Better health through plant-based functional foods

By

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Abstract

The concept of food as medicine has existed for centuries. The focus of this dissertation is on plant-based functional foods that contain unique bioactive compounds with properties beyond basic nutrition. The health benefits of plant-based food consumption are supported by large epidemiological studies and randomized controlled trials demonstrating that the intake of fruits, vegetables, and whole grains are inversely associated with the rate of chronic disease morbidity and mortality. Two functional foods, sorghum and red wine, were studied. Chapter I provides a historical overview on the use of food as medicine and the application to modern times, with an emphasis on sorghum and red wine. Chapter II presents three probe studies that detail the postprandial plasma amino acid and glucose responses in healthy adult men following the intake of extruded or conventional sorghum flour. Chapter III explores the effects of Hokkaido Zweigelt red wines from different vintage years on cardiovascular health outcomes in healthy adult men. Chapter IV reviews the results of clinical research studies on red wine and vascular health, discussing factors that contribute to variable results, and proposes considerations for future studies. Chapter V provides perspectives and insights for future clinical nutrition research. Additionally, Appendix A describes the role of strawberries for weight management in adolescents and offers

dietary recommendations. Appendix B summarizes the results of clinical studies with fruits, vegetables, nuts, and legumes on skin health outcomes. Taken together, the body of work emphasizes the importance of plant-based functional foods as key elements to support health promotion and disease prevention.

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Chapter I: Introduction to food and health

Introduction

The value of botanicals as traditional medicine has been documented or passed down through generations in many civilizations[1]. Aristotle, the ancient Greek philosopher, proposed the idea of using food beyond essential nutrition according to the quotes, "*Let thy food be the medicine and let medicine be thy food*" and "*Food moves in two directions. On the one hand, it works to regenerate the body while also limiting disease progression*"[2]. Later, the definitions of food and medicine were disconnected. The United States (US) Food and Drug Administration (FDA) defines a drug as "*A substance other than food intended to affect the structure or any function of the body*"[3]. However, food is a complex matrix that contains macronutrients, vitamins, and minerals for basic nutrition, as well as phytochemicals which can promote health through anti-inflammatory, antioxidant, and immune-modulatory properties[4]. No consensus term was used to define food that is used for therapeutic purposes until the 1980 Japanese academic community definition as "food that has physiological functions beyond nutrient functions, such as regulation of biorhythms, the nervous system, the immune system, and bodily defense"[5].

Functional foods are commonly defined as food that contains compounds "beyond basic nutrition," which can cause controversial interpretations. Various organizations, including the United Nations Food and Agriculture Organization (FAO) and the US Academy of Nutrition and Dietetics (AND) provide slightly different terms[6]. The definition provided by the FAO[7] is "*a food stuff that provides a health benefit beyond basic nutrition, demonstrating specific health or medical benefits, including the*

prevention and treatment of disease" while the 2013 position statement from the AND[8] noted *"…whole foods along with fortified, enriched, or enhanced foods that have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis at effective levels based on significant standards of evidence*".

Three levels of disease prevention exist: primary, secondary, and tertiary[9]. Nutrition can help prevent illness before the development of the disease (primary), maintain health conditions after the diagnosis (secondary), and alleviate the symptoms of the disease (tertiary)[9]. Functional food containing bioactive compounds other than basic nutrition is mainly used for primary and secondary prevention, including cardiovascular diseases (CVD) and type II diabetes[9,10].

Whole foods such as grains, fruits, and vegetables are functional foods[11]. Large epidemiological studies, such as the EPIC study[12], and the combined analysis from the Nurses' Health study and the Health Professional study[13] reported significant inverse associations between the consumption of plant-based foods (whole grains, fruits, vegetables, and products such as red wine and olive oil) and mortality and morbidities (e.g. cardiovascular diseases (CVD). Furthermore, large randomized controlled trials (RCTs), including the PREDIMED[14] and COSMOS[15] studies, showed that habitual consumption of plant-based foods rich in phenolic compounds (e.g., red wine, olive oil, and cocoa) significantly delayed the progression of chronic diseases and prolonged the individual lifespan, which was measured as reduced mortality.

Findings from observational studies such as cross-sectional, case-control, and cohort models help predict the relationship between food and health outcomes[16]. However, causality cannot be implied from observational studies; their findings can help

establish hypotheses for future RCTs with a robust study design[16]. Prior to starting a randomized controlled trial (RCT), the food or its bioactive components should be validated in preclinical studies to assess potential toxicity and identify an appropriate amount for testing in humans[17]. Preclinical studies are experimental processes before the application of food or products in humans, which includes *in vitro* animal or human cells and *in vivo* animal studies[17,18]. Clinical trials can be categorized into four phases: I, II, III, and IV[19]. Phase I is conducted to examine the pharmacokinetic and pharmacodynamic effects of a food; phase II is used to verify the effective amounts and the dose-response relationship, and phase III is conducted to evaluate the efficacy and safety of the food[19]. Phase IV will be conducted mostly in drug trials after the drug is approved in order to follow up for potential adverse events or interactions after a long-time use[19].

In this dissertation, two functional foods, sorghum and red wine were studied. Sorghum and wine are both ancient foods rich in phenolic compounds, but their journeys to being classified as functional foods differ. Wine has been used for medicinal purposes since ancient times[20]. Sorghum has been consumed as a staple food for centuries[21] and only recently have its health benefits for on gluten sensitivity[22], weight management and blood glucose control[23-27].

Sorghum

History

Sorghum (*Sorghum bicolor L.*) was the fifth most consumed grain worldwide in 2022/23, at 58.54 million metric tons[28]. The United States is the top producer at 11.375 million metric tons[29], of which exports 4.699 million metric tons were exported in 2021/2022[28,30]. Among the unique characteristics of sorghum compared to other grains are that it is resistant to drought and heat and can provide high yields even in harsh conditions[31].

Sorghum is an ancient grain that has been consumed since 8,000 BCE based on the historical evidence that seeds have been found in archeological sites in Egypt and Sudan[21]. Sorghum is thought to have traveled from Africa to India and then to China[32]. The ancient Aztec civilization also appear to have consumed sorghum[33]. Sorghum is currently a staple food in many parts of Africa, Asia, and South America[34], where it is consumed as porridge, bread, flatbread, or in fermented products[35].

In 1981, a lower mortality rate of esophageal cancer was reported in countries where sorghum was predominantly consumed (e.g., Nigeria, Uganda, Namibia, Mozambique, and Shantung province in China) compared to countries where major grains consumed were wheat or corn (e.g., Iran, Kazakhstan, Zimbabwe, and Linxian province in China)[36]. The idea that a staple food, rich in riboflavin, nicotinic acid, magnesium, and zinc was associated with a lower risk of esophageal cancer was novel at that time[36]. The research inspired others to further explore nutrients and bioactive compounds in sorghum that might lead to other health benefits[37].

In the past decade, sorghum has gained attention as a gluten-free alternative to wheat, barley, and rye[22], as well as its potential ability to help control blood glucose[26], reduce oxidative stress[38,39], and support the immune system[40]. These purported health benefits may be due to a relatively slow digestibility of carbohydrates and protein in the grain, as well as from the unique polyphenolic compounds such as tannins and anthocyanins[41]. Although polyphenolic compounds are favorable for modulating the health of individuals with cardiometabolic diseases, these compounds can bind to nutrients such as protein, making proteins less available for body utilization[42]. Other compounds in sorghum that bind to protein, including phytic acid, starch, and non-starch polysaccharides[43], can also reduce protein availability[44,45].

Nutrients and bioactive compounds

Sorghum is composed of 70-80% carbohydrates, 8-18% protein, 1-5% fats, and 19% dietary fiber[46]. Sorghum contains an appreciable amount of minerals, including calcium, potassium, magnesium and phosphorous, and vitamins such as niacin, riboflavin, thiamin, pyridoxine, and vitamin E[47]. The profiles of macronutrients and micronutrients in the grain can vary, depending on genetic, varietal, and environmental factors[48]. For example, red and black sorghums grown in a Mediterranean environment in Italy contained higher mineral contents of magnesium, potassium, and iron compared to white sorghum grown in the same area[49].

Sorghum is considered as a high-protein grain because it contains up to 18% protein[50], similar to the content in maize[51]. Proteins in sorghum are located in the endosperm, the largest part of the grain[44] (**Figure 1**). and exist as protein bodies, which are freely distributed or bound to carbohydrates[45] (**Figure 2**). Tight bonds between the

protein body and carbohydrate complex are thought to be responsible for low digestibility[52]. The major protein structure in sorghum is prolamin, which is rich in proline and glutamine[45,50]. The specific types of prolamin protein in sorghum are kafirins, which can be categorized as α, β, γ, and δ[53]. Kafirins are alcohol-soluble and heat resistant[45,50,54]. Cooking sorghum with heat and water decreases kafirin solubility, making sorghum porridge less digestible compared to porridge from other grains such as wheat, maize, and rice[50,52,53,55].

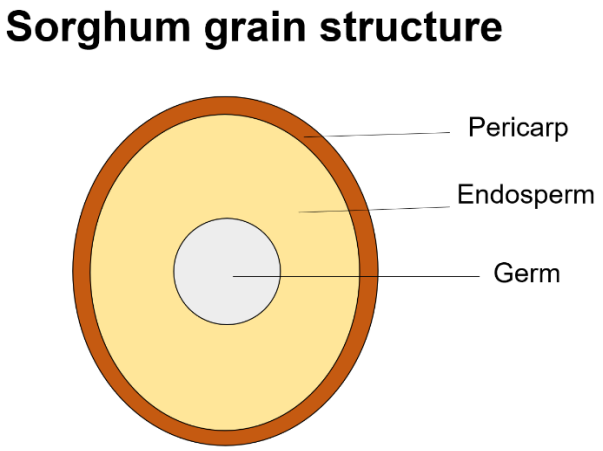

Figure 1. Sorghum grain structure (adapted from Mejia et al.[51])

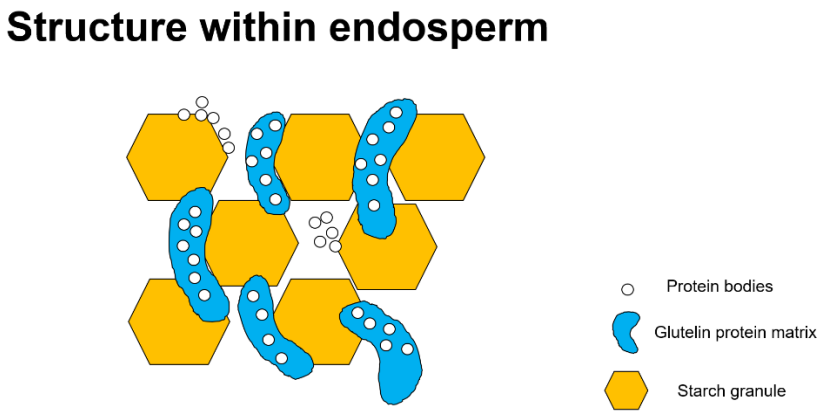

Figure 2. Sorghum protein bodies and starch granules within the endosperm (adapted from de Mesa-Stonestreet et al.[50])

In addition to essential nutrients, sorghum also contains polyphenols, secondary plant metabolites that are produced in response to environmental stress[56]. The types and concentrations of sorghum polyphenols vary depending on the varietal and environmental factors such as water, temperature, and soil conditions[56]. Polyphenolic compounds in sorghum also vary based on the color of the pericarp and the pigment in the testa, a structure between the pericarp and endosperm[57]. The types of sorghum can be categorized based on the color of the grains, which include white, lemon-yellow, red, brown, and black[58]. The predominant polyphenols in sorghum are procyanidins (i.e., tannins), phenolic acids, and anthocyanins[37]. A review on sorghum and health effects[37] reported that studies conducted in many species (rats[59], pigs[60,61], and rabbits[62,63]) observed significant weight loss after feeding tannins in sorghum, which might be due to low digestibility. This suggestion is supported by *in vitro* studies that note sorghum tannins form complexes with protein and carbohydrates which inactivate digestive

enzymes[37]. Furthermore, when compared to corn, male[64] and female[65] cattle fed with sorghum resulted in the lower starch digestibility.

Potential use of sorghum as a functional food

Following the 1981 report that sorghum may be a healthier grain option compared to wheat[36], research in the 1990s used sorghum as a key ingredient in a grain-based electrolyte solution. This mixture was verified for safety in children under five years of age with diarrhea and showed comparable effects to the standard oral rehydration solution in resolving diarrhea-related outcomes in countries including Nigeria[66], Pakistan[67,68], Kenya[69], and Sudan[70].

Since sorghum is a staple food in many under-developed countries, it has been researched as an option to alleviate malnutrition. One of the first studies explored the effects of four different varieties of whole sorghum (each provided 100-150 kcal/kg; 6-8% protein) on nitrogen absorption and retention in 13 preschool children (6-30 months) compared to a casein control. No differences in nitrogen absorption and retention were observed between the two groups, and sorghum intake was actually associated with undesirable outcomes such as increased weight loss compared to the control [71]. The same authors then conducted another study comparing the effects of whole sorghum and extruded sorghum in a similar aged population. Nitrogen assimilation was relatively poor in the whole sorghum group, with 46% absorption and 14% retention throughout 26 six-day sorghum feeding periods[72]. In contrast, the protein quality and digestibility of decorticated, extruded sorghum fed to nine children (seven to 24 months of age) increased protein absorption to 81% and maintained nitrogen retention at 21% of intake

when combined with high-protein ingredients such as casein, suggesting that the combination of extruded sorghum flour with casein may improve the digestibility of sorghum[72]. Extrusion employs temperature, pressure, and shear stress, which are thought to break the bond of kafirins with other compounds[53], while the traditional wet cooking has been shown to reduce protein digestibility[45] by 40-60% compared to the protein content of the raw grain[73].

Red wine

History

Wine is thought to originate as a food in the Neolithic period, when humans started domesticating plants and animals for consumption[74,75]. The oldest available archeological evidence for grape wine and viniculture was discovered from the biomolecular analysis of organic compounds absorbed into pottery from an excavation site in the South Caucasus region approximately 6,000–5,000 BC[74]. Wine consumption increased as the practice of wine grape cultivation and wine production knowledge were distributed throughout the Mediterranean and other regions, with evidence from areas of what are now France, Greece, Italy, and Iran[20,76]. The uses of wine grape alone or the mixture of it with other fruits, honey, and herbs as medicine were documented in ancient Greece, Mesopotamia, Egypt, and China[77-79]. In ancient China, wine was consumed for socialization and religious purposes[79]. Wine was also the medium in Christian rituals, influencing the distribution of viniculture (planting wine grapes in vineyards) and wine processing throughout the world[20].

In the 1980s, epidemiologists observed that the mortality rate of ischemic heart disease (IHD) in people from France was the lowest among 18 developed countries (Australia, Austria, Belgium, Canada, Denmark, England and Wales, Finland, France, Germany, Ireland, Italy, Netherlands, New Zealand, Norway, Scotland, Sweden, Switzerland, and the United States of America) regardless of similar saturated fat consumption. This difference in IHD was proposed to be related to the relatively higher wine consumption in French people compared to those from other countries[80]. The conclusions were controversial, since they were consistent with other epidemiological studies that observed an inverse relationship between alcohol consumption and CVD mortality, but seemed to undervalue or ignore outcomes from other studies[80]. In the 10-year prospective cohort from the World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease (WHO MONICA) project involving 41 centers, including most European countries, Australia, New Zealand, Canada, the US, China, and Japan[81,82], a 40% lower mortality rate from coronary heart disease (CHD) in France compared to other countries with proportional saturated fat intake was observed, which the authors termed the “French paradox”[83]. The authors further explored the rationale behind the French paradox and suggested that the amount of alcohol intake, primarily from wine, along with other dietary habits in Southern France (Toulouse), might contribute to the lower CHD rates in France compared to other countries with similar saturated fat intake and mean serum cholesterol, mean systolic blood pressure, and cigarette smoking[83]. Compared to other MONICA centers in France (Strasbourg and Lille), people in Toulouse had lower CHD mortality, while the HDL cholesterol was similar. When considering their diet, people in Toulouse consumed more whole grain

bread, vegetables, fruits, vegetable fat, and wine (i.e., a Mediterranean diet), while their cheese intake was higher[83]. This finding inspired scientists to explore the health benefits of red wine and its bioactive components.

