 results separated by diet and sex in Healthy Omnivore and Vegetarian Participants

Variable	Omnivores		Vegetarians		*P*		
	Men	Women	Men	Women	Diet	Sex	Diet*Sex
Total HEI-2015 score	62.4 ± 10.3	64.9 ± 12.3	62.7 ± 7.3	72.8 ± 7.8	0.185	**0.041**	0.212
Adequacy components							
Total Fruits	2.8 ± 1.1	3.3 ± 1.8	3.8 ± 1.9	4.3 ± 1.1	**0.034**	0.235	0.974
Whole Fruits	3.8 ± 1.3	3.9 ± 1.7	3.9 ± 1.6	4.7 ± 0.8	0.215	0.330	0.347
Total Vegetables	4.3 ± 1.3	4.4 ± 0.9	3.7 ± 1.2	4.6 ± 0.9	0.656	0.156	0.210
Greens and Beans	4.1 ± 1.5	4.6 ± 1.1	3.7 ± 1.7	4.6 ± 0.9	0.613	0.077	0.544
Whole Grains	2.2 ± 1.4	2.5 ± 1.3	2.9 ± 1.5	3.4 ± 1.9	0.118	0.456	0.820
Dairy	6.2 ± 2.8	6.2 ± 3.1	6.3 ± 4.7	4.4 ± 3.4	0.418	0.322	0.354
Total Protein Foods	4.8 ± 0.4	4.6 ± 1.1	3.7 ± 1.7	4.3 ± 1.0	**0.029**	0.557	0.200
Seafood and Plant Proteins	3.6 ± 1.6	4.3 ± 1.2	4.1 ± 1.4	4.9 ± 0.3	0.070	**0.033**	0.934
Fatty Acids	4.8 ± 3.1	5.5 ± 3.5	4.7 ± 4.9	7.3 ± 3.3	0.410	0.138	0.365
Moderation components							
Refined Grains	8.0 ± 1.5	8.2 ± 2.2	5.0 ± 3.8	7.9 ± 2.7	**0.032**	**0.049**	0.081
Sodium	4.0 ± 2.0	3.4 ± 1.9	5.2 ± 2.8	5.1 ± 3.1	0.083	0.659	0.753
Added Sugars	8.6 ± 2.7	9.1 ± 1.1	9.6 ± 0.9	9.4 ± 1.2	0.202	0.717	0.493
Saturated Fats	5.1 ± 2.5	5.1 ± 3.2	5.9 ± 3.0	7.8 ± 2.6	**0.047**	0.251	0.264

Values are means ± SD. HEI, healthy eating index.

Table S3. Blood Pressure and Hemodynamic Measures separated by Diet and Sex in Healthy Omnivore and Vegetarian Participants

Variable	Omnivores		Vegetarians		*P*		
	Men	Women	Men	Women	Diet	Sex	Diet*Sex
Heart rate, bpm	58 ± 11	61 ± 8	62 ± 5	64 ± 8	0.242	0.318	0.806
Central SBP, mm Hg	104 ± 12	97 ± 8	108 ± 4	98 ± 8	0.452	**0.003**	0.599
Central DBP, mm Hg	72 ± 5	68 ± 7	75 ± 3	70 ± 7	0.264	**0.019**	0.554
Central MAP, mm Hg	82 ± 9	79 ± 8	88 ± 3	81 ± 7	0.183	**0.043**	0.432
Central PP, mm Hg	35 ± 4	29 ± 3	33 ± 5	29 ± 4	0.310	**<0.001**	0.483
AP, mm Hg	2 ± 4	3 ± 4	2 ± 4	3 ± 4	0.766	0.504	0.992
AIx, %	4.4 ± 12.5	9.1 ± 12.2	6.8 ± 12.1	9.2 ± 14.0	0.749	0.379	0.780
AIx75, %	-3.9 ± 16.3	0.8 ± 16.2	0.4 ± 11.6	3.8 ± 14.1	0.433	0.382	0.890
Forward wave amplitude, mm Hg	27 ±3	24 ± 2	26 ± 4	23 ± 2	0.352	**<0.001**	0.799
Backward wave amplitude, mm Hg	12 ± 2	11 ± 2	13 ± 1	11 ± 3	0.862	0.053	0.969
Reflection Magnitude, %	45.5 ± 6.3	46.6 ± 6.7	48 ± 8	47.9 ± 9.7	0.398	0.911	0.731
Pulse wave velocity, m/s	5.9 ± 0.7	5.3 ± 0.8	5.8 ± 0.7	5.2 ± 0.8	0.651	**0.022**	0.869

Values are means ± SD. AP, augmentation pressure; AIx, augmentation index; AIx75, augmentation index normalized to 75 bpm; MAP, mean arterial pressure; PP, pulse pressure.

Table S4. Endothelial function measures separated by Diet and Sex in Healthy Omnivore and Vegetarian Participants

Variable	Omnivores		Vegetarians		*P*		
	Men	Women	Men	Women	Diet	Sex	Diet*Sex
Brachial Artery FMD							
Baseline diameter, mm	4.4 ± 0.5	3.3 ± 0.4	4.1 ± 0.5	3.4 ± 0.7	0.812	**<0.001**	0.383
Peak diameter, mm	4.6 ± 0.5	3.5 ± 0.4	4.5 ± 0.7	3.6 ± 0.7	0.894	**<0.001**	0.652
Absolute FMD response, mm	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.083	0.076	0.119
FMD, %	4.9 ± 1.8	6.7 ± 3.4	9.2 ± 5.5	6.4 ± 2.7	0.060	0.828	0.058
AUC shear rate to peak, a.u.	1451 ± 5200	16307 ± 5643	18177 ± 2889	16429 ± 5616	0.234	0.885	0.261
Passive Leg Movement							
Baseline flow, mL/min	247 ± 140	196 ± 74	142 ± 74	162 ± 68	**0.044**	0.866	0.409
Peak flow, mL/min	821 ± 446	540 ± 254	463 ± 279	514 ± 223	0.058	0.249	0.098
ΔLBFpeak, mL/min	575 ± 344	344 ± 209	321 ± 211	352 ± 191	0.127	0.214	0.104
Percent change, mL/min	234 ± 85	175 ± 84	214 ± 91	262 ± 211	0.649	0.439	0.270
AUC LBF, mL	164 ± 109	104 ± 791	135 ± 82	123 ± 77	0.864	0.208	0.397

Values are means ± SD. ΔLBFpeak: peak change in leg blood flow; A.U.C.: area under the curve; FMD: flow-mediated dilation

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APPENDIX B

Adolescent Obesity: Diet Quality, Psychosocial Health, and Cardiometabolic Risk Factors

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Introduction

Adolescence is a critical period of development, defined by navigating challenging social circumstances and cementing identity as youth transition into emerging adulthood.[1] Adolescence is also a time of immense growth—second only to the first year of life—and as such, nutrient requirements increase substantially.[2] Data from the National Health and Nutrition Examination Survey (NHANES), which surveys representative samples from varying age groups of the United States, are collected every fiscal year, referred to as a cycle, on an array of health-related topics including overweight and obesity status.[3] In youth, overweight and obesity status is commonly classified by age- and sex-specific body mass index (BMI) percentiles, determined by growth charts developed by the Centers for Disease Control and Prevention using historical data from national surveys.[4] Measurement at or above the 85th percentile and below the 95th percentile on the age- and sex-specific growth charts indicates overweight status, while measurement at or above the 95th percentile indicates obesity.[5,6] Severe obesity in children and adolescents is defined by a BMI percentile at or above 120% of the 95th percentile.[6] As identified by NHANES, rates of childhood and adolescent obesity have more than tripled since the 1970s and severe obesity rates have more than quintupled within the same timeframe.[7,8] Data collected from the 2015–2016 NHANES cycle indicated that 18.5% of youth aged 2–19 years in the United States were obese, of which 5.6% were classified as severely obese.[7] Adolescents aged 12–19 years, the age range utilized throughout this review to define adolescence, had the highest prevalence of obesity at 20.6%, compared to 18.4% for youth aged 6–11 years and 13.9% for children aged 2–5 years.[7] Even more concerningly, youth from different ethnic groups are disproportionally obese, with Mexican American, Hispanic, and non-Hispanic black youth having above average prevalence of obesity.[7,8]

Consistently, overweight or obese children and adolescents are more likely to have elevated BMIs as adults.[6,9,10] Adolescents with BMIs above the 85th percentile are more likely to be obese by age 35 than their normal weight counterparts.[6] The probability of being overweight or obese as an adult increases with both youth BMI percentile and age, with obese adolescents being at the highest risk for obesity during adulthood.[6,9,11,12] In particular, youth who are obese during their teenage years have an over 90% likelihood of being overweight or obese at 35 years.[9] A study that combined several national datasets to model obesity trajectories from childhood through to middle age found that overweight or obesity at age 18 increased the risk of being obese in adulthood and that risk for adult obesity was more accurately assessed in adolescents rather than younger age groups.[12]

Similar to adults, obesity in adolescents impacts all major organ systems and often contributes to morbidity.[13,14] Adolescent obesity promotes inflammation and increases the risk of chronic disease development into and throughout adulthood.[15] Compared to those who are of normal weight, adolescents who are obese are at increased risk for adverse health effects, including cardiovascular risk factors such as hypertension, dyslipidemia, and endothelial dysfunction,[16-21] and metabolic risk factors including insulin resistance and hyperglycemia.[16,22-26] These risk factors persist throughout adolescence and into adulthood.[14,16,21,25-31] The diminished cardiometabolic health status that often digresses throughout adolescence is associated with the adoption of poor dietary and physical activity behaviors.[27]

Factors Contributing to Adolescent Obesity

Diet Quality

Diet quality can be measured to better understand overall eating patterns. One common method for assessing diet quality is through the generation of Healthy Eating Index (HEI) scores, from either food frequency questionnaires or 24 h recalls.[32] Currently NHANES utilizes two 24 h recalls to collect dietary information; however, prior to 2002, only one 24 h recall was collected.[33,34] The HEI allows for the assessment of overall diet quality as well as individual dietary components, including those recommended to be limited in the diet.[35,36] Higher HEI scores indicate an eating pattern more congruent with dietary recommendations than lower scores, and are based on a coordinating edition of the Dietary Guidelines for Americans, which is updated in accordance every five years.[32,35] The current method of the HEI was first created to coincide with the Dietary Guidelines for Americans, 2005 and MyPyramid recommendations.[37] This version of the index assessed diet quality through adequacy, or moderation, of 12 components as a ratio of overall energy intake.[37] Many of these components were carried forward into the subsequent version of the HEI in 2010, although milk, meats and beans, and calories from solid fats, alcoholic beverages, and added sugars were renamed to dairy, total protein foods, and empty calories, respectively.[35] The remaining components were modified in this update to better reflect updated dietary recommendations, including the inclusion of seafood and plant proteins and adding refined grains as a component to limit.[35] For the most recent update, HEI-2015, the only component change was the splitting of empty calories into saturated fat and added sugars.[36] Therefore, HEI-2015 is a composite score of 13 components to assess how well individuals meet key recommendations outlined in the 2015–2020 Dietary Guidelines

for Americans.[32,36,38] As with previous versions, an overall HEI-2015 score ranges from 0 to 100, with 100 representing an eating pattern exactly aligned with recommendations.[32,36]