A study by Frankel et al. assessed the effects of phenolic compounds in red wine on low-density lipoprotein (LDL) oxidation by conducting two *in vitro* experiments utilizing plasma LDL from two non-smoking participants with normal blood lipids (aged 41 and 64 years) [84]. The first experiment compared the effects of wine extracts at two different concentrations (500 and 1,000-fold dilutions) on LDL oxidation induced by Cu^{2+} and gas headspace[84]. The results showed that the higher concentration (500-fold dilution) inhibited LDL oxidation completely (100%), while the 1,000-fold extract was lower at 86%[84]. The second experiment compared the effect of 1,000-fold dilution to other antioxidants, including 10 μmol/L of alpha-tocopherol (vitamin E) and quercetin in the presence of Cu^{2+} (20-80 μmol/L)[84]. They found that diluted red wine could inhibit LDL oxidation almost 100% at all ranges of copper concentration and the antioxidant activity was similar to that of quercetin but higher than vitamin E[84], suggesting that the non-alcoholic components of red wine (phenolic compounds) might play important roles in reduction of CHD mortality through the reduction of oxidized LDL.

Further studies were conducted to understand the mechanisms behind the health benefits of red wines and/or similar phenolic compounds found in other foods such as fruits, vegetables, and grains. One such study was the European Prospective Investigation into Cancer and Nutrition (EPIC) study, a prospective cohort model that assessed the relationship between diet and chronic conditions, including cancer and

cardiovascular disease, involved 531,457 healthy adults aged 35 to 70 years from 23 centers in Europe[12]. The recruitment was conducted from 1993-1999, and participants were followed until 2004[12]. One of the key findings was that the combination of smoking cessation, being physically active, consumption of at least 5 servings per day of fruits and vegetables, and moderate alcohol intake were associated with 14 additional years of life[85]. The scientists from this project further explored the relationship between alcohol consumption and CVD risks and found that moderate alcohol intake (12 g/d or higher) was associated with reduced non-fatal CHD risks but increased risks of stroke subtypes[86]. A J-shaped curve of the relationship between alcohol consumption (as the x-axis) and CHD incidents (as the y-xais) was also observed in the selected population[86]. A sub-analysis of the EPIC study investigated the dietary polyphenol intake in Europe that described the main contributors to the health in a Mediterranean diet to be coffee, tea, and fruits, with the most abundant polyphenols being phenolic acids and flavonoids (caffeoylquinic acid, proanthocyanidin oligomers and polymers, tyrosol, hydroxytyrosol, and oleuropein-aglycone[87]. Two others' large cohort studies in the US, the Nurses' Health Study (NHS) and Health Professionals Study (HPS) also found results consistent with the EPIC study about the relationship between moderate alcohol consumption and lower CVD mortality[88,89]. The NHS was a prospective cohort study of only females recruited from 1980-2012 and 1989-2011, respectively[88]. One key finding was that moderate alcohol consumption (up to 1 drink per day; 10.8-15.1 g alcohol) was associated with reduced risks of CVD, gallstones, cognitive decline, and all-cause mortality but increased risks for breast cancer and bone fractures[88]. The HPS was another prospective cohort study, with the participants being males[89]. One of the

sub-analysis in the follow-up study of the HPS investigated the associations between long-term alcohol intake (before or after MI diagnosis) and all-cause and CVD mortality among myocardial infarction (MI) survivors[89]. Alcohol consumption was estimated from the questionnaire that asked the intake frequency, which could be categorized into four groups based on approximate daily alcohol serving (g alcohol/day), including 0 (0 g/d), 1 (0.1-9.9 g/d), 2 (10-29.9 g/d) and >2 (≥ 30 g/d). The results showed that long-term moderate alcohol consumption (up to two drinks per day; 0.1-29.9 g alcohol/d) was inversely associated with all-cause and CVD mortality with a U-shaped curve observed, similar to the J-shaped curve noted in the EPIC study. The association between moderate wine intake and CVD mortality was strongest among those who had less impaired cardiac function compared to those with more severe impairment[89].

As causality cannot be drawn from most observational studies[90], subsequent RCTs were conducted to assess the relationship between alcoholic beverage intake and CVD outcomes. The PREDIMED study is one of the largest parallel-arm RCTs that included red wine consumption as a factor in the intervention. This study randomly assigned participants to either a Mediterranean diet supplemented with extra virgin olive oil, the same diet with mixed nuts replacing the extra oil, or a low-fat control regimen[14]. The Mediterranean diet instructions encouraged appropriate servings of olive oil, nuts, fresh fruits, vegetables, seafood, legumes, sofrito, white meat, and wine (only to those who already regularly consume alcohol as a part of a meal) and discouraged consumption of soda, bakery goods, spread fat, and red and processed meat[14]. The mean intervention period of the study was 4.8 years with the total of 6,633 participants were included in the final analyses[14] (originally 7,447 people before the retraction due to

the issues with randomization and statistical analyses[91]). The main findings were that the groups following the Mediterranean diet (both the olive oil and nut groups) had 29% lower major CVD events compared to the low-fat control[14]. In a further cross-sectional analysis involving 3,897 older adults in their 60s or older, which focused on the effect of alcohol consumption on metabolic syndrome-related outcomes (triglycerides > 150 mg/dL, HDL ≤ 40 or 50 mg/dL (female or male, respectively), blood pressure > 130/85 mmHg, and fasting glucose >100 mg/dL)[92]. Participants were categorized into three groups: 1) non-drinker; 2) ≥ 1 serving/day; 3) > 1 serving/day (one serving was 100 mL of red wine, 250 mL of beer, 65 mL of liquors, 32 mL of spirits, or 10 g pure alcohol) and their alcohol intake was assessed using a food frequency questionnaire[92].The results showed that the group with moderate intake was associated with reduced risks of metabolic syndrome, including a reduction in HDL and blood pressure, which were consistent with other large cohort studies[92].

Red wine contains phenolic compounds in addition to alcohol. The large cohort studies noted above and the PREDIMED studies have consistently reported inverse associations between fruit and/or vegetable intake and total or CVD mortality, which may be due, in part, to the phenolic compounds in these foods. The main finding of the EPIC study was that higher fruit and vegetable intake was associated with decreased risks from all-cause mortality[12] while another sub-analysis found an association between higher flavonoids and lignan consumption and lower gastric carcinoma risk in women[93]. A combined analysis of the NHS and HPS projects showed an inverse association between five servings of fruit and vegetable intake and total mortality[13], which is consistent with the results from the EPIC study and the current dietary guidelines for

American 2020-2025[94]. In another cross-sectional analysis of the PREDIMED study, people who consumed three or more servings of fruits per day were associated with reduced waist circumference, fasting glucose and LDL, but increased systolic and diastolic blood pressure compared to those who consumed less than one serving[95]. Interestingly, cardiometabolic risks differed depending on the color of the fruits[95]. Researchers involved in the PREDIMED study also explored which foods were the major sources of polyphenols in a typical Spanish diet and ranked the top five as coffee (18% of total dietary polyphenols), orange (16%), apples (12%), olives and olive oil (11%), and red wine (6%)[96]. However, these results are general for polyphenols and more details are needed. When considering polyphenol subclasses, red wine contains proanthocyanidins, anthocyanins, catechins, hydroxybenzoic acids, dihydroxyflavonols, stilbenes, and other polyphenols such as hydroxytyrosol[96]. A cross-sectional exploration of the PREDIMED study also reported several polyphenol metabolites, including resveratrol and hydroxytyrosol, were associated with wine intake[97,98].

Nutrients and phenolic compounds

Red wines typically consist of water (86%), alcohol (12%), glycerol and polysaccharides or other trace elements (1%), acids (0.5%), and volatile compounds (0.5%)[99]. Although the main components of red wines are similar across different types and different countries, the bioactive compounds in red wines, such as alcohol percentage and polyphenolic profiles, vary[100].

Volatile compounds typically comprise less than 1% of red wine by volume and polyphenols are part of this portion[99]. Polyphenols can be categorized as phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid), coumarins, flavonoids (flavonols,

flavones, flavan-3-ols, flavanones, anthocyanidins, and isoflavones), stilbenes, and lignans[101]. The highest concentration of polyphenols in red wine are present as flavonoids, particularly anthocyanins (malvidin-3-O-glucoside at 99.7 mg/L) , procyanidin dimers (94.7 mg/L), and flavan-3-ols [(+)-catechin (68.1 mg/L) and (-)-epicatechin (37.8 mg/L]). Resveratrol is not a flavonoid in red wine and has been studied for the health benefits[102], but the actual concentration in the red wine is low[103]. Ellagitannins are another non-flavonoid in red wine that can be absorbed from the wood during barrel aging[104] and may also contribute to bioactivity[105].

Anthocyanins are the pigment compounds that impart red, blue, and purple colors to red wine. The major anthocyanins in red wine are malvidin, cyanidin, and delphinidins. Mavidin-3-glucoside is the main anthocyanin that imparts color[106]. Anthocyanin consumption is associated with improved cardiovascular health, which is curious since bioavailability appears to be low[107].

Flavan-3-ols and proanthocyanins are derived from the seeds and skin of the grape and contribute to the quality of the wines, including an indicator of ripeness of the grapes and the bitterness/astringency of the red wine[108]. Among the four flavan-3-ol isomers, (-)-epicatechin has most bioactive effects on vascular function[109]. Proanthocyanins are polymers of flavan-3-ols and can be categorized as type A, B, and C[110]. Procyanidin dimers B3 and B4 are the most common types of proanthocyanidins in red wine[111].

Resveratrol is a stilbene polyphenol that is produced in response to environmental stresses, including physical damage to plants, microbial infection, UV and heat exposures, and pathogenic conditions[112]. The skin is the source of resveratrol in wine

grapes (50-100ug/g)[113], which contributes to significantly higher concentrations compared to white wines[62] and the alcoholic beverage made from other raw materials[111]. The trans-resveratrol isomer exerts biological activity related to cardiovascular health both *in vitro*, *in vivo* and clinical studies[102].

Ellagitannin is a polymer of ellagic acid, the dimer of gallic acid[110] that can be found in red wine that comes from aging of red wine in oak or other wood barrels[104]. Ellagic acid and ellagitannins from food are metabolized into bioactive urolithins by the gut microbiota, *Gordonibacter spp*.[114].

Digestion, absorption, and metabolism of bioactive compounds

Red wine contains alcohol and phenolic compounds, which either individually or synergistically contribute to the health benefits. As each compound has different optimal conditions for metabolic processing, understanding the digestion, absorption, and metabolism is helpful when designing a clinical study exploring the effects of red wine on health outcomes.

Absorption and metabolism of alcohol

Once alcohol is consumed, absorption occurs directly throughout the gastrointestinal tract[115]. The rate of absorption is fast if alcohol is consumed alone, but absorbed slower in the presence of food[115]. Alcohol is minimally absorbed in the mouth and esophagus and slightly higher in the stomach at approximately 20% of the alcohol consumed[116]. The major site of absorption of alcohol is the small intestine, where 70% of alcohol is absorbed[116]. The majority of absorbed alcohol (~90%) circulates through

the body, interacts with epithelial cells of different organs, and is transported via the portal vein to the liver for further metabolism, while the remaining portion is directly excreted through the skin, urine, and breathe[117]. The liver is the main site of metabolism of alcohol, which involves two main enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)[118]. The ADH enzyme converts ethanol into acetaldehyde via an oxidation process using nicotinamide adenine dinucleotide (NAD+) as the intermediate electron carrier[118]. Acetaldehyde is further oxidized by ALDH, mainly ALDH2, with the assistance of NAD+, which results in acetate as the product[118]. Acetate is not reactive or toxic, which allows it to circulate in the bloodstream, interact with other cells (e.g., skeletal muscle and heart cells), and be metabolized into carbon dioxide[118]. The rate of alcohol metabolism varies depending on individual genetics, with variation of genes related to ADH and ALDH, especially among East Asians[116]. An ALDH2 deficiency is associated with allergy-like symptoms such as skin flushing, headache and vomiting due to slow alcohol clearance[119]. This deficiency was found and extensively studied in East Asian populations[120]. New variants of ALDH2 that lead to similar symptoms were also observed people of Latino, African, and South Asian origins[119]. With excessive alcohol consumption or metabolism of alcohol in other organs such as the brain, an alternative pathway utilizing cytochrome P450 and catalase enzymes are involved, resulting in the production of reactive oxygen species that can cause oxidative stress[117], a core mechanism of many chronic diseases[121].

Digestions, absorption, and metabolism of phenolic compounds

Generally, small phenolic compounds such as phenolic acids[122] and some flavan-3-ols (e.g., epicatechin[123]) can be absorbed in the small intestine, where they are metabolized in the liver via Phase I and II enzymes. The metabolites are transported in the blood and interact with cells throughout the body. Larger phenolic compounds, such as anthocyanins[124], proanthocyanins[125], and ellagitannins[126] are primarily metabolized and absorbed in the large intestine. Polyphenols with large chemical structures, including anthocyanins[127], proathocyanins[128], ellagitannins[126], and resveratrol[129], are minimally absorbed in the oral cavity, stomach, and small intestine. In the oral cavity, proanthocyanins[128] and anthocyanins[127] can be biotransformed when exposed to salivary amylase. Anthocyanins can also be metabolized by an oral microbiome that contains beta-glucosidase to smaller molecules such as phenolic acids[127]. In the stomach, the large polyphenols interact with hydrochloric acid, which results in the biotransformation or hydrolysis of the large compounds into smaller molecules. Anthocyanins are hydrolyzed into glucose and anthocyanidins, which are transported into the liver via the organic anion transporter, bilitranslocase[127]. If the transporter is saturated, more anthocyanins are metabolized into phenolic acids[127]. In the small intestine, a small amount of ellagitannins are hydrolyzed, releasing bislactone ellagitannins, while depolymerization of proanthocyanins can slightly occur[126]. Similarly, the majority of anthocyanins consumed are minimally absorbed and metabolized in the small intestine. Approximately 1% of anthocyanins consumed are metabolized into the glucuronidated-, sulfated-, or methylated metabolites via phase I and II enzymes in the small intestine[127]. The remaining anthocyanins enter the colon, where gut microbiota

metabolism creates the primary absorption of anthocyanins[127], ellagitannins[126], proanthocyanins[128], and resveratrol[129].

The metabolites of anthocyanins vary[130], and a number of compounds have been observed after consumption, including gallic acid, vanillic acid, protocatechuic acid, 4-hydroxybenzoic acid, and syringic acid[127]. Syringic acid can also be found in red wine as an intermediate product of malvidin degradation[131]. Proanthocyanidins can be hydrolyzed into smaller monomers including catechin and epicatechin which are then further metabolized into γ-valerolactones[125]. Ellagic acid and ellagitannins are extensively metabolized in the large intestine into urolithin A (3,8-dihydroxy-6H-dibenzopyran-6-one) or urolithin B, a similar version to urolithin A with only one hydroxyl group attached to the benzene ring[132,133]. Individuals can be categorized into three different metabolic types depending on the urolithin production, including non-producers and producers of urolithin A and B as metabotype O, A, and B, respectively[133]. Resveratrol's main metabolite is dihydroresveratrol, which has high antioxidant and anti-inflammatory activity *in vitro* [134].

The effects of grape genetics, growing environments, and wine processing methods on red wine polyphenolic profiles

Red wines are diverse. They can be categorized by the grape cultivars, location of the wine production, processing methods, vintage years, among other factors[135]. Although red wines from different locations have similar contents of water and alcohol, slight differences in polyphenols can significantly affect sensory attributes, including aroma, taste, and color[136]. Although many studies note that health benefits of red wines

are derived in large part from the type and amount of polyphenols, no human study has directly compared the effects of red wines with different polyphenol profiles on cardiovascular health. However, several studies[137] have reported that polyphenols and volatile compounds, including anthocyanins and epicatechin[136] are different, depending on grape cultivars (genetics)[138,139], planting location [100,140], and vintage years[141,142]. All red wines have unique polyphenolic profiles, which contribute to differences in sensory evaluation[104] and in *vitro* activity[143], and potentially produce different effects on physiological functions.

Internal factors (genetics)

The polyphenolic profiles of red wine are significantly affected by the genetic profiles of the grapes[138,139]. The polyphenolic profiles of two types of grapes that are used to produce commonly consumed red wines in Poland (and in Hokkaido, Japan)[144], Zweigelt, and Rondo, can vary significantly in polyphenol profiles, including anthocyanins and epicatechins[138]. This is an important factor to consider in interpreting results of clinical studies with red wine, since different amounts of anthocyanins[145,146] and epicatechin[109] can result in significant differences in vascular function, one of the parameters for cardiovascular health.

Environmental factors

Production of anthocyanins can be stimulated by various environmental stresses, such as ultraviolet radiation, blue light, high-intensity light, plant wounding, pathogen attack, drought, sugar, and nutrient deficiency[135]. One *in vitro* study showed that the same type of red wines that was produced in different countries had different amounts of total polyphenols[143]. This study suggested that the location and environment of grape

cultivation and possibly wine processing (e.g., variations in yeasts used for fermentation or bacteria in the air or in wood barrels) may differentially influence the production of polyphenols in red wine[143]. The planting location of the grapes has several elements that may contribute to the differences in polyphenols, including variations in climate, temperature, sunlight exposure, water and soil[135]. The term “terroir” is used to encompass factors that affect the geography, geology, altitude, and environmental factors of the grape vine[104,147]. Differences in terroir characteristics influence the taste profile and phenolic composition of the grapes, and subsequently, the red wine. Such differences may then be factors that contribute to discrepancies in the health effects noted in research studies[104].

Environmental temperature is another factor that influences chemical composition of wine grapes[108,135,148,149]. The optimum temperature for anthocyanin production ranges from 20 to 25 degrees Celsius, which tends to be in cooler climate regions[108]. Interestingly and perhaps importantly, the same cultivar of wine grape grown in temperatures warmer or colder than this optimum range have shown reduced amounts of anthocyanins compared to grapes grown in the 20 to 25 degree Celsius range[100,150]. Water stress can also affect the concentration of anthocyanins in grapes[104,135]. When Cabernet Sauvignon grapes were planted in different regions with varying levels of drought, total polyphenol and anthocyanins content were significantly different[100].

Wine processing

Maceration and fermentation

Maceration is the process of extracting the juice and phenolic compounds from the pulp, skins, seeds, and stems of the grapes[104,108]. Maceration can be divided into three

stages: 1) Pre-fermentation; 2) Fermentation; 3) Post-fermentation[104]. With longer exposure of the grape juice to the skins, seeds, and stems more compounds leach into the liquid, resulting in the higher intensity of red wine color[104]. However, excessively long maceration times, typically exceeding 20 days, can reduce the volume of anthocyanins as these compounds can be metabolized by the yeast, resulting in enhanced ethanol production[104]. A cold temperature during maceration tends to preserve the anthocyanins[104].

Wine aging

Ellagitannins and phenolics from the wood barrel

In this process, the wine is in contact with a wood barrel and further ages[104]. Gentle oxidation may occur during wine aging, which decreases astringency, concentrates color, and stabilizes pigment structures[104]. Soluble oak extracts, such as tannins, can also diffuse into the wine, contributing to sensory characteristics[104]. Ellagitannins are hydrolyzable tannins, which are present in oak bark and are associated with bitterness and astringency in wine[104]. Toasting the oak wood at light temperature yields the highest levels of ellagitannins[104]. With more toasting of oak wood, more lignin bonds are broken, releasing vanillin, syringaldehyde, guaiacol, and furfurals, all of which are volatile compounds responsible for organoleptic properties of the wine[104] and possibly impacting human physiological functions. Other types of woods (ash, chestnut, cherry) with different polyphenolic profiles than oak can be used in wine production[104].

A clinical study in people with hypertension investigated the vasodilatory effects of red wines aged in different environments (a small oak barrel, large wood barrels, and steel tanks) and compared the effects with white wine aged in steel tanks[151]. The results

showed that intake of red wine aged in both small oak and large wood barrels resulted in significantly greater potent vasodilation than either of the wines aged in steel tanks, assessed by a biopsy of small arteries in subcutaneous fat[151]. The authors noted that the red wines aged in barrels had increased polyphenol content compared to wines aged in the steel tanks, which showed relatively lower concentration of polyphenols as a possible explanation for the vasodilation effects[151].

Anthocyanin and derivatives during wine aging

Sensory evaluations of red wines consist of visual, taste, odor, and aroma[136]. The color of red wine can influence consumer acceptance[148]. Monomeric anthocyanins contributed to young red wine color[141,148]. During wine maturation and bottle aging, the concentration of monomeric anthocyanins in red wines declines due to degradation and oxidation, which irreversibly forms more complex and stable anthocyanins resulting in more color expression. One of the most stable proanthocyanins is visitin A[148], which is formed from malvidin-3-O-glucoside, one of the main types of anthocyanins in red wine, and pyruvic acid, a product from yeast fermentation[148]. Visitin B is synthesized from malvidin-3-O-glucoside and acetaldehyde, another product of yeast fermentation[148]. Pinotin A is another anthocyanin that is synthesized during aging[148]. The synthesis of pinotin A tends to be observed in the bottle from 2.5 to 4 years old wines when the malvidin-3-O-glucoside had decreased significantly[148]. Polymeric anthocyanins are more stable than monomeric anthocyanins, which help stabilize wine color and contribute to the mouthfeel of red wines[148].

Oxygen is important for the evolution of anthocyanins in red wines, especially during aging[148]. As anthocyanins interact with oxygen[150], micro-oxygenation can be used to

promote the evolution of anthocyanins in red wines minimizing the oxidation reaction to anthocyanins[148].

Select effects of polyphenols in food on vasodilation

Although evidence exists from agricultural sciences about the amounts and types of polyphenols in red wines, as discussed above, differences in polyphenolic profiles of red wines have not been well studied at the clinical level. Given the epidemiological evidence discussed above, a better understanding of the mechanism(s) by which red wine can impact vascular function would be helpful.

In vitro study

An *in vitro* study explored the effects of red wines from different grape cultivars, growing areas, and the vinification/fermentation process on the ability to stimulate human endothelial nitric oxide synthase (eNOS) activity[143]. Results showed that polyphenol extracts from wines of specific origin and grape cultivars produced variable results. Red wines from France had significantly higher total polyphenol content than those from other regions in Europe[143]. The red wine with the highest total polyphenols was about four to five times higher than the red wine with the least total polyphenols[143,145,146].

Clinical studies

No clinical studies appear to exist that directly compares that directly compares the effects of red wines with different polyphenolic and anthocyanin profiles, so studies with other anthocyanin-rich foods must be used to infer or extrapolate the effects to red wine. Blueberries are rich in anthocyanins and have shown a significant difference in vascular function improvement and other cardiovascular health-related parameters compared to

a calorie- and carbohydrate-matched control[145]. Results from this study noted that intake of 150 g of blueberries (equivalent to 1 cup) daily for six months, containing 879 mg phenolics and 364 mg anthocyanins significantly improved vascular function as assessed by flow-mediated dilation while 75 g (equivalent to ½ cup) of blueberries [containing 439 mg phenolics and 182 mg anthocyanins] showed no difference in vascular function or other cardiovascular health-related parameters[145]. Another study compared the effects of six different amounts of blueberries, containing 316, 637, 766, 1278, or 1791 mg total blueberry polyphenols (129, 258, 310, 517, 724 mg anthocyanins, respectively) and a macro/micronutrient-matched control[146] reported that the FMD increased dose-dependently when the total blueberry polyphenol intake was 766 mg or less[146], while the measure plateaued at the two higher concentrations[146].

Objectives

Plant-based functional foods such as sorghum and red wine have been traditionally consumed as food and more recently have been studied for their health benefits beyond basic nutrition. As the phenolic compounds in plant-based food are sensitive to environmental changes and food processing, food produced under different conditions may provide different health benefits.

Therefore, the objectives of this dissertation are:

i) to explore the effects of sorghum produced by different processing methods (conventional milling vs. extrusion) on health outcomes, including plasma amino acids and glucose patterns.

ii) to investigate the effects of Hokkaido red wines produced in different vintage years (2015 vs. 2018) on vascular responses, including blood pressure, platelet aggregation, augmentation index, and reactive hyperemia index.

iii) to review evidence for factors that contribute to the discrepancies of results across studies assessing the effects of red wine on vascular function.

iv) to offer insights and suggestions for future clinical nutrition research

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Chapter II: Effects of extruded and conventional sorghum flour on postprandial plasma amino acid and glucose patterns in adult men

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Abstract

Sorghum is a nutrient-rich grain shown to improve growth and alleviate malnutrition in clinical studies; however, starch-protein interactions can limit its protein digestibility. Extrusion can help to improve protein availability from some foods. Three probe feeding studies were conducted to assess amino acid availability from extruded sorghum flour using postprandial plasma amino acid concentrations. For each study, a randomized crossover design with a one-week washout period was used to determine responses in healthy men aged 21-34 yr following intake of either extruded (EX) or conventional (CON) sorghum flour. In probe 1 (P1) and probe 2 (P2), men consumed 34 g (n=2) or 68 g (n=3) of flour, with plasma amino acid concentrations determined every 30 min for 180 min. A third probe (P3) provided 68 g (n=4) of flour, and samples for both plasma amino acids and glucose were collected every 15 min for 90 min. Responses were calculated as both the area-under-the-curve (AUC) and the incremental AUC (iAUC). In all three probes, amino acid responses were similar between the flours. The plasma glucose AUC was significantly greater from EX compared to CON, but the iAUCs between them were not significantly different. In these initial probe trials, a small sample size, along with individual variability in responses may explain the lack of differences in patterns of postprandial amino acids. Additional research on extrusion techniques and response measures is warranted.

Keywords: sorghum, extrusion, plasma amino acids, protein availability, plasma glucose, gluten-free

Introduction

Sorghum is currently the fifth most consumed grain in the world [1], being consumed in particularly high amounts in Africa, Asia, and South America, where the crop is a staple food and can be considered a sustainable source of protein [2,3]. On a weight basis, the grain contains an average of 70% carbohydrates, 11% protein, 11% fiber, 3% fat, a variety of vitamins and minerals [4] and is gluten-free [5]. This nutrient-rich grain has been used as one of the primary ingredients in complementary foods for dietary interventions with infants and toddlers suffering from acute malnutrition [6–10]. Collectively, these studies report that a child's recovery rate after consuming sorghum-based products has comparable responses to that of milk- or peanut-based products [6–8] and show a better growth response compared to the intake of control foods such as a maize-soy mixture or rice [9,10]. However, the protein digestibility of sorghum may limit its use as a primary dietary protein source. Illustrative of this, 26 Peruvian children, six to 30 months of age, were given 6.4% protein as whole grain sorghum (11-15.63 g sorghum/100kcal) or a similar amount of protein from casein (1.86 g Casec® /100 kcal) for a 59-day period [11]. When the children consumed the sorghum diet, they had significantly lower nitrogen absorption (46%) and retention (14%) compared to when they ingested the casein diet (81% nitrogen absorption and 38% retention) [11].

The majority of proteins in grains are found in a storage form, with prolamins and glutelins predominating [12]. Gluten is the main storage protein in wheat, rye, and barley and is responsible for triggering hypersensitivities that can result in celiac disease [12]. Sorghum is devoid of gluten and instead contains kafirin proteins that are rich in the amino acids proline and glutamine [13]. However, kafirin proteins can bind tighly to the

surrounding carbohydrate and polyphenol matrices, limiting the availability of amino acids during digestion from sorghum [14,15].

Food technology has been used to improve the protein digestibility of sorghum, including malting [16], fermentation [17], and extrusion, resulting in novel products [18]. Extrusion is a method of food processing that employs temperature, pressure, and shear stress [19], and is useful in improving the protein digestibility of sorghum, as noted from *in vitro* models [20,21]. In humans, 27 days of daily extruded sorghum intake in Peruvian infants and toddlers showed nitrogen absorption and retention values similar to those seen for casein (81% and 21% for extruded sorghum and 84% and 27% for casein, respectively) [22]. While promising, no washout period between the two interventions was used in the above study, so residual carry-over effects between treatments could not be determined, which may have confounded the results.

In addition to protein considerations, sorghum has been reported to improve glucose control. Two studies in well-nourished adults reported that sorghum intake modulated the postprandial blood glucose response and aided in weight loss more effectively than wheat [23,24]. A crossover study among overweight men noted that extruded sorghum intake (40 g/d) for two eight-week periods was significantly associated with reduced body fat compared to consumption of calorie-matched extruded wheat (38 g/d) [25]. Another crossover study that assessed the response to muffins made with either whole wheat or conventional sorghum flours (50 g each) reported that lower mean glucose and insulin responses were observed over a 180-minute period following ingestion of the sorghum, but not the whole wheat product [24]. To

our knowledge, no published studies have compared the effects of extruded versus non-extruded sorghum on blood glucose concentrations.

The primary aim of the current study was to determine if ingestion of extruded sorghum flour could increase protein availability to a greater extent than flour produced from a conventional milling process. Plasma amino acid levels were used as markers of protein availability [26]. The second aim of this study was to compare the postprandial blood glucose responses from the two sorghum flours. Three probe studies were conducted, with the blood glucose concentrations being measured only in the third probe. The conventionally milled sorghum was not cooked as porridge like in previous studies, as many *in vitro* reports note that wet cooking decreases digestibility and absorption of sorghum, which could potentially affect the availability of the protein[14,15,27–29].