Youth in the United States do not meet dietary recommendations and adolescent diet quality is of particular concern. An expansive analysis of NHANES data covering seven cycles, from 1999 to 2012, included over 17,000 adolescents aged 12–18 years, out of a total of over 38,000 youth aged 2–18 years.[33] This study assessed adolescent diet quality utilizing HEI-2010, as well as analyzing trends over time.[33] Consistent with other analyses,[33,39-42] overall diet quality was shown to decrease with age as adolescents persistently had significantly lower overall HEI scores compared to younger youth.[33] Furthermore, results indicated that adolescents 12–18 years had an average overall HEI score of 48.4 out of 100 in the 2011–2012 NAHNES cycle, which was a significant increase from the average overall score of 40.4 observed during the 1999–2000 cycle.[33] On trend with overall diet quality improving over time,[33] the overall HEI-2015 score for adolescents 12–18 years was 52.0 in a more recent analysis.[42] Although overall diet quality scores have significantly improved over time for adolescents, the current scores are still considered low.[33,42]

Many countries, including the United States, have food-based dietary guidelines that include recommendations for food group consumption.[43] The majority of countries that have food-based dietary guidelines use five food groups, including starchy staples (grains for MyPlate in the United States), fruits, vegetables, dairy foods, and protein foods. While these guidelines may be more understandable to the general public, they do not provide recommendations for the consumption of specific nutrients. Most recent analyses of dietary data collected from NHANES

show that, in general, adolescents are able to meet recommendations for protein including having better consumption of seafood and plant sources of protein compared to younger age groups.[42] However, these analyses also suggest that adolescents are not meeting recommendations for fatty acids.[42] The HEI-2015 scoring for fatty acids is based on a ratio of polyunsaturated and monounsaturated fatty acid relative to saturated fatty acid intake.[36] With an average score of only 3.7 out of 10, it is likely that adolescents generally consume higher levels of saturated fatty acids in comparison to unsaturated fatty acids.[42] Additionally, the overall poor diet quality of adolescents is driven by the inadequate consumption of components considered more healthful, such as fruits, vegetables, and whole grains.[39-42,44] Analyses suggest that adolescents only consume about half the recommendations for fruits and vegetables[39,41,42] and with an average HEI score of 1.32 out of a possible 10, were consuming below the whole grains recommendation in one analysis.[39] The inadequate intake of these food groups perpetuated dietary fiber to be recognized as a nutrient of concern in 2015–2020 Dietary Guidelines for Americans.[45] Another recommendation outlined in the 2015–2020 Dietary Guidelines for Americans is to shift away from consuming added sugars.[45] Before becoming a singular category in HEI-2015,[36] added sugars were included in the HEI-2010 "Empty Calories" component, along with solid fats and alcohol, and are calculated negatively into the overall score.[35] While data from NHANES 2005–2010 suggested that adolescents had high consumption of empty calories,[39] a separate analysis utilizing data from NHANES 1999–2012 showed a substantial decrease in empty calorie consumption over time.[33] Although this trend was an improvement, empty calorie consumption in adolescents still exceeded recommendations in both studies[33,39] and adolescents were only meeting about half the recommendation for reducing added sugar consumption in a more recent study using HEI-2015.[42]

Socioecological Influences

In addition to developmental changes, adolescence is a period of social change, with adolescents progressing toward increased autonomy, and perhaps may result in the establishment of dietary habits.[2,46] The Social-Ecological Model describes that food choices can be influenced from several different levels, spanning from intrapersonal factors to policy and systems.[47,48] These sectors of influence can have differential effects on an individual's risk for overweight or obesity.

Ethnicity and socioeconomic status (SES) are two factors that are associated with youth obesity rates. The prevalence of obesity is higher in Hispanic and non-Hispanic black youth compared to non-Hispanic white youth within the same age group.[49] In 2016, the prevalence of obesity for Hispanic and non-Hispanic black adolescents aged 12–19 years were 25.9% and 25.0%, respectively, which was substantially higher than the 17.2% observed in non-Hispanic white adolescents.[49] The prevalence of severe obesity was also highest among these groups, with 11.6% of Hispanic adolescents and 11.5% of non-Hispanic black adolescents being considered severely obese, compared to only 6.7% of non-Hispanic white adolescents.[49] In line with these values, data collected through NHANES suggest that non-Hispanic black adolescents typically have the lowest overall diet quality scores compared to other youth.[33,41,42] However, Mexican-American and Hispanic adolescents tend to have the highest overall diet quality and component scores compared to other groups,[33,41,42] which is surprising given the high prevalence of overweight and obesity observed in Hispanic youth.[7] Similar results were found in a study assessing the diet quality of high school students utilizing HEI-2010, with Hispanic students having higher overall HEI scores compared to non-Hispanic white youth.[50] One potential