Methods

Study participants

Healthy men, 21 to 50 years old, were recruited through flyers at the University of California, Davis (UC Davis) and via the Department of Nutrition website. Before enrollment, all volunteers were interviewed by telephone. Inclusion criteria required a body mass index between 18.5 and 36 kg/m^2 and weights greater than 49.9 kg (110 pounds). Exclusion criteria included fruit consumption greater than two cups per day, vegetable consumption greater than three cups per day, fatty fish intake greater than three servings per week, coffee or tea intake greater than three cups per day, dark chocolate intake higher than 75 g (three ounces)/day, consumption of a non-traditional diet (e.g., vegetarian, vegan, gluten-free, intermittent fasting), alcohol intake of more

than three drinks/week (one drink defined as one bottle of beer, one glass of wine, or one shot of distilled spirits), and dislike of, or allergy to, sorghum. Additional exclusion factors included self-reports for use of daily anticoagulation agents including aspirin or other non-steroidal anti-inflammatory drugs, restriction of physical activity due to a chronic health condition, routine high-intensity exercise, diabetes, blood pressure ≥ 140/90 mm Hg, renal or liver disease, heart disease (including cardiovascular events and stroke), malabsorption or cancer within the past five years, currently taking prescription drugs except for a stable amount of thyroid medication for at least six months, use of a multivitamin and mineral supplement other than a general formula providing up to a maximum of 100% of the US Daily Value, use of botanical or oil supplements within one month prior to study enrollment, indications of substance or alcohol abuse within the last three years, or current participation in another clinical research study. Volunteers were excluded if they had abnormal liver values outside of the reference range from a comprehensive metabolic panel (CMP), if determined to be clinically significant by the study physician.

Study designs

Three probe studies were conducted using randomized, two-treatment crossover designs to investigate the postprandial plasma amino acid and glucose patterns, as well as short-term safety and tolerability of extruded and non-extruded sorghum flour consumed under fasting conditions. Each probe employed either varying amounts of sorghum flour or assessed amino acid levels over different time courses. A one-week washout was included in all designs.

Extruded (EX) and non-extruded conventional (CON) sorghum flours were produced in a licensed food-grade facility (GHL International, Cedarberg, WI). The microbial and heavy metal concentrations were below acceptable upper limits. Extrusion processed the sorghum at 12-14% water content with no additional water added, and used a single screw and pressure >1,000 PSI [30]. The final product was 4-10% water and became water soluble at <60 degree Celsius [30].

For probe one (P1), two participants consumed 34 g (0.25 cup) of each sorghum flour, with plasma amino acid levels assessed at baseline and every 30 minutes for 180 minutes. For probe two (P2), three participants consumed 68 g (0.5 cup) of each flour, and plasma amino acid levels were assessed every 30 minutes for 180 minutes. For probe three (P3), four participants consumed 68 g (0.5 cup) of each flour, and plasma amino acid levels were assessed every 15 minutes for 90 minutes. For P3, a CMP was also collected. Since proline and glutamic acid are major amino acids in the kafirin proteins in sorghum, the plasma levels of these two amino acids were of particular interest and were used as primary indicator amino acids. Additionally, essential amino acids were measured. For each probe, the final amount of flour was based on a 70-kg male as the standard and then adjusted according to each participant's metabolic size, (body weight in kilograms to the three quarter power [$kg^{3/4}$]).

Study procedures

The intervention was conducted at the Ragle Human Nutrition Research Center, Department of Nutrition on the UC Davis campus. The protocol, forms, and advertisements were approved by the UC Davis Institution Review Board and all participants provided written informed consent prior to entry.

At each study visit, participants arrived at the facility after an overnight 12-hour fast. Anthropometric data (height, weight, body mass index) was collected, seated blood pressure was measured, and baseline blood collection was performed by a registered nurse using an indwelling catheter placed in the antecubital vein. Participants then consumed a mixture of the extruded or non-extruded sorghum flour along with 237 ml (one cup) of bottled water. Serial blood samples were collected at the time points described above.

Blood samples were centrifuged at 4^{o}C for 15 minutes at 3,500 g. The resultant plasma was combined with 6% sulfosalicylic acid (1:1) for deproteinization and the mixture was centrifuged at 16,100 g for 25 minutes. The supernate was processed through a 0.45 mm syringe drive PTFE filter and the final solution was analyzed with a Biochrom 30 amino acid analyzer at the Amino Acid Laboratory, UC Davis School of Veterinary Medicine. The CMP analysis, including blood glucose, sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and other liver and kidney markers was performed at the UC Davis Department of Pathology and Laboratory Medicine.

Statistical analyses

Data were calculated as the area-under-the-curve (AUC) and incremental AUC (iAUC) and are presented as their mean and standard deviation (SD). The AUC values were calculated from the plasma amino acid levels over the time course of the assessment by summing the trapezoid area between each time point on the absorption curve. The iAUC was calculated by considering only the area in which the plasma concentration was higher than the baseline value for each trapezoid between the time points on the absorption curve. Evaluating the AUC alone could overestimate the actual responses since the plasma concentration from some participants went below their baseline levels following the flour intake. As the baseline values may be varied among the participants, the mean baseline was used to check for the differences at baseline and reported as mean(SD). Additionally, the maximum concentration (Cmax) was determined for plasma glucose.

The RStudio statistical package version 4.0.3 (RStudio, Boston, Massachusetts, USA) was used to calculate the AUC and iAUC values for individual plasma amino acids and glucose. SigmaPlot version 14.0 (Systat Software, Inc., San Jose, California, USA) was used to perform statistical analyses including paired t-tests to assess the differences in AUC and iAUC of plasma amino acids between EX and CON in P1, P2, and P3, and to assess the differences in the AUC and iAUC of plasma glucose in P3. The Wilcoxon signed rank test was used for data that was not normally distributed.

Results

Complete plasma amino acid data was obtained from eight participants and complete CMP data was obtained from four participants in probe 3. Data from one participant was removed from the amino acid calculations due to analytical errors, but was included in the glucose analysis. The participant demographics are shown in Table 1. No adverse events were reported.

Table 1: Participant characteristics, n=8

Demographics	**mean(SD; range)**
Age (years)	28(4; 22-34)
Weight (kg)	77(20; 61.5-115.8)
Height (cm)	174(8; 163.1-180.4)
BMI* (kg/m^2)	25(5; 20.7-35.5)
Race	**N (%)**
White	4 (50)
Asian	3 (37.5)
African/Black	1 (12.5)

SD, standard deviation; N, number of participants

Plasma amino acids

There were no significant differences for any of the measured plasma amino acids at baseline for all three studies. In P1, P2, and P3, all AUCs were similar between EX and CON [Tables 2-4]. In P2, the baseline level of proline was significantly higher in the CON group than the EX group ($p=0.004$), which was reflected in a trend of higher AUC of CON relative to EX ($p=0.073$) [Table 3]. For a number of amino acids, iAUC plasma levels were not detectable with the intake of 34 g of either flour (Table 2). This includes the primary indicator amino acid proline. Apart from tryptophan in P2, all plasma amino acids achieved detectable levels after 68 g of flour intake. However, even with 68 g of flour intake, the iAUCs of EX and CON were not significantly different.

Table 2: Probe 1 area-under-the-curve (AUC) and incremental area under the curve values for plasma amino acids after consuming 34 g and assessing the responses every 30-minute over a 180-minute time course

Amino acids	Calculation Method	CON Mean(SD)	EX Mean(SD)	p-value (two-tailed)
Primary indicator AAs				
Proline	Baseline	252 (49)	240 (70)	0.57
	AUC	38,153(15,220)	37,440(10,607)	0.86
	iAUC	0	0	1.00†
Glutamic acid	Baseline	27 (7)	22 (2)	0.36
	AUC	6,773(689)	6,398(499)	0.22
	iAUC	1,973(668)	2,468(201)	0.57
EAAs				
Histidine	Baseline	86 (3)	84 (1)	0.34
	AUC	15,180(615)	14,895(191)	0.52
	iAUC	0	90(42)	0.21
Isoleucine	Baseline	76 (7)	62 (1)	0.18
	AUC	11,895(1,358)	10,463(711)	0.51
	iAUC	195(106)	45(64)	0.43
Leucine	Baseline	142 (12)	128(6)	0.18
	AUC	24,833(2,386)	23,865(148)	0.69
	iAUC	1,095(1,039)	300(382)	0.57
Lysine	Baseline	172 (41)	176 (45)	0.40
	AUC	29,752(7,881)	29,745(5,282)	1.00
	iAUC	270(42)	0	0.07
Methionine	Baseline	29 (4)	30 (1)	0.50
	AUC	4,598(562)	4,635(424)	0.77
	iAUC	0	0	1.00†
Phenylalanine	Baseline	66 (4)	57 (2)	0.10
	AUC	10,905(615)	9,923(817)	0.09
	iAUC	75(106)	15(21)	0.63
Threonine	Baseline	134 (10)	131 (10)	0.87
	AUC	21,608(32)	21,765(870)	0.84
	iAUC	15 (21)	15 (21)	1.00†
Tryptophan	Baseline	64 (1)	54 (6)	0.28
	AUC	10,448(435)	10,208(435)	0.50†
	iAUC	15(21)	758(1,071)	0.51
Valine	Baseline	194 (24)	252 (16)	0.09
	AUC	48,255(5,240)	44,400(1,782)	0.36
	iAUC	225(149)	345(488)	0.83

CON, conventional sorghum flour; EX, extruded sorghum flour; AUC, area-under-the-curve; iAUC, incremental AUC;† Wilcoxon signed rank test was used; n=2

Table 3: Probe 2 area-under-the-curve and incremental area under the curve values for plasma amino acids after consuming 68 g and assessing responses every 30-minute over a 180-minute time course

Amino acids	Calculation Method	CON Mean (SD)	EX Mean (SD)	p-value (two-tailed)
Primary indicator AAs				
Proline	Baseline	164 (28)	132 (25)	0.004*
	AUC	29,360(6,017)	25,540(4,817)	0.073
	iAUC	625(394)	1,945(1080)	0.14
Glutamic acid	Baseline	29 (12)	37 (3)	0.25†
	AUC	5,505(861)	4,715(1,137)	0.25
	iAUC	855(977)	10(17)	0.27
EAAs				
Histidine	Baseline	72 (8)	71 (9)	0.50†
	AUC	13,045(1,592)	13,110(1,581)	0.84
	iAUC	380(114)	470(48)	0.36
Isoleucine	Baseline	73 (5)	69 (10)	0.68
	AUC	11,645(906)	11,015(1,605)	0.62
	iAUC	200(46)	120(104)	0.43
Leucine	Baseline	132 (21)	145 (9)	0.48
	AUC	27,415(1,439)	25,315(2,997)	0.39
	iAUC	1,700(293)	1,790(686)	0.83
Lysine	Baseline	185 (65)	171 (51)	0.22
	AUC	32,765(10,110)	31,735(8,905)	0.29
	iAUC	650(693)	1,230(676)	0.40
Methionine	Baseline	28 (7)	26 (6)	0.46
	AUC	4,685 (862)	4,550 (831)	0.67
	iAUC	80 (114)	110 (121)	0.48
Phenylalanine	Baseline	60 (8)	58 (4)	0.62
	AUC	10,940 (630)	11,075 (775)	0.80
	iAUC	400 (568)	635 (372)	0.31
Threonine	Baseline	120 (25)	115 (21)	0.21
	AUC	21,165 (4,173)	20,625 (3,500)	0.30
	iAUC	210 (182)	260 (171)	0.83
Tryptophan	Baseline	55 (5)	52 (6)	0.45
	AUC	8,390 (98)	7,715 (1,315)	0.47
	iAUC	0	0	1.00†
Valine	Baseline	281 (31)	255 (45)	0.52
	AUC	48,905 (4,974)	44,285 (6,319)	0.45
	iAUC	610 (348)	440 (75)	0.472

CON, conventional sorghum flour; EX, extruded sorghum flour; AUC, area-under-the-curve; iAUC, incremental AUC;*$p<0.05$; † Wilcoxon signed rank test was used; n=3

Table 4: Probe 3 area-under-the-curve and incremental area under the curve values for plasma amino acids after consuming 68 g and assessing responses every 15-minutes over a 90-minute time course

Amino acids	Calculation Method	CON mean (SD)	EX mean (SD)	p-value (two-tailed)
Primary indicator AAs				
Proline	Baseline	143 (5)	145 (42)	0.93
	AUC	14,050 (2,112)	13,735 (2,927)	0.72
	iAUC	1,513 (1,671)	780 (727)	0.64
Glutamic acid	Baseline	30 (20)	41 (33)	0.28
	AUC	2,928 (2,188)	3,410 (2,501)	0.15
	iAUC	358 (380)	38 (46)	0.29
EAAs				
Histidine	Baseline	66 (3)	47 (42)	0.49
	AUC	6,113 (67)	6,330 (469)	0.45
	iAUC	355 (180)	2,178 (3,199)	0.41
Isoleucine	Baseline	67 (21)	70 (25)	0.80
	AUC	6,283 (1,866)	6,073 (1,695)	0.74
	iAUC	388 (123)	168 (264)	0.43
Leucine	Baseline	116 (40)	125 (41)	1.00†
	AUC	11,725 (3,531)	11,870 (2,740)	0.92
	iAUC	1,398 (236)	723 (927)	0.41
Lysine	Baseline	135 (28)	154(47)	0.40
	AUC	13,265 (3,237)	13,800 (3,452)	0.39
	iAUC	1,360 (1094)	345 (379)	0.35
Methionine	Baseline	22 (2)	22 (1)	1.00†
	AUC	2,093 (133)	1,970 (178)	0.08
	iAUC	135 (40)	65 (113)	0.48
Phenylalanine	Baseline	44 (20)	63 (6)	0.15
	AUC	5,202 (607)	5,848 (96)	0.1
	iAUC	1,284 (1,212)	243 (394)	0.16
Threonine	Baseline	97 (14)	109 (24)	0.61
	AUC	9,813 (1,847)	9,782 (1,596)	0.98
	iAUC	1,328 (1,622)	248 (278)	0.42
Tryptophan	Baseline	54 (7)	49 (10)	0.57
	AUC	5,165 (604)	4,768 (288)	0.35
	iAUC	453 (397)	392 (615)	0.92
Valine	Baseline	171 (138)	269 (90)	0.39
	AUC	22,250 (5,410)	24,435 (6,722)	0.32
	iAUC	7,130 (10,212)	698 (1,041)	0.41

CON, conventional sorghum flour; EX, extruded sorghum flour; AUC, area-under-the-curve; iAUC, incremental AUC;*$p<0.05$; † Wilcoxon signed rank test was used; n=3

Plasma glucose

Baseline fasting plasma glucose levels in P3 were in the normal range (<100 mg/dL), and were not significantly different between EX and CON. Postprandial glucose levels for three of the four participants had a similar pattern with EX generally higher than CON (Figure 1). The mean AUC of plasma glucose after EX intake was significantly greater than CON (Table 5). The mean iAUC of plasma glucose between EX and CON was not significantly different. The plasma glucose levels for most participants reached their maximum concentration (Cmax) at 30 to 45 minutes following consumption of either flour (Table 5, Figure 1). The maximum concentration of plasma glucose following EX intake was significantly greater than CON (149±14 vs. 121±11, respectively; p=0.011; Table 5).

Table 5: Probe 3 mean AUC, mean Cmax, and Tmax of plasma glucose following EX and CON intake; *p<0.05; n=4.

Glucose	CON Mean (SD)	EX Mean (SD)	p-value (two-tailed)
Baseline	88 (8)	91 (4)	0.61
AUC	9,559 (716)	11,239 (1,304)	0.012*
iAUC	1,646 (948)	3,107 (932)	0.073
Cmax	121 (11)	149 (14)	0.011*
Tmax	45 (21)	34 (8)	0.44

CON, conventional sorghum flour; EX, extruded sorghum flour; AUC, area-under-the-curve; iAUC, incremental AUC; Cmax, maximum concentration; Tmax, time at maximum concentration;*p<0.05; n=4

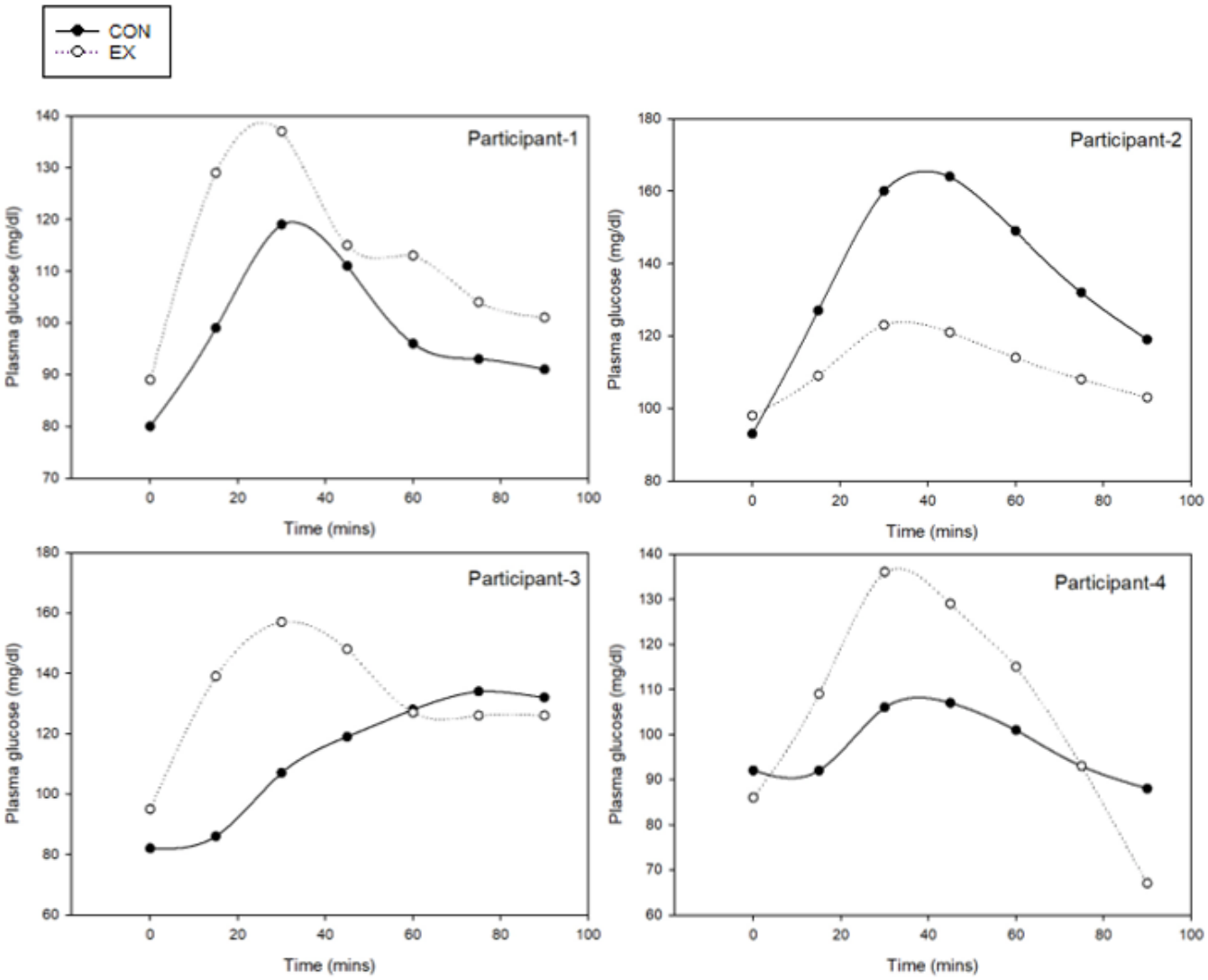

Figure 1: Plasma glucose patterns over 90 minutes for each of four individuals in P3; CON, conventional sorghum flour; EX, extruded sorghum flour; n=4

Discussion

Three probe studies were designed to provide exploratory data on the relative changes in plasma levels of indicator and essential amino acid levels following the intake of conventionally processed and extruded sorghum flours. The AUCs and iAUCs of postprandial plasma amino acid levels of EX and CON in P1, P2, and P3 were similar. A significant increase in plasma glucose AUC was noted for EX compared to CON, and a similar, non-significant trend was also observed for the iAUC values. This may be due to an increase in starch digestibility and glucose availability after the extrusion process [31]. Most of the elevated glucose values returned to a normal range within the 90-minute test period.

The glucose response to sorghum intake can be influenced by numerous factors. Lower plasma glucose and insulin responses were reported after ingestion of sorghum porridge compared to sorghum flatbread [32]. When comparing sorghum intake to other grains such as wheat and maize, plasma glucose and insulin AUC levels tended to be lower for sorghum [24,33]. The polyphenol content of sorghum may also influence postprandial glucose responses. In a trial comparing three different extruded sorghum formulas of varying polyphenol compositions (proanthocyanidins [PAC] and 3-deoxyanthocyanidins [3-DXAs]; 3-DXAs; sorghum control [no PAC and 3-DXAs]), postprandial glucose AUCs were significantly lower with PAC- and 3-DXAs-rich sorghum compared to the control [34]. Since the extrusion conditions and type of sorghum used in this study were different from the current project, a direct comparison of the results is limited.

Although AUCs were considered as the primary outcome measures, iAUCs were also assessed to account for the differences in individual baseline values. As the AUC does not account for the difference in each person's baseline, a higher baseline value generally resulted in a greater AUC, which may not represent the actual change throughout the measurement period. The differences at baseline can be reflected in the AUC differences as seen in a trend towards significance of proline AUCs, while the proline iAUCs were not affected [Table 2]. Differences between AUC and iAUC calculations have also been reported for plasma glucose results among men following an exercise regimen [35]. Another study concluded that iAUC was a more representative calculation than AUC for triglyceride responses to an oral fat load in healthy and diabetic individuals [36].

A report investigating the effects of whole grain sorghum intake on plasma amino acid levels in preschool children observed a significant increase in plasma essential amino acids three hours after consumption [11]. The trends returned toward baseline values by four hours. However, the researchers noted that sorghum had a lower digestibility compared to that of wheat, rice, potato, maize, or casein [11]. Direct comparison of these results to the current study is difficult, due in part to differences in calculation methods. Moreover, the study in children averaged individual plasma amino acids at each time point, while the current study used AUC and iAUC.

A number of factors limit the comparison of our results with other published work, including differences in the population assessed, the use of uncooked versus cooked sorghum flour, and specific details on the methods of extrusion, and the controls used in most trials utilized high-quality, complete protein sources (e.g., beef, dairy, and dish)

[37,38]. Compared to maize, sorghum contains a higher amount of phenolic and polyphenolic compounds [14], which often bind to and precipitate the kafirin proteins present in sorghum [39]. The extrusion process typically generates heat, and some *in vitro* studies report that an increase in temperature during extrusion can reduce the digestibility of sorghum protein by 40 to 60 percent compared to unprocessed sorghum [14,21]. This may be explained, in part, by the Maillard reaction, which increases the cross-links between amino acids and glucose, thus reducing protein digestibility and availability[40]. Differences in other extrusion conditions, such as pressure and pore size of the sieve can also contribute to different properties of the final product [19,40].

Future studies are needed to investigate the effect of extrusion on bioavailability of other nutrients including vitamins, minerals, and polyphenols, which are known to have health benefits. Previous studies reported that different extrusion conditions can result in products with different polyphenol profiles [33,34,41] and while the present study did not assess the polyphenolic profiles of extruded and non-extruded sorghum flour, such investigation would be of interest. Sorghum polyphenols have been reported to improve markers of diseases such as glucose intolerance, inflammation, and cancer in several *in vitro* [42], animal [43,44] and human [34,45] studies. More clinical trials about extruded sorghum and markers of disease are clearly warranted.

Sorghum is drought tolerant, environmentally sustainable, and an affordable food source, as well as a gluten-free grain [46]. Consumer demand for gluten-free products will encourage advances in food technology to enhance the nutritional and sensory attributes of sorghum [13,47]. When tested among patients with celiac disease using both *in vitro* organ culture of duodenal biopsies as well as a five-day feeding trial of baked

goods created with sorghum flour, no evidence of immunological reactivity was found [48]. The unique composition and properties of different sorghum cultivars [49] provide a range of new options for the creation of food products. Novel processing methods designed to improve the nutritional value and sensory attributes of sorghum will further promote the consumption of this important grain.

Conclusions

Probe studies exploring differences in postprandial plasma amino acid patterns following intake of extruded and conventional sorghum flour showed no statistically significant changes under the conditions tested. Plasma glucose levels were generally greater after extruded sorghum intake compared to conventional sorghum flour. Future studies are indicated to explore different extrusion methods in order to produce improved protein availability.

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Chapter III: The effect of Hokkaido red wines on vascular outcomes in healthy adult men: A pilot study

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Abstract

Moderate red wine intake has been associated with lower cardiovascular mortality, due in part to the intake of polyphenols and anthocyanins, whose content can vary from varietal and year of harvest. This study assessed the vascular effects in response to a single intake of 2015 and 2018 Zweigelt red wines from Hokkaido, Japan. Healthy men were randomly assigned to consume 240 mL each of a red wine, or a sparkling white grape juice as a control in a randomized three-arm cross-over design with a 7d washout between arms. The augmentation index (AI; a measure of arterial stiffness) and AI at 75 beats/min (AI75), reactive hyperemia index, systolic and diastolic blood pressure (SBP and DBP, respectively) and platelet reactivity were assessed at baseline and two and four hours after each beverage intake. Changes from the baseline were analyzed using a linear mixed model. Significant treatment effects (p=0.02) were observed, with AI 13% lower after the intake of the 2015 or 2018 vintages compared to the control. Intake of the 2018 vintage reduced SBP and DBP (-4.1 mmHg and -5.6 mmHg, respectively; p= 0.02) compared to the 2015 wine and the control drink. The amount of hydroxytyrosol in the 2018 wine was almost twice the amount as in the 2015 wine, which may help explain the variable blood pressure results. Future studies exploring the vascular effects of the same red wine from different vintage years and different phenolic profiles are warranted.

Keywords: red wine; vascular function; blood pressure; polyphenols; anthocyanins; arterial stiffness; augmentation index; hydroxytyrosol

1. Introduction

Moderate intake of red wine has been associated with beneficial effects on cardiovascular health[1,2]. The bioactivity of red wine is thought to be due in part to the intake of polyphenols[3,4]. Anthocyanins, a group of polyphenols known for their beneficial effects on cardiovascular health, strongly influence red wine color and hue[5-7]. Higher anthocyanin intake has been associated with reduced arterial stiffness and blood pressure in women 18-75 years old[8]. However, major red wine anthocyanins, such as malvidin glucoside (MG), can vary between the year of production, be higher in younger wines, and is affected by vintage years[9-11]. The polyphenolic content of wine can also be influenced by environmental factors such as temperature, humidity, light exposure, and soil and growing conditions[12,13]. Similarly, yearly variations in yeast biodiversity may contribute to differences in the polyphenolic profiles for different red wine vintages[14,15]. Changes in polyphenolic characteristics such as hue and color intensity have been noted in the same wine produced in different vintage years[16-18]. Differences in anthocyanin concentration and the color appearance were observed in the same cultivars planted in two areas in China with contrasting geographies and climates[19].

Given the above, the polyphenolic profile can vary substantially between red wines produced from the same grape varietal in different years and location[20,21]. Whether the variation in anthocyanin content, along with other polyphenols can result in different cardiovascular health outcomes, is unknown. Therefore, this study explored the effects of a single intake of the same red wine varietal produced in a similar geographic area, but from different vintage years on indices of vascular function, blood pressure, and

platelet aggregation in healthy adult men. A Zweigelt red wine grape varietal grown in Hokkaido, Japan, was selected. In Hokkaido, vineyards are typically covered with heavy snow during the winter (**Figure 1**), and grape production occurs during a short summer season with fewer hours of daily sunlight compared to varietals from warmer climates and longer days of summer sunlight that produce a majority of the world's wine[22].

2. Materials and methods

2.1 Recruitment

Healthy men 50 to 70 years old were recruited through flyers, newspapers, and online resources at the University of California, Davis (UC Davis). Criteria for inclusion were a body mass index (BMI) of 18.5 - 40 kg/m^2, body weight ≥ 110 pounds (49.9 kg), self-reported stable dose of prescription medication for the past six months (if taking any), non-smoker, and regular consumer of alcoholic beverages (between two drinks/week to two drinks/day). One standard drink of alcoholic beverage was defined as 355 mL (12 oz.) of beer (5% alcohol), 237 mL (8 oz.) of malt liquor (7% alcohol), 148 mL (5 oz.) of wine (12% alcohol), or 1.5 oz. of 80-proof distilled spirits or liquor (40+% alcohol). Exclusion criteria were daily use of aspirin or non-steroidal anti-inflammatory drugs, dislike of wine, grapes, or alcohol, following a non-traditional diet (e.g., vegan or vegetarian), fruit consumption ≥ 364 gm (2 cups)/day, vegetable intake ≥ 546 gm (3 cups)/day, consuming fatty fish or coffee/tea ≥ three portions/week, or eating, dark chocolate ≥ 85 gm (3 oz)/day. Self-reported restriction of physical activity or

chronic/routine high-intensity exercise were also exclusions, as were blood pressure ≥ 140/90 mm Hg, disorders that could affect vascular function (e.g., diabetes mellitus, renal or liver diseases, and cardiovascular events or stroke), or indications of substance or alcohol abuse. Volunteers were asked to refrain from using multivitamin and mineral supplements other than a general formula that met up to 100% of the United States recommended dietary allowance, and if applicable, were required to discontinue the intake of supplements containing botanical ingredients or fish oil for at least a month before study enrollment. Abnormal values from a comprehensive metabolic panel (CMP) and complete blood count (CBC) were exclusions unless approved by the study physician. The University of California Davis Medical Center's Department of Pathology and Laboratory Medicine performed the CMP and CBC analyses. The Institutional Review Board of the University of California, Davis approved the study protocol, with the study registered on ClinicalTrials.gov: NCT05138939.

2.2 Study design and procedures

Those eligible for enrollment were randomized into a three-arm, controlled cross-over study. Twenty-four hours prior to the study day, participants were instructed to refrain from soda, sports drinks, flavored water, and polyphenol-rich food, particularly olives, berries, apples, beans, citrus, onions, nuts, herbs, coffee, tea, beer, wine, cocoa, and chocolate products or beverages as these foods might confound the outcomes. Participants fasted for at least 12 hours, with the measurements of vascular function, blood pressure, and blood sampling performed at baseline and two and four hours after beverage consumption. After baseline measurements, the participants were

provided in a single-blinded fashion 240 mL (8 oz) of one of the two Zweigelt red wines from the 2015 or the 2018 vintages or a sparkling white grape juice (Welch's, USA) as a control. The beverages were provided along with a small snack consisting of low-moisture part-skim mozzarella string cheese (Galbani-Dal 1882, USA; 160 kcals, 12 g fat, 14 g protein, and 0 g carbohydrate) and 16 crackers (200 kcal; Carr's table water crackers, UK; 4 g fat, 4 g protein, and 40 g carbohydrate). Hokkaido Wine Co., Ltd. produced the wines from Zweigelt grapes (also known as Rotburger[23]) grown in Hokkaido, Japan. The wine was dispensed using a system that placed a probe through a cork in the neck of the wine bottle, and after each wine pour, argon gas was injected into the headspace in order to preserve freshness and chemical composition (Coravin, Bedford, MA, USA). The nutritional composition of the sparkling white grape juice was 110 kcal containing 28 g of total sugar, with an additional 24 g (80 kcal) of granulated sugar added by the investigators in order to match the caloric content of the red wines, which was approximately 190 kcal per serving[24].