explanation for this observation is the lack of physical activity opportunities for adolescents from some ethnic/racial minority groups and communities of lower SES. Analyses of NHAHES 2007–2016 data showed that adolescents from low-income families participated in less physical activity than more affluent adolescents.[51] This association may be the result of reduced access to parks, playgrounds, and exercise facilities, which is more prevalent in less advantaged communities[52]; a problem that is even more prevalent in communities where the population is predominantly of an ethnic/racial minority group.[53] A nationally representative study found that neighborhoods primarily comprising ethnic/racial minority and low SES groups were half as likely to have access to a physical activity facility on their block.[53] This is a substantial disadvantage given that the assessment also found that access to one of these facilities significantly decreases the odds of adolescent overweight.[53]

An analysis that included 10 years of NHANES data sought to better characterize the role SES plays in modifying diet quality of Mexican-origin youth.[54] For this study, high or low SES was estimated with consideration for education and income-to-poverty-ratio.[54] As in other analyses of NHANES data,[33,41,42] Mexican-origin youth of the same generation as non-Hispanic white youth had higher overall diet quality, as determined by HEI-2010 scores.[54] Interestingly, the average HEI score for overall diet was significantly lower in third-generation Mexican-origin youth from low SES families compared to first and second generations.[54] This decrease in overall diet quality as generation progressed was perceived to be from acculturation and the increased consumption of empty calories, as is more customary in a typical American diet.[54] The trend in later generations having poorer diet quality was attenuated by SES as no significant differences in diet quality were observed between generations from high SES families.[54] Unlike the

association found with Mexican-origin youth, overall diet quality scores from NHANES data have either shown no difference between the highest and lowest income youth[41] or were occasionally significantly associated with income level, but the direction of this association was not consistent over time.[33] Despite this, lower income households tend to have a higher prevalence of obesity than higher income households.[55] Similarly, there is, generally, an inverse relationship between head of household education attainment and youth obesity.[55] In 2016, youth obesity prevalence was highest for those whose head of household did not receive a high school diploma.[49]

Youth from lower SES families are also more likely to experience food insecurity.[56-58] Food security can be categorized into one of four ranges: very low, low, marginal, and high food security.[59] Classification into one of these ranges is determined by how often a family or individual experiences distress involving food selection or alters eating patterns due to insufficient resources to obtain food.[57,59] The United States Department of Agriculture monitors food insecurity rates utilizing an annual survey. Most recent estimates have shown a continuous decline in the percentage of food insecure households since 2011.[57] While low-income families and households with children, in particular non-Hispanic black and Hispanic households, remain at percentages above the national average,[57] this shift in prevalence is promising given that household food insecurity is related to overweight and obesity in youth.[56,58,60,61]

Adolescence is marked by increased autonomy and a transition from spending the majority of time with parents to away from home with peers.[46,62] While parents still provide guidance on certain matters, peers assert more influence on superficial concerns, especially as adolescents

enter teenage years.[62] This influence in regard to eating behaviors may be perpetuated by a desire to fit into a particular peer group, among other complex factors.[63] Peer influence is evident in adolescent selection and consumption of food,[63] with mixed observations on whether the tendency is toward encouragement or discouragement of consuming healthy foods.[63] Peers, especially friends, can have a beneficial effect on adolescent eating patterns. One study found that adolescent diet quality scores were positively related to healthy food choices made by peers.[46] Another study found that healthful aspects of best friends' eating patterns can be influential for adolescents and result in consumption of significantly more vegetables, whole grains, and dairy.[64] While statistically significant, the increases observed in this analysis were not substantial, with adolescents consuming an additional 0.09, 0.14, and 0.08 servings of vegetables, whole grains, and dairy, respectively.[64] In practice, the 0.08 serving increase in dairy would be roughly equivalent to 0.5 ounces of fluid dairy or about one tablespoon of milk. A cross-sectional study assessing youth and adolescent diet quality, observed no relationship between overall HEI-2010 score and friend support for eating healthy or unhealthy foods.[65]

Despite increased autonomy, parents still play a role in shaping adolescent eating. Parents influence adolescent eating patterns through food procurement and by modeling and supporting healthy eating behaviors.[46,66-69] In the cross-sectional analysis mentioned previously, parental offering of food considered unhealthy was associated with decreased diet quality scores.[65] However, 40% of the sample also indicated that their parents rarely or never offered unhealthy foods, thus modifying their availability and accessibility.[65] If high-fat foods and sweets are not being offered, then consumption may be limited allowing for higher adolescent diet quality.

Furthermore, the availability of fruits and vegetables in the home is correlated with adolescent fruit and vegetable consumption.[67,70]

Adolescent Stress and Adiposity

Physiological Stress

Adolescence is known to be a stressful developmental period, and emerging research supports the need to address psychosocial stress as a factor in obesity prevention and management.[71-74] The psychosocial stress arising from poor body image and social ostracization, especially associated with adolescent obesity, may further promote stress and corresponding health-compromising coping mechanisms.[75] Stress is broadly defined as the body's response to a real or perceived threat beyond the ability to cope.[76] A perceived threat activates the neuroendocrine hypothalamic-pituitary-adrenal (HPA) axis, ultimately resulting in the secretion of cortisol from the fasciculata of the adrenal cortex.[77] Cortisol binds to receptors found in the peripheral and central nervous system, where its objective is to mobilize and redistribute energy stores to maintain homeostasis and minimize incurred damage to the individual until the threatening stimulus has passed.[78] Outcomes of chronic HPA-axis activation include effects on gluconeogenesis and glycogenolysis,[79] lipolysis,[80] insulin resistance,[81,82] and compromised reproductive functions.[83]

Cortisol is essential for organism survival.[84] However, the effects of chronic stress are systemically deleterious, as glucocorticoid receptors are ubiquitously spread throughout body tissues, such that nearly every organ system is affected.[85] Under chronic stress, the characteristic negative-feedback nature of the HPA-axis may become dysfunctional, which increases the risk

of developing a host of metabolic and affective disorders.[85] Prolonged stress contributes to allostatic load, where the body develops new "set points" including, but not limited to, higher blood glucose, stress sensitivity, and reactivity.[86] Chronic psychosocial stress promotes metabolic derangement including adiposity, as well as abnormal eating behaviors including over- or under-eating, and preferentially selecting highly palatable foods.[87] Furthermore, prolonged stress also confers increased risk for developing numerous chronic diseases, including metabolic syndrome,[88] diabetes mellitus,[89] cardiovascular disease,[90] obesity,[71] and mental health disorders.[91]

Adolescent Stress

Adolescents are especially vulnerable to the negative effects of stress, at least partially due to the sensitization of the HPA-axis that occurs during this period.[92] Adolescence is a developmental period marked by heightened stress reactivity and sensitivity, increased emotionality, and increased incidence of both risk-taking and harm-avoidant behaviors.[93] Adolescents typically experience heightened stress sensitivity and prolonged reactivity in a sex-dependent manner, with basal and stress-responsive cortisol typically higher in females.[94] Many of the affective and behavioral signatures typical of adolescence can be explained by rapid gonadal hormone development and non-linear neurodevelopment.[95] In adolescents, limbic brain regions involved with motivation, instant gratification, and reward develop much more rapidly than do cortical regions involved in inhibitory control.[93] Thus, limbic brain circuitry is more likely to predominate over less mature cortical regions during emotionally salient contexts.[96] The effects of stress on metabolism and food choice plus the psychosocial stress experienced by adolescents with obesity are critical points for consideration.

Stress-Motivated Eating Behavior

Both animal and human studies have demonstrated that the majority of individuals preferentially select highly palatable foods when stressed, whether-or-not they exceed their caloric requirements.[87,97] This once conferred evolutionary advantage, as additional calories increased the likelihood of escaping from or fighting—and thus surviving—what were historically acute physical threats.[98] Modern stress is largely chronic and psychogenic in nature rather than physical.[98] These chronic stressors, coupled with a more sedentary modern lifestyle, result in an evolutionary mismatch; the body employs conserved response mechanisms to psychosocial stress, which involve increased drive to seek out palatable foods meant to aid in fighting or fleeing a threatening situation.[76] Repeated exposure to psychosocial stressors, and the resultant consumption of such highly palatable foods in our modern environment may, ultimately, increase the risk of developing overweight and obesity.

Adolescents are at increased risk of partaking in unhealthy behaviors, especially in emotionally salient contexts.[95] Maturation in brain regions involved in reward seeking may underpin the drive for palatable food consumption in adolescence.[99] In fact, the repeated consumption of palatable foods in this critical window of neurodevelopment may derail normal maturation processes, thus predisposing the adolescent brain to abnormal eating behaviors.[99] Palatable foods eaten under stress are typified by sweet taste and tend to be foods high in rapidly digesting, simple carbohydrates.[100] The physiologic signals that arise from consuming palatable foods rich in simple carbohydrates orchestrate cognitive, metabolic, and behavioral responses to stress, which, over time, may increase obesity risk.[101,102] Importantly, sweet taste is instantly rewarding, and may promote reinforcement learning—even in the absence of post-prandial metabolic signals,

which can also contribute to overconsumption and obesity.[103] This was exemplified when rats given oral administration of sucrose solution demonstrate reduced stress responses, whereas intragastric gavage of sucrose had no such effect.[104] In humans, this attenuation of stress in response to consuming palatable foods high in simple carbohydrates has been shown when exogenous carbohydrate consumption before a combined mental and physical stress challenge mitigated effects of stress.[105]

Metabolic Effects of Palatable Food Consumption

Stress-related emotional eating in the absence of hunger involves the motivation and reward-associated brain networks that override homeostatic feeding cues originating from the hypothalamus.[106] The post-ingestive metabolic signals arising from continually exceeding caloric requirements for weight maintenance promote increased energy storage and reduced expenditure, and these effects are exacerbated under stress.[107] Postprandial effects of consuming palatable foods include blood glucose elevation, which is met by an increase in insulin secretion.[108] Effects of insulin in tandem with the effects of cortisol on disruption of glucose and insulin homeostasis, further promote energy storage, especially in the visceral region.[109] In addition to promoting glucose homeostasis, insulin also interacts with neuropeptides to increase energy expenditure and reduce food intake in the absence of stress.[110] The neuroendocrine axes orchestrating stress and energy balance overlap, with notable neuropeptides and hormones involved in energy balance also influencing stress regulation.[111]