2.3 Chemical composition and polyphenolic profiles

The basic chemical characteristics of the wines were provided by the manufacturer. Independent analyses of the polyphenolics (ETS Laboratories. St. Helena, CA) for the two red wines were conducted prior to the planned start of the intervention in 2019 and again at trial completion in 2022, which also included the sparkling white grape juice control. In 2022, the total polyphenol content (TPC in mg gallic acid equivalents [mg GAE]) of all three beverages was measured in triplicate according to the manufacturer's instructions (Zen-Bio, Durham, NC, USA). Briefly, the beverages were diluted in water

at a ratio of 1:10, then 10 µL of the diluted samples were incubated with a 10% Folin-Ciocalteu reagent for two hours and absorbance was measured at 765 nm using a Synergy H1 plate reader (BioTek, Winooski, VT).

Tyrosol (Tyr) and hydroxytyrosol (HT) concentrations were measured from each of the two Hokkaido red wines. Briefly, the wine was filtered through a 0.45µM nylon filter and then analyzed in duplicate using a direct liquid chromatography method with a diode array detector as previously described [25,26].

2.4 Assessment of vascular function

Prior to the vascular function measurement, participants rested in a seated position for 15 minutes, after which systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured three times, five minutes apart, with a digital blood pressure device (Welch Allyn, NY, USA). Data were calculated as the average of three readings.

Peripheral arterial tonometry (PAT; Endo-PAT2000; Itamar Medical, Israel) was used to monitor changes in digital pulsatile arterial volume[27]. Participants rested in a supine position for 10 minutes prior to a supine blood pressure measurement required for the system settings. After baseline collection for six minutes, a five-minute occlusion was performed by inflating a lower arm blood pressure cuff 60 mmHg above the supine SBP. Reactive hyperemia is the phenomenon of reperfusion of blood to the ischemic area following pressure cuff release[28,29]. The system software automatically calculated the reactive hyperemia index (RHI) as the ratio of the average pulse wave amplitude

(PWA) during a one-minute period following 90 seconds of reactive hyperemia to the average PWA during a three-to-five-minute baseline period, with the same ratio in the non-occluded arm serving as a control[30]. The RHI measures peripheral microvascular function in the digital vasculature that reflects, in part, nitric oxide-dependent vasodilation[31-33]. Relationships between the RHI response and circulating nitrate and epoxyeicosatrienoic acid levels have been reported[34,35]. The natural logarithmic transformation of the RHI ratio during data collection from 90 to 120 seconds after the release of the occlusion was calculated along with the Framingham reactive hyperemia index (fRHI), which has been correlated with cardiovascular risk factors[36]. An RHI value of less than 1.67 has been correlated with endothelial dysfunction and an index higher than this number represents normal endothelial function[27].

The Augmentation Index (AI), a measure of peripheral arterial stiffness, was calculated from the baseline PAT waveform as the difference between the first (P1) and second (P2) peaks of the central arterial waveform (i.e., [P2-P1]/P1 x 100%). The AI was also standardized to a heart rate of 75 beats per minute (AI75). A lower AI value represents greater elasticity in the arteries (i.e., less stiffness).

2.5 Platelet aggregometry

Optical platelet aggregometry was performed in citrated blood using a two-channel Chrono-Log 700 device (Havertown, PA, USA). Fifteen minutes after blood collection, platelet-rich plasma (PRP) was separated from whole blood by centrifugation (200 x*g* for 10 minutes at 25^{o} C). The upper 75% of the PRP layer was aliquoted into a separate

tube, and then platelet-poor plasma (PPP) was obtained by further centrifugation of the whole blood tubes at 1500 x g for 10 minutes at 25° C. After resting the PRP for a minimum of 15 minutes, the platelet aggregation testing commenced. Aliquots of 500 µL of PRP were incubated at 37° C for a minimum of three minutes prior to stimulation with three agonists: collagen at a concentration of either one or three µg/mL or 10µM adenosine diphosphate (ADP). The aggregation tests were performed in duplicate at a stirring speed of 1200 rpm and showed intra-assay mean and standard error of 10 ± 2%. After 10 minutes of data collection, the software generated values for area-under-the-curve (AUC), maximal aggregation (maxA), and slope from the response of activated samples.

2.6 24-hour dietary recall

Participants completed five 24-hour dietary recalls during the course of the study using the Automated Self-Administered dietary assessment tool (https://asa24.nci.nih.gov/). A recall was taken during each study visit, representing the days participants were asked to restrict polyphenol-rich foods, to check for compliance. The other two dietary recalls were completed by the participants at their convenience, which represented their usual intake.

2.7. Statistical analyses

A linear mixed model was used to assess changes from baseline in vascular outcomes, blood pressure and range-scaled platelet function using time and

intervention groups as the main effects and individual participants as the random effect (JMP, version 16; Cary, NC, USA). Post-hoc analyses were conducted using significant effects of time, treatment, or their interactions with Tukey's test. A one-way ANOVA assessed reported differences in food intake using the intervention group as the main factor. Similarly, a one-way ANOVA was used to assess differences in baseline values of each parameter among different interventions. Data not normally distributed were adjusted using Johnson's transformation prior to analysis. Unless indicated otherwise, data are reported as mean $\pm$ standard deviation (SD). The least square mean (LSM) of the observed values that showed significant differences between intervention groups was used to illustrate the data in bar graphs.

3. Results

3.1 Chemical composition and polyphenolic profiles

Table 1 presents the basic chemical characteristics of the two red wines. Both wines had similar specific gravity, alcohol content, acidity, and pH, with the Zweigelt 2015 34% lower in total sulfur dioxide (SO_2), the sum of molecular, free, and bound SO_2, (2015: 98 and 2018: 149 ppm) and 38% higher in sugar (2015: 5.6 and 2018: 3.5 g/L) than Zweigelt 2018.

Table 2 presents the polyphenolic profiles from an initial analysis conducted in 2019 and a subsequent analysis conducted in 2022 following a 17-month clinical laboratory closure due to the COVID-19 pandemic. While the 2018 Zweigelt wine was substantially higher in total anthocyanin content in 2019, it declined 76% by 2022. A

decrease in monomeric anthocyanin content primarily influenced this observation, which was approximately 64 mg in 2019 but reduced to 8.4 mg in 2022. Even with these reductions, the total and monomeric anthocyanin content for the 2018 wine was still 34 and 45%, respectively, higher than the 2015 Zweigelt, with the later wine having a decline in total anthocyanin and monomeric anthocyanin content by 49% and 71%, respectively. In contrast, the TPC content of the 2015 Zweigelt was 16% greater in 2022 compared to the 2018 wine (674 vs. 583 mg GAE, respectively), with both wines having a substantially higher TPC content compared to the sparkling white grape juice (104 mg GAE).

The amount of Tyr and HT in the 2015 and 2018 Zweigelt red wines is shown in **Table 3**. The amount of HT was almost twice as large in the 2018 Zweigelt red wine compared to the 2015 vintage, while the amount of Tyr was approximately 45% greater in the 2015 wine compared to its 2018 counterpart.

3.2 Demographics and baseline characteristics

Ten men completed the study, which spanned from September 2021 to June 2022 (**Figure 2)**. Their baseline demographic, glucose, platelet count and vascular function characteristics are shown in **Table 4.** Their baseline dietary characteristics are shown in **Table S1**. Eight participants self-reported their race as Caucasian or White, one as African-American or Black with European ethnicity (French, Spanish, and Greece), and one self-reported as Spanish-American or Latino. On average, the participants were 58.6 years of age, in the overweight range for BMI, with their CBC and CMP values

within the normal reference ranges. At baseline, participants' glucose levels and platelet counts were within the normal range of 98.60±10.81 mg/dL and 234.05±87.60 K/MM^3, respectively. On average, the participants were considered pre-hypertensive with a SBP of 124.47±2.70 mmHg, while DBP and heart rate (HR) were 82.20±1.23 mmHg and 63.5±2.68 bpm, respectively (mean±SEM). The AI and AI75 values were 17.62±6.46 (% pulse pressure) and 7.70±5.99 (% pulse pressure), with a normal RHI and fRHI at 2.25±0.10 and 0.78±0.11 (mean±SEM).

3.3 Arterial stiffness

The changes from baseline at two and four hours after beverage intake for vascular outcomes, including the AI and AI75 values (measures of arterial stiffness) are shown in **Table 5**. No significant interactive effects were observed. A significant effect for the red wine (0.002) was observed, with the overall change in AI lower after the intake of the 2015 and 2018 vintages compared to the control values (-12.98±3.20% and -13.34±3.20%, respectively vs. control -5.38±3.20%; $p<0.05$; **Figure 3a**). Significant time effects included a lower two-hour change in AI with the intake of the 2018 vintage (-18.27±3.58%) compared to the control (-7.97±2.67%; $p<0.05$). Similarly, significant time ($p=0.01$) and intervention group ($p=0.04$) effects for AI75 were noted, with intervention group trends for lower AI75 with the intake of the Zweigelt 2015 (-10.92±3.13; $p=0.09$) and 2018 (-11.28±3.13; $p=0.06$) wines compared to the sparkling white grape juice control (-5.41±3.13; **Figure 3b**).

3.4 Reactive hyperemia index

No significant interactive or intervention group effects for RHI or fRHI were observed. A significant change (P=0.003) in RHI for time was noted with the intake of the Zweigelt 2015 wine over the entire four-hour period (0.74±0.24) compared to the first two hours (-0.04±0.13), with similar trends observed for fRHI (**Table 5**).

3.5 Blood pressure

Significant treatment effects were observed for both SBP and DBP, with the overall changes lower following intake of the 2018 vintage compared to the 2015 vintage or the control beverage (-4.1 SBP and -5.6 DBP mmHg; p=0.02) **(Figures 4a and 4b)**. No significant interactive or time effects for SBP and DBP were noted. A strong time effect was observed for the HR, with the two-hour change greater after consumption of the 2018 wine compared to the other groups (p= 0.051; **Figure 5**). No other significant changes in HR were observed.

3.6 Platelet aggregation

Significant time effects were observed for the overall changes in MaxA, slope, and AUC following stimulation with the 3 µg/mL collagen agonist, with the two-hour change in platelet reactivity significantly higher than the four-hour change after consumption of each beverage (**Supplementary table 2**). No significant interactive or intervention

group effects were noted for other platelet function parameters (**Supplementary table 3**).

3.7 Dietary intake

No significant differences were noted in reported dietary intake between the three interventions. However, when comparing dietary intakes among study visits, the consumption of fats (saturated, total, and solid fats), iron, and folate was significantly reduced in the second and third visits compare to the first study day (**Supplementary tables 4 and 5**).

4. Discussion

The objective of this study was to assess the influence of the intake of two red wines of the same varietal and region, but from different vintage years on vascular and platelet function. The main finding was that intake of either the 2015 or 2018 Zweigelt red wines, along with a small snack providing 360 kcal (40% of calories from fat), lowered AI compared to the intake of a sparkling white grape juice control. In addition, SBP and DBP were significantly reduced in the postprandial period with the 2018 wine compared to the 2015 wine or the control beverage.

With frequent consumption of excessive calories, fats, and refined sugars in the modern Western diet, a postprandial state that typically lasts 6-12 hours can extend to more than 16 hours[37,38]. The prolonged postprandial state can increase cardiovascular

risk through increased exposure to elevated plasma glucose, triglyceride-rich VLDLs, chylomicrons and their remnants, which induce inflammation, oxidative stress, immune imbalances, and endothelial dysfunction to promote atherosclerosis[37,39]. Atherosclerosis, along with vascular aging, endothelial dysfunction, and structural remodeling, results in increased arterial stiffness, which can be measured by pulse wave velocity (PWV) and AI[40,41].

In the postprandial state, vascular function responses, including PWV, AI, and BP, may vary depending on food composition. Imbalanced macronutrients such as high calories, saturated fat, and simple carbohydrates can induce unfavorable postprandial responses, including inflammation, oxidative stress, and endothelial dysfunction[42,43]. In the current study, a reduction in blood pressure and AI and a trend toward increased HR following beverage and food intake are consistent with the aforementioned postprandial studies that report a decrease in AI two[28] and four hours after a high fat meal[44] or a standardized breakfast[45]. The significantly greater reduction in AI with the intake of the 2015 or 2018 red wines compared to sparkling white grape juice, all consumed with a snack, could be due to the presence of additional bioactive compounds in the meal, including alcohol and certain polyphenols such as anthocyanins that were not present in the control. Light to moderate alcohol consumption (15 g for women and 30 g for men) has been associated with lower arterial stiffness[40], while polyphenols in red wine, including anthocyanins, flavan-3-ols, phenolic acids, ellagitannins, and resveratrol, have been reported to have anti-inflammatory, anti-oxidative, and vasodilating properties[46,47]. The individual and interactive effects of alcohol and polyphenols may help explain the more favorable

vascular response in red wine compared to other beverages. In two separate studies, the intake of red wine with a meal (a slice of white bread [30 g] and 30 g of cottage cheese [4% fat]; 107 kcal, 6 g protein, 2 g fat , 15 g carbohydrate) significantly improved flow-mediated dilation (FMD) and AI[48,49]. Interestingly, the increase in FMD response was greater when red wine was combined with the intake of green olive oil[48]. A similar response was not observed with white wine intake, suggesting that the phenolic content in the red wine was a key to an improved vascular response[49]. The results from our study of an improved vascular response with red wine in contrast to a sparkling low phenolic white grape juice control are in agreement with these results.

The significant reduction in SBP and DBP following intake of the 2018 vintage compared to the 2015 red wine or the white grape juice control suggests differences in bioactive constituents between the two vintages. For the most part, the 2015 and 2018 red wines were similar in alcohol content and polyphenolic profiles (**Table 2**). However, the 2018 red wine contained almost twice as much HT compared to the 2015 wine, which is. of interest since hydroxytyrosol has been shown to be better absorbed than Tyr in *in vitro* models[50], as well as more bioavailable than Tyr in human studies[51]. Hydroxytyrosol supplementation has been shown to reduce blood pressure in a diabetic rat model[52] and counteract endothelin-1 expression, a hypertensive agent[53].

Apart from red wine, HT is a major olive oil phenolic[54]. A sub-analysis of the Prevención por Dieta Mediterránea (PREDIMED) study showed a positive association between participants' urinary HT and alcohol consumption that was primarily from red wine[55]. Additionally, metabolites of phenolic compounds found in wine, including

resveratrol[56,57] and HT[58] have been associated with alcohol intake. Hydroxytyrosol can also be produced endogenously as a metabolite of tyramine from dopamine metabolism[59]. Researchers from the PREDIMED study subsequently conducted two randomized, cross-over, controlled clinical trials to understand the disposition of HT by comparing the 24-hour pharmacokinetics from one study following a single intake of red wine (250 ml; 0.35 mg HT) and another study with extra virgin olive oil (EVOO: 25 ml; 1.7 mg)[59]. The results showed that urinary HT levels after red wine intake were significantly higher than those from EVOO[59], suggesting the unique properties in red wine that might promote endogenous production of HT. In another study, urinary HT concentration was assessed over a six-hour period after intake of a single serving (147 mL) of vodka, red wine, dealcoholized red wine, or water in 28 healthy male adults (average age of 26.6 years). Urinary levels were significantly greater for those consuming red wine, dealcoholized red wine, or vodka than from the water group, suggesting that alcohol and/or phenolic compounds aid in *de novo* HT generation[60]. Another sub-analysis from the PREDIMED study reported a significant association between the higher concentration of the HT metabolite, homovanillyl alcohol, and the lower mortality rate and less CVD burden[61]. Since HT can be absorbed and metabolized within four to six hours, as observed in the studies utilizing red wine[60] and olive oil[62], its presence in higher amounts in the 2018 Hokkaido red wine than in the 2015 vintage might help explain the discrepancies in blood pressure observed in the present study.

In addition to HT, anthocyanins and ellagitannins, the primary polyphenols in red wines, may also influence vascular outcomes. Since the current study assessed the effects of red wines on vascular outcomes over four hours, the absorption and

metabolism of anthocyanins and ellagitannins would likely be minimal[63,64]. Future studies of longer duration should also consider a more precise differentiation of compounds within these two categories, since previous studies have reported favorable cardiovascular outcomes depending on the major subtypes of anthocyanins[65,66] or different ellagitannin profiles of red wine from different vintage years[67].

No significant differences between the Zweigelt red wines and white grape juice were noted for platelet reactivity. The results are similar to two clinical studies that compared the responses following the consumption of red and white wines using similar methods (light transmission aggregometry (LTA) with agonists)[68,69]. A crossover randomized controlled trial assessing the effect of four-week intake of 180 mL/d of red or white wine on platelet aggregation, using thrombin and collagen as the agonists, reported no significant change in platelet aggregation from baseline for each of the wines[68]. Another randomized crossover controlled trial in 24 health participants assessed the effects of 300 to 350 mL/d of red or white wines on platelet aggregation, using collagen and ADP as the agonists, found no significant differences between the two beverages[69]. In contrast, a two-week randomized parallel-arm controlled trial in 20 healthy individuals assessed the effects of 300 mL/d red or white wine on platelet aggregation, assessed by LTA using collagen as the agonist, showed a significantly lower platelet aggregation response following red wine consumption, while no change from baseline was observed in the white wine group[70]. A four-week study providing 180 mL/d of red or white grape juice to individuals with hypercholesterolemia noted a significant decrease in platelet aggregation with white but not red grape juice[71]. An *in vitro* study compared the effects of five phenolic compound classes extracted from red

and white wine on platelet aggregation, assessed by anti-platelet activity on peripheral blood mononuclear cells (PBMCs) using five agonists including platelet-activating factor, ADP, thrombin receptor activating peptide, collagen, or arachidonic acid. Although the study did not identify the type of phenolic compounds extracted in the study, the results suggested that red wine and white wine have different bioactivities in anti-oxidant and anti-inflammatory potentials using these *in vitro* methods[72]. The results from this study may suggest that different phenolic compounds in red and white wines or grape juices might reduce platelet aggregation at comparable levels, though the application to humans is unclear given the method of assessment. Humans have digestive tracts and livers, which typically modify parent compounds into metabolites, dynamics that are not captured by placing the parent compound directly onto cells.

A strength of this study is the assessment of clinical responses from the same type of wine, vinted from the same grape cultivar, grown in a similar geographic region, but from two different years. This novel study design has not been employed previously, to our knowledge. Numerous studies have reported significant differences in the content and type of polyphenols in red grapes or red wines produced under different environmental stresses[15,20,73,74], and it is reasonable to hypothesize that these chemical differences may produce differences in vascular outcomes. Another strength is the detailed polyphenolic profiles of the red wines and sparkling white grape juice. Many previous studies provide little or no compositional information about the red wines tested[75-79]. Future studies should provide a detailed chemical profile of the test wine(s) to better enable interpretation of results and make more accurate comparisons between studies.

The red wine in the current study was produced in Hokkaido, Japan, where grapevines are covered by snow during the winter and experience a short, cool summer season[22]. A higher concentration of phenolic compounds has been reported in cultivars grown in climates of long winters, low temperatures and possible snowfall[80]. However, the total polyphenolic content of the wines tested here are similar as the vast majority of studies on vascular function that have assessed wines from warmer climates with longer growing seasons[81]. Unfortunately, the influence of any particular polyphenol on vascular or other physiologic responses cannot be evaluated from most other studies, which report little detailed analysis of the test wines. Chemical profiling is also of interest when comparing the same red wine across different vintage years. Such detail is important since results from the present study demonstrate that the 2018 red wine, relatively rich in HT, produced a significant reduction in blood pressure, while the 2015 vintage (with approximately half of the HT concentration), did not. Overall, the cool climate Hokkaido Zweigelt red wines produced vascular responses comparable to the existing body of research to date regarding the vasculoprotective effects from wines produced in warm climate regions across the world.

The data presented here has some limitations. The relatively small sample size is noted, but for a pilot study using the novel study design, is reasonable. Interpersonal variability is another limitation, since substantial variability in postprandial responses to food consumption were also observed in the Personalised REsponses to Dietary Composition Trial (PREDICT) study that included 1,002 adult participants. The results of the PREDICT study showed highly variable postprandial responses in interleukin-6, glycoprotein acetylation, blood triglyceride, glucose, and insulin following a breakfast

(86 g carbohydrate, 54 g fat, 16 g protein) and a lunch (71 g carbohydrate, 22g fat, 10 g protein; consumed at the four-hour point) over six hours[82,83]. Individual profiles, including gut microbiome and genetic variants, greatly contributed to the variable postprandial outcomes[83]. Variations in personal genetic and gut microbiome profiles may influence personal ability to absorb and metabolize polyphenols such as hydroytyrosol[61,84], anthocyanins[85-87] and ellagitannins[88], and no gut microbiome profiles were assessed in the present study. The participants in this study were healthy adult males aged 50-70 years, and females or males of different age ranges, or those with elevated blood pressure or other vascular dysregulations were not assessed. The experimental design tested a single intake of red wine, and the results may not be generalizable to a longer duration of consumption. Sparkling white grape juice with added sugar was used as the control beverage in an attempt to mirror caloric and simple sugar content of the red wines. Although the sparkling white grape juice did not contain anthocyanins, other bioactive compounds such as gallic acid, tannins, and caftaric acid were present, albeit in very small amounts, which may have slightly impacted the outcome measures. Finally, inaccurate reporting of food intake is common in nutrition research[89] and the dietary changes noted in the second and third study visits may have influenced vascular function measures. However, the initial red wine group assignment was randomized, and when the treatment order was assessed as a factor that might influence the outcome measures, no evidence was found that the reported changes in diet were significant (Supplementary table S4 and S5).

5. Conclusions

A single intake of Hokkaido Zweigelt red wine produced in 2015 or 2018 resulted in a significant reduction in arterial stiffness in healthy adult males, while a sparkling white grape juice control beverage showed no effect. Consumption of the 2018 vintage significantly lowered SBP and DBP compared to the 2015 vintage or the control. Future studies with a larger sample size, detailing the red wine polyphenolic profiles including hydroxytyrosol, and comparing different vintage years are warranted.

Supplementary Materials:

Table S1: Reported baseline dietary intake assessed by a health habit questionnaire at the screening visit; Table S2: Changes in platelet aggregation assessed by Light Transmission Aggregometry (LTA) using 1 and 3 ug collagen and 10mM ADP as the agonists from baseline to 2 hr and from baseline to 4 hr after beverage consumption; Table S3: Changes in platelet aggregation for each intervention group; Table S4: Participants' 24 hour recall dietary intake categorized by intervention; Table S5: Participants' 24 hour recall dietary intake categorized by study visit.

Author Contributions: Conceptualization, P.C. and R.M.H.; methodology, P.C. and R.R.H; formal analysis, P.C.; data curation, P.C., investigation, P.C.; resources, T.S., T.S., and R.M.H.; writing—original draft preparation, P.C.; writing—review and editing, P.C., R.M.H, , R.R.H., C.L.K, N.S., T.S., and T.S; visualization, P.C.; supervision, R.M.H; project administration, P.C. and R.M.H; funding acquisition, R.M.H. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: RMH has received honoraria to present lectures in Global Food Resources at Hokkaido University under the supervision of T.Sone. The other authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Tables and figures

Table 1: Basic characteristics of the Hokkaido 2015 and 2018 Zweigelt red wines

Item	**Zweigelt 2015**	**Zweigelt 2018**
Vineyard	Tsurunuma	Kitajima
Specific gravity	0.994	0.993
Alcohol (%)	12.34	12.54
Total acidity (g/L as tartaric acid)	5.23	5.73
pH	3.67	3.56
Total sulfur dioxide (SO_2) (ppm)	98	149
Sugar (g/L)	5.6	3.5

Total SO_2 is the sum of molecular, free, and bound SO_2; ppm, part per million

Table 2: Polyphenolic profile of a single serving of Hokkaido 2015 and 2018 Zweigelt red wine from the analyses conducted in 2019 and 2022 (prior to and at completion of the intervention, respectively) and white grape juice from the 2022 analysis.

Polyphenols (mg/240mL)	Zweigelt 2015		Zweigelt 2018		White grape juice
	2019 analysis	2022 analysis	2019 analysis	2022 analysis	2022 analysis
Gallic acid	9.12	8.64	4.80	5.52	0.17
Catechin	3.84	5.52	3.12	4.08	<0.05
Epicatechin	5.76	7.20	4.08	5.04	n.d.
Tannin	75.6	81.60	86.40	96.48	0.77
Caftaric acid	3.84	3.36	9.12	7.92	0.77
Caffeic acid	3.36	4.08	1.68	2.40	<0.05
Quercetin glycosides	2.64	2.64	2.64	1.68	<0.05
Quercetin	0.24	0.48	0.48	0.72	n.d.
Malvidin glucoside	4.80	0.72	31.92	2.64	n.d.
Polymeric anthocyanins	5.76	6.48	6.96	8.40	n.d.
Total anthocyanins	21.60	11.04	70.56	16.80	n.d.
Monomeric anthocyanins	15.84	4.56	63.60	8.40	n.d.
Resveratrol (cis+trans) (HPLC)	0.31	0.48	0.48	0.60	n.d.
Total polyphenol content (mg GAE)	455.28	674.00	NA	582.88	104.07

n.d.: not detected.; NA: not available; HPLC: high performance liquid chromatography; GAE: gallic acid equivalent

Table 3. Tyrosol and hydroxytyrosol concentrations in the 2015 and 2018 Zweigelt red wines

Wine sample		**Hydroxytyrosol (mg/L)**	**Tyrosol (mg/L)**
2015 Zweigelt	Sample 1	8.81	84.57
	Sample 2	8.97	85.14
Average (Mean±SD)		**8.89±0.11**	**85.86±0.40**
2018 Zweigelt	Sample 1	15.24	49.42
	Sample 2	15.31	49.63
Average (Mean±SD)		**15.28±0.05**	**49.53±0.15**

Table 4: Baseline characteristics of participants

Demographics	**Mean (SD), range [min-max]**
Age (years)	58.6 (6.10), [51-69]
Weight (kg)	87.5(13.85), [71-114]
Height (cm)	178.32(6.84), [167-189.5]
BMI (kg/m2)	27.46(4.02), [22.7-34.0]
Waist circumference (cm)	100.45(14.02), [86-129]
Selected CMP and CBC parameters	**Mean (SD), reference range**
Glucose (mg/dL)	98.60(10.81), 74-109
Platelet count (K/MM^3)	234.05(87.60), 130-400
Vascular function parameters	**Mean ± SEM**
RHI	2.25±0.10
fRHI	0.78±0.11
AI (% pulse pressure)	17.62±6.46
AI75 (% pulse pressure)	7.70±5.99
SBP (mmHg)	124.47±2.70
DBP (mmHg)	82.20±1.23
HR (bpm)	63.5±2.68

RHI, reactive hyperemia index; fRHI, Framingham reactive hyperemia index; AI, augmentation index; AI75, augmentation index adjusted to 75 bpm; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; SEM, standard error of mean; CMP, comprehensive metabolic panel; CBC, complete blood count

Table 5: Changes in vascular outcomes from the baseline (T0) to two (T2) and four (T4) hours after each beverage consumption

Outcomes (Change from baseline)	Intervention group						P-value	
	Zweigelt 2015		Zweigelt 2018		White grape juice		Time	Treatment
	T2-T0	T4-T0	T2-T0	T4-T0	T2-T0	T4-T0		
	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM		
AI	-16.32±1.64	-9.64±3.61	-18.27±3.58†	-8.40±3.85	-7.97±2.67	-2.79±5.30	0.001	0.002
AI75	-13.20±1.37	-8.64±3.29	-15.27±3.82	-7.28±3.74	-7.35±2.65	-3.47±5.46	0.01	0.04
SBP	0.13±3.37	6.77±3.08	-5.13±3.67†	-3.13±3.21	3.37±2.33	2.33±1.35	0.28	0.02
DBP	-3.63±2.84	1.70±1.83	-8.10±1.60	-3.13±1.75	-0.13±1.12	-2.33±1.39	0.07	0.02
HR	6.33±2.06	1.50±2.41	10.17±1.67	3.57±1.08	4.40±1.64	1.57±1.31	0.001	0.051
RHI	-0.04±0.13	0.74±0.24‡	0.09±0.10	0.33±0.21	0.07±0.13	0.35±0.14	0.003	0.62
fRHI	-0.08±0.07	0.27±0.10	-0.03±0.06	0.14±0.15	0.00±0.07	0.18±0.08	0.003	0.88
MaxA 1 µg collagen*	-0.45±0.25	-0.10±0.30	0.59±0.33	-0.30±0.34	0.35±0.28	-0.10±0.29	0.24	0.37
MaxA 3 µg collagen	-0.05±0.13	-0.21±0.08	0.23±0.10	-0.10±0.08	0.13±0.11	-0.02±0.14	0.02	0.17
MaxA 10 µM ADP*	0.08±0.35	-0.04±0.29	-0.11±0.26	-0.53±0.28	0.48±0.34	-0.22±0.22	0.12	0.36

*Data transformed by Johnson's transformation to achieve normal distribution prior to the linear mixed model analysis

†Significantly different from control at the same time point, $p<0.05$ upon post-hoc analysis of treatment effect

‡Significantly different compared to T2-T0 value in the same group, $p<0.05$ upon post-hoc analysis of time effect

All data are reported as Mean±SEM; SEM, standard error of mean; RHI, reactive hyperemia index; fRHI, Framingham reactive hyperemia index; AI, augmentation index; AI75, augmentation index adjusted to 75 bpm; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; MaxA, Maximal aggregation; ADP, adenosine diphosphate

Figure 1. Winter vineyard pruning in Hokkaido Japan (photo used with permission from Hokkaido Wine Co.)