Leptin is an adipocyte-derived hormone with anorectic effects and has been shown to dampen HPA activity associated with chronic stress.[112] Leptin has both central and peripheral targets,

where combined effects with insulin and other anorexigenic hormones result in, but are not limited to, alterations in food intake, glucose and lipid metabolism, pancreatic islet B-cell secretion, reproductive function, immunity, and energy expenditure.[113,114] In the fed state, centrally acting leptin is secreted from the arcuate nucleus of the hypothalamus, then activates neurons associated with increased satiety and energy expenditure, and inhibits neurons associated with increased food intake and weight gain.[115] Circulating leptin concentrations are often high in individuals with obesity, thus suggesting a state of leptin resistance.[112,116,117] Whereas leptin deficiency can be corrected with exogenous recombinant leptin administration, leptin resistance is not attenuated with the introduction of additional hormones.[118] With respect to stress, one study showed that a seven-day glucocorticoid treatment intervention resulted in increased food intake despite increased serum leptin levels.[119] Conversely, another study demonstrated that high glucocorticoids after a social stressor were associated with transient increases in plasma leptin, thus resulting in temporarily suppressed appetite and food consumption under stress.[120] Hypercorticism is oftentimes observed in obesity, and glucocorticoids are known to restrain the effects of leptin.[121] Thus, psychogenic stress promotes metabolic dysregulation directly and indirectly through its influence on hormones and neuropeptides involved with energy balance.

Obesity-Associated Psychogenic Stress in Adolescents

Obesity can be a stressful state due to weight stigma[122] and adolescents who experience stress related to social ostracization are more likely to rely on food-related coping mechanisms.[123] This behavior is immediately rewarding and may contribute to temporary solace and improved mood,[124] however, repeated intake in excess of caloric needs will result in weight gain, thus

perpetuating the cycle.[125] Psychogenic stress as a result of weight stigma may contribute to disordered eating habits in adolescents.[126] A prospective cohort study collected 10 waves of data from 1420 participants and found that victims of bullying in childhood and adolescence had an increased likelihood of developing anorexia nervosa and bulimia.[127] Furthermore, adolescents experiencing weight-related stigma are at increased risk of engaging in secretive eating, characterized by eating in solitude to avoid being seen by others.[128] Secretive eating is correlated with binge eating and the onset of other eating disorders, and may be related to depression and poor body image.[128] In a cross-sectional study examining 577 youth, those endorsing secretive eating experienced greater eating-related psychopathology.[128] Additionally, it was found that adolescents experience more dietary restraint and purging than younger youth.[128]

Sex differences, personality types, cultural and familial normative beliefs, self-worth, and learned coping mechanisms all inform the extent to which an individual internalizes and copes with psychosocial stress.[129] For example, neuroticism partially accounted for associations between depression and chronic life stress in 603 adolescents in a study exploring risk factors for emotional disorders.[130] Furthermore, depression was associated with chronic life stress in females only, and low extraversion partially accounted for associations between social phobia and chronic life stress.[130] With regard to sex differences, female sex hormones contribute to higher stress sensitivity and sustained stress responses.[94] Adolescents are at an increased risk for dieting with the goal of weight loss,[131] and those who experience personal factors such as weight concern, body dysmorphia, and depression are more likely to develop disordered eating behaviors 10 years later.[132] One study found that body image dissatisfaction in adolescent females was associated with self-esteem.[133] Females are also at higher risk of developing eating

disorders.[134] Finally, restrained eaters, those who consciously elect to restrict intake of food quantity or food types,[135] are at higher risk of emotional eating compared to unrestrained eaters.[136]

Obesity and Cardiometabolic Disease in Adolescents

Cardiovascular Disease

Cardiovascular disease (CVD) is the number one cause of death in the United States.[137] By the year 2030, the percentage of the population suffering from CVD is projected to approach 44%.[138] Although CVD is generally perceived as a disease of adulthood, studies suggest that atherosclerosis often begins in childhood or adolescence.[26,139,140] Cardiovascular risk develops as a culmination of the atherogenic process over the lifespan.[20,26,28,141] Progression of atherosclerosis is related to the number and intensity of cardiovascular risk factors, which develop in childhood and track into adulthood[21,26,142] and may be independent of adult weight.[143] It is estimated that 70% of obese children and adolescents ages 5–17 years have at least one cardiovascular risk factor.[144] Risk factors—for example, hypertension and dyslipidemia—are directly, positively associated with the presence and severity of early atherosclerotic lesions in adolescents and young adults.[20,140] Obese children have been observed to have significantly impaired arterial elasticity and endothelial function.[21] In addition, obesity in youth is associated with increased cardiac mass and intima-media thickness in adulthood.[145-147] Out of the cardiovascular risk factors, obesity is the most predictive of future disease[22,142] and adolescent obesity is projected to yield an increase in coronary heart disease in adulthood.[26]

The Bogalusa Heart Study, a long-term epidemiologic study of cardiovascular disease risk factors beginning in childhood, assessed cardiovascular risk factors, including serum lipid concentration, blood pressure, and BMI, in children and adolescents, following them from youth into adulthood.[22,141,142] Findings suggest that intensity of cardiovascular risk in youth predicts subclinical atherosclerosis and adult morbidity and mortality.[27,142] The Pathobiological Determinations of Atherosclerosis in Youth Study, another large-scale study of atherosclerosis in adolescents and young adults (15–34 years) also assessed the presence and extent of early atherosclerotic lesions in relation to cardiovascular risk factors in subjects who underwent autopsy.[148] Their results were in agreement with those from the Bogalusa Heart Study; intimal lesions were present in the aorta of all subjects aged 15–19 years and severity increased with age.[148] Other studies report that specifically an android fat distribution, or central adiposity, is correlated with dyslipidemia and arterial stiffness in youth.[20,21] Tounian et al.[21] suggested that android fat distribution, dyslipidemia, and insulin resistance may be primary contributors to these vascular impairments. Obese youth had significantly higher levels for each of these parameters,[21] which aligns with several other studies examining lipid profile, blood pressure, and glucose and insulin concentrations in obese youth.[144,149-151]

Severity of obesity is also relevant.[14,152] In a large-scale, cross-sectional study utilizing data from NHANES 1999–2012, researchers observed that all cardiometabolic risk factors were elevated as severity of obesity increased in adolescents.[153] When controlling for age, race/ethnicity, and sex, greater severity of obesity yielded increased risk of dyslipidemia, hypertension, and elevated glycated hemoglobin level.[14]

Type 2 Diabetes Mellitus

Similar to CVD, insulin resistance and type 2 diabetes mellitus (DM) are obesity-related complications previously thought to develop in adulthood that are becoming increasingly more prevalent in younger populations.[25,154,155] As with adults, central adiposity in youth is associated with insulin resistance.[21] The first metabolic abnormality seen in obese youth is hyperinsulinemia.[156] The decrease in insulin sensitivity that occurs with puberty further compounds insulin resistance in obese adolescents.[23] In addition to the inflammatory response, adiponectin is considered to partially explain the relationship between obesity and type 2 DM.[157] Due to its negative association with insulin resistance,[158] adiponectin has been considered an insulin-sensitizing adipokine[157,159] and is inversely related to adiposity.[150,160] Obesity is also strongly, negatively correlated with adiponectin level in adolescents, as well as in children and adults.[161,162] Lower levels of adiponectin are associated with increased levels of insulin resistance in obese adolescents[163] such that most youth with insulin resistance are overweight or obese.[158] In addition to obesity and insulin resistance, low adiponectin levels in youth are also associated with hypertension and dyslipidemia and may therefore predict the clustering of these symptoms of metabolic syndrome.[24,164] The presence of these risk factors in obese children and adolescents compounds the risk for the development of subsequent type 2 DM and CVD in youth.[150]

Compound Risk: Obesity, Diabetes, and Cardiovascular Disease

The diagnosis of DM is an established risk factor for vascular disease and the early development of CVD.[26] The metabolic abnormalities in energy utilization that are associated with DM cause diabetic dyslipidemia.[165] Also, the chronic hyperglycemia often seen in combination with obesity results in damage to the vasculature.[166] For this reason, adolescents with obesity are at

significantly increased risk for accelerated atherosclerosis.[26] Children with the described cluster of metabolic abnormalities were more likely to have type 2 DM and clinical cardiovascular events after a follow-up of 25 years.[30,31] Even in absence of the metabolic abnormalities, there is a strong association between obesity in adolescence and subsequent development of the metabolic syndrome cluster in adulthood,[26] which may be attributed to obesity-induced chronic inflammation.[167] Pro-inflammatory adipokines—for example, leptin—have been implicated in the development of both obesity-related type 2 DM and CVD.[154] Obesity-induced insulin resistance is also associated with increased carotid intima-media thickness[168] and endothelial dysfunction in obese adolescents.[169,170]

Intervention Opportunities

Without intervention, it is projected that most youth will be overweight or obese and likely suffering from chronic diseases in adulthood given current expected trajectories.[11,12,25] Diet and lifestyle modification in adolescence or earlier is essential in the prevention of the development of chronic diseases in adulthood. The concept that youth are in the subclinical stages of cardiometabolic disease, which may be exacerbated by stress, emphasizes the need for early intervention.[14,18,27,150,171-173] The abnormal accumulation of lipids in the vascular wall is a reversible stage in the atherogenic process,[26] making the early stages of atherosclerosis, which often appear in youth, an ideal opportunity for intervention. Early identification and intervention may attenuate clinical manifestation and improve long-term health outcomes.[26] Analyses of results from several prospective longitudinal cohort studies found that risk for hypertension, dyslipidemia, atherosclerosis, and type 2 DM in obese youth who became non-obese by adulthood were similar to those who were never obese.[142,147,174,175] These findings suggest that

weight management in youth, adolescence, and young adulthood may at least partially diminish cardiometabolic risk in adulthood.[29] Weight loss coupled with lifestyle modifications, including stress reduction, increased physical activity, and improvements in diet, is often sufficient to improve insulin sensitivity and can thereby assist in the prevention or control of type 2 DM without the need for exogenous insulin administration.[154,176,177]

According to the United States Burden of Disease Collaborators, the primary risk factor related to disease burden was found to be suboptimal diet.[178] It has been recommended that interventions geared toward improving adolescent dietary behaviors are thoroughly planned in advance to ensure that they are designed, implemented, and monitored appropriately for the targeted population.[179] In designing interventions, the most successful have been developed in line with a theoretical framework, most commonly the Social Cognitive Theory (SCT).[179] The SCT is utilized in nutrition interventions due to its consideration for improvement of individual factors, such as self-efficacy and knowledge, as well as environmental factors, when facilitating behavior change.[180] Utilizing SCT as the guiding theoretical framework also aids in designing behaviorally-focused interventions that modify the environment while also being developmentally appropriate for the intended participants, which have also been implicated as elements of successful nutrition interventions.[179]

Nutrition interventions for adolescents are frequently implemented through comprehensive school-based interventions and multicomponent programming, as recommended by the Academy of Nutrition and Dietetics, Society for Nutrition Education and Behavior, and School Nutrition Association.[181] Multicomponent school-based interventions have shown promise for improving

dietary intake and health status of children and adolescents.[182] Multicomponent programs commonly include nutrition education implemented in the classroom; modifications to school policies and the food environment; and methods for parental involvement.[182]

Further recommendations include adjustments to the school environment in order to facilitate acquisition of healthful behaviors and the promotion of evidence-based nutrition education that includes opportunities for youth to grow and prepare food.[181] Programs that incorporate garden and cooking components are important given their potential for translatable and long-term effects. Gardening experience during childhood is valuable as it has been associated with significantly higher fruit and vegetable consumption during late adolescence compared to older adolescents with no prior gardening experience.[183] Furthermore, frequent gardening is beneficial in that gardening weekly or even monthly has been associated with high fruit and vegetable consumption compared to infrequent or no gardening.[183] A recent survey of high school students found that adolescents who had a home garden or experience with community gardening or farming were significantly more likely than others without experience to try new fruits and vegetables.[184] Additionally, adolescents with a home garden were more likely to consume adequate amounts of vegetables.[184] As for inclusion of cooking, a review of programs that incorporate cooking found that participation in these programs has the potential to beneficially impact youth knowledge, skills, and behaviors related to nutrition in addition to cooking.[185] It has been found that participation in food preparation during adolescence was associated with a continuation of enjoyment and involvement in food preparation as an emerging adult.[186]

In accordance with these recommendations, several recent interventions targeting youth dietary habits have included garden components[187-193] and cooking components.[189,191-195] While the age range in most of these studies included ages prior to adolescence,[187,188,191,193,194,196] methods utilized and findings from these studies may have application for adolescents. Compared to controls, programs that included a gardening component resulted in greater willingness to try vegetables,[187,188,197] preferences for vegetables,[187,188,197] and reported vegetable consumption.[187,190] Similarly, participants in a nutrition program geared toward cooking had improvements in reported fruit and vegetable post-intervention consumption.[194] This program also resulted in increased reported nutrition knowledge, cooking self-efficacy, and cooking at home after completing the intervention.[194] Programs that included both gardening and cooking components observed that, compared to controls, participants had significantly higher fruit and vegetable[193] and nutrition knowledge.[196] Additionally, youth participating in the programs were significantly more likely correctly identify vegetables,[196] consume fruits and vegetables daily,[193] and be willing to try new foods.[191]

The emerging concept of food literacy takes recommendations for the incorporation of growing and preparing food further. Broadly, food literacy is defined as the interconnection between the knowledge, skills, and behaviors necessary for procuring, planning, and preparing healthful food.[198] Food literacy is quite complex, encompassing several components including elements of nutrition, health, agriculture, food systems, food safety, and cooking.[198-200] Three reviews of adolescent food literacy programs have been conducted recently.[201-203] Two of these reviews highlight the need for a reliable and validated questionnaire to assess food literacy as a whole, given that none of the studies reviewed supplied such an assessment.[201,203] Varying

interpretations of food literacy prior to establishment of a definition[198] limited the ability to develop an assessment to encompass the complexity of food literacy. With this and contrasting study designs, all three reviews noted difficulty in determining inclusionary criteria for articles and interpreting results.[201-203] Given the limitations of previous adolescent food literacy programs, Brooks and Begley compiled a list of recommendations for future programs, including the development of adaptable school-based food literacy programs for older adolescents.[202]

Future Directions

Interventions are needed to aid in the reduction and prevention of adolescent obesity. As obesity is a complex health concern with numerous contributing factors, effective intervention strategies may require a multifactorial approach aimed at reducing stress and cardiometabolic risk factors while also empowering adolescents with the knowledge and skills necessary to make informed and healthful dietary choices. Some studies have suggested that mindfulness interventions for adolescents may be feasible for decreasing distracted eating[204] and reducing stress and depressive symptoms,[205] which can aid in reducing and preventing obesity. Additionally, interventions that include exercise and calorie restriction components can be effective at reducing obesity and cardiometabolic risk factors.[206,207] These methods—in combination with multicomponent school-based interventions and skill-building health education programs, such as those that promote food literacy—warrant further research. However, a thorough review of the literature consistently demonstrates a gap with respect to adolescent food literacy education. Given that food literacy education is a comprehensive approach to target the upstream behaviors leading to obesity and related comorbidities, the timeliness of a program to combat this issue is critical. Aligning with the above recommendations,[202] guided by SCT,[180] and the definition established

by Vidgen and Gallegos,[198] a food literacy curriculum for high school-aged adolescents has been developed.[208] The curriculum, Teens CAN: Comprehensive Food Literacy in Cooking, Agriculture, and Nutrition (Teens CAN), includes experiential lessons within twelve modules that comprise opportunities to advance food literacy.[208] Teens CAN[208] will be incorporated into an existing multicomponent program, the Shaping Healthy Choices Program,[189] to provide an intervention aimed at improving diet quality and the overall health status of children and adolescents. While this is one suggested approach, the ultimate goal is to mitigate the effects of childhood and adolescent obesity. Resources including time, money, and effort should be allocated toward this type of obesity prevention programming.

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