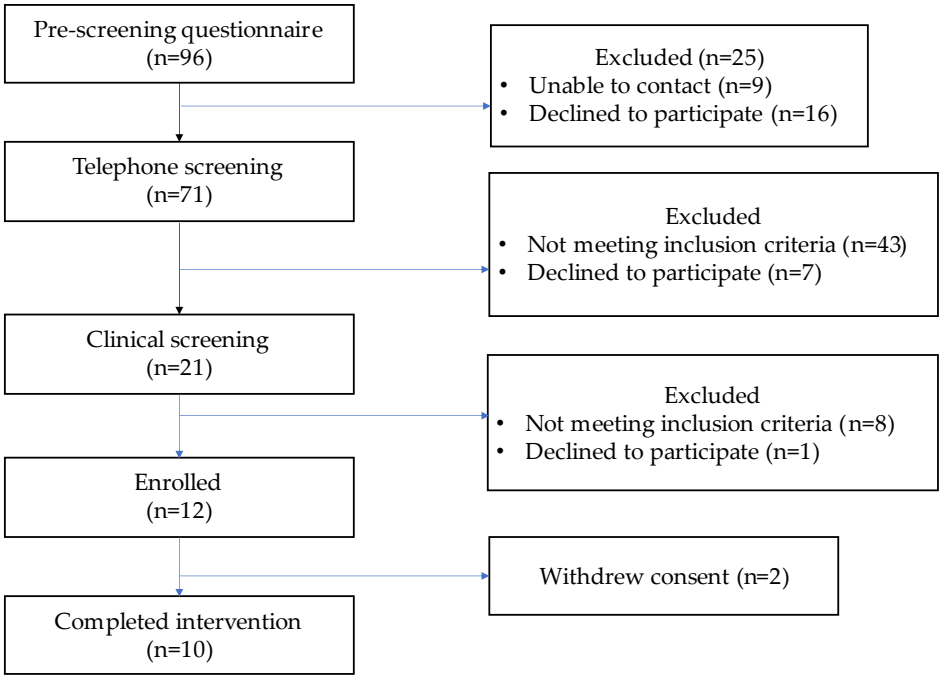
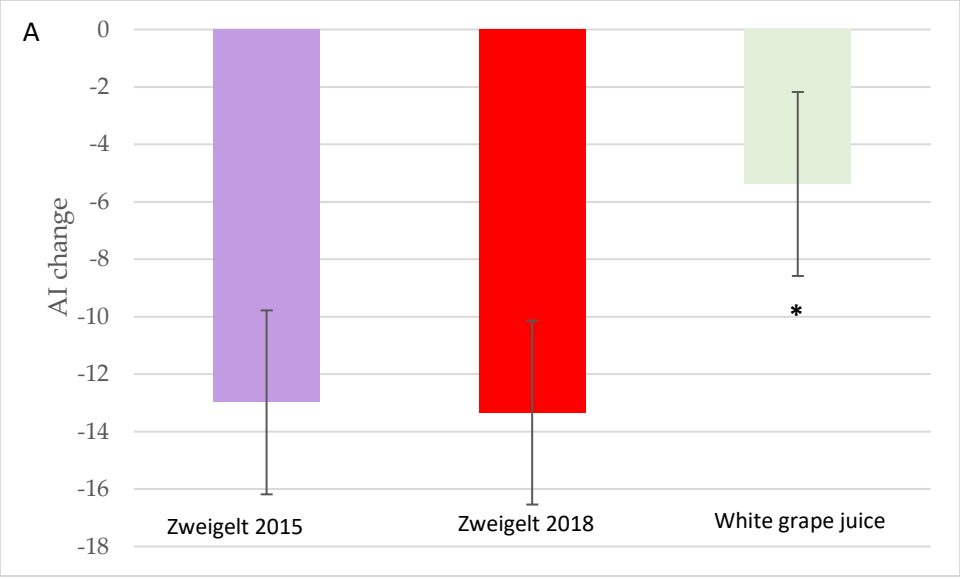
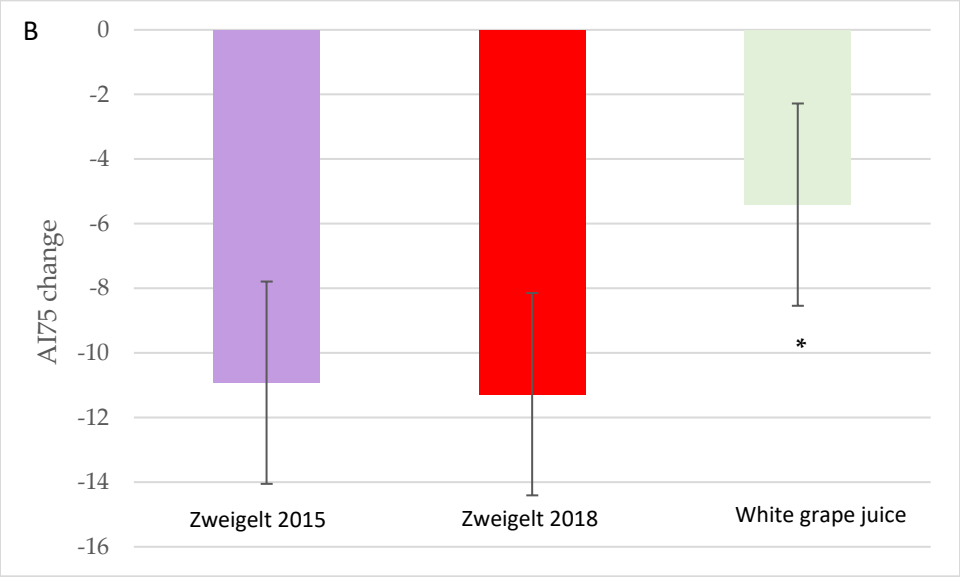


Figure 2: Recruitment and enrollment

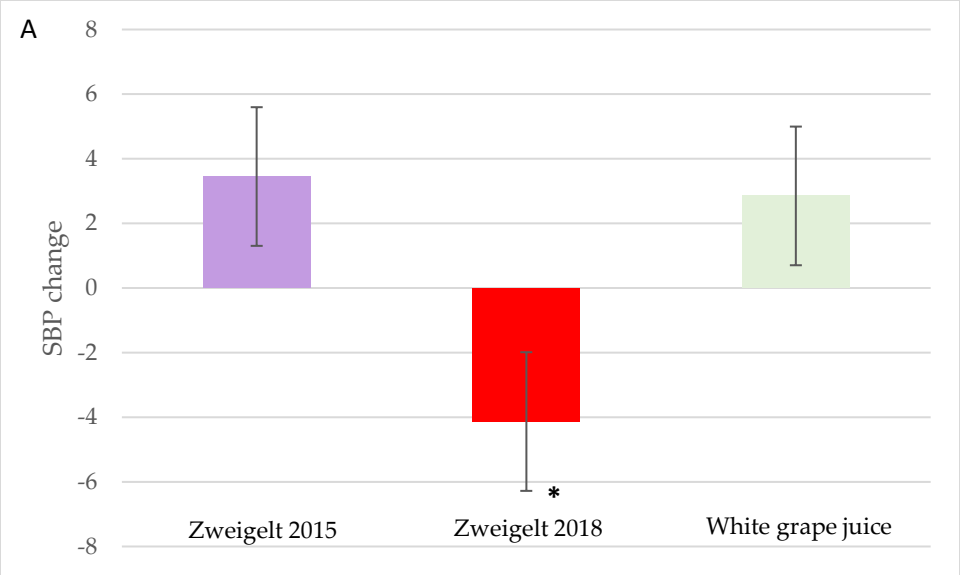


(a)



(b)

Figure 3: Least square means (LSM) plot of the intervention group effects for the overall change from baseline for (2a) augmentation index; and (2b) augmentation index adjusted to 75 bpm. A linear mixed model was used to assess changes from baseline using time and intervention groups as the main effects and individual participants as the random effect. Data are the LSM ± SEM; * Significantly different at the $p<0.05$ level after Tukey's post-hoc testing. AI, Augmentation Index; AI75, Augmentation Index adjusted to 75 bpm; bpm, beats per minute



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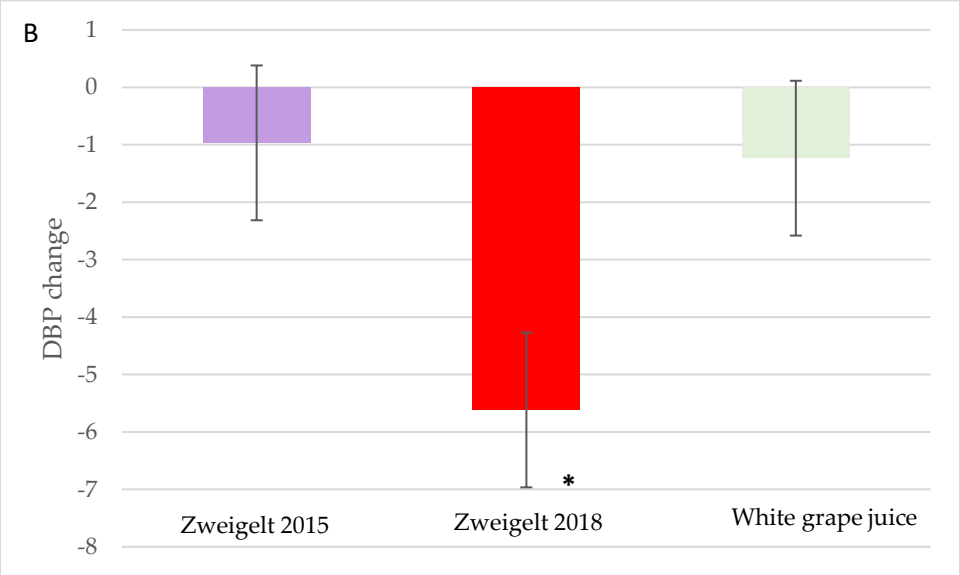

(b)

Figure 4: Least square means (LSM) plot of the intervention group effects for the overall change from baseline for (3a) systolic; and (3b) diastolic blood pressure. A linear mixed model was used to assess changes from baseline using time and intervention groups as the main effects and individual participants as the random effect. Data are the LSM ± SEM; * significantly different at the $p<0.05$ level after Tukey's post hoc testing. SBP, systolic blood pressure; DBP, diastolic blood pressure

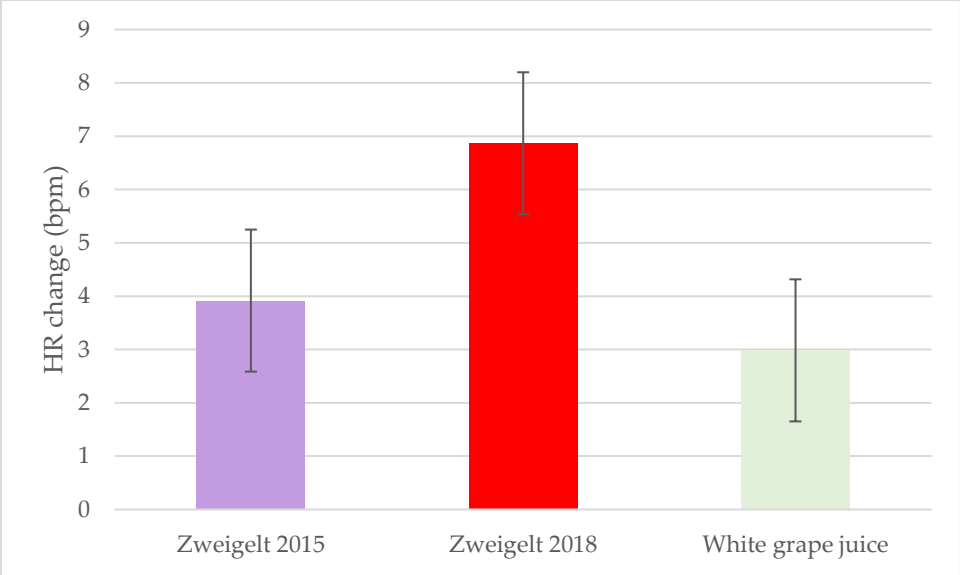

Figure 5: Least square means plot of overall change from baseline of heart rate. A linear mixed model was used to assess changes from baseline using time and intervention groups as the main effects and individual participants as the random effect. Data are the LSM ± SEM; HR, heart rate; bpm, beats per minute

Supplementary tables and figures

TableS1: Reported baseline dietary intake assessed by a health habit questionnaire

Baseline dietary intake characteristics	**Mean**	**SD**
Alcohol consumption (drinks/week)	4.6	2.8
Red wine (drinks/week)	2.8	1.5
White wine (drinks/week)	1.1	1.7
Total wine (drinks/week)	3.9	2.7
Beer (drinks/week)	1.6	1.5
Hard liquor (drinks/week)	1.2	1.6
Fruit intake (cups/week)	1.1	0.8
Vegetable intake (cups/week)	1.6	0.9
Tea intake (cups/day)	0.5	0.6
Coffee intake (cups/day)	0.9	0.7

Table S2: Changes in platelet aggregation assessed by Light Transmission Aggregometry (LTA) using 1 and 3 ug collagen and 10mM ADP as the agonists from baseline to 2 hr and from baseline to 4 hr after beverage consumption

Variable	Zweigelt 2015 (mean(SD))		Zweigelt 2018 (mean(SD))		White grape juice (mean(SD))		p-value (time effect)
	T2-T0	T4-T0	T2-T0	T4-T0	T2-T0	T4-T0	
10μM ADP MaxA	0.08(1.11)	-0.04(0.93)	-0.11(0.81)	-0.53(0.83)	0.48(1.07)	-0.22(0.70)	0.1264
10μM ADP Slope	-0.07(0.42)	-0.06(0.40)	0.01(0.30)	-0.07(0.22)	0.07(0.37)	0.10(0.39)	0.9400
10μM ADP AUC	0.08(1.12)	0.15(0.96)	-0.01(0.79)	-0.45(0.81)	0.49(1.08)	-0.08(0.82)	0.2443
1μg Collagen MaxA	-0.45(0.80)	-0.10(0.89)	0.59(1.05)	-0.30(1.09)	0.35(0.89)	-0.10(0.93)	0.2921
1μg Collagen Slope	-0.05(0.27)	0.01(0.31)	0.1(0.46)	-0.05(0.37)	0.04(0.36)	-0.14(0.30)	0.2111
1μg Collagen AUC	-0.14(0.33)	0.01(0.32)	0.16(0.40)	-0.08(0.34)	0.12(0.28)	-0.08(0.35)	0.2872
3μg Collagen MaxA	-0.05(0.40)	-0.21(0.24)	0.23(0.31)	-0.10(0.25)	0.13(0.33)	-0.02(0.45)	0.0378*
3μg Collagen Slope	0.25(0.31)	-0.01(0.24)	0.04(0.35)	-0.18(0.31)	0.02(0.42)	-0.13(0.36)	0.0241*
3μg Collagen AUC	0.08(0.36)	-0.06(0.21)	0.12(0.35)	-0.21(0.23)	0.19(0.32)	-0.12(0.43)	0.0038*

ADP, Adenosine diphosphate; MaxA, Maximal aggregation; AUC, area under the curve; Superscript * shows a significant difference between time points at $p<0.05$.

TableS3: Changes in platelet aggregation for each intervention group

Data	Zweigelt 2015		Zweigelt 2018		White grape juice		p-value (treatment)
	Mean	SD	Mean	SD	Mean	SD	
10µM ADP MaxA	0.0069	0.37386	-0.0765	0.30741	0.06581	0.32856	0.3582
10µM ADP Slope	-0.0641	0.39722	-0.0238	0.26324	0.08671	0.37021	0.4030
10µM ADP AUC	0.01369	0.38164	-0.0747	0.30233	0.05731	0.3445	0.3899
1µg Collagen MaxA	-0.0986	0.31214	0.04541	0.40565	0.04826	0.33046	0.3218
1µg Collagen Slope	-0.0199	0.27962	0.06755	0.42075	-0.0486	0.33326	0.5645
1µg Collagen AUC	-0.0667	0.32271	0.03877	0.38036	0.02462	0.32416	0.6250
3µg Collagen MaxA	-0.1249	0.337	0.06624	0.32289	0.0524	0.3915	0.2217
3µg Collagen Slope	0.12649	0.30305	-0.068	0.34266	-0.0522	0.38828	0.2169
3µg Collagen AUC	0.0123	0.2989	-0.0451	0.3376	0.03339	0.40441	0.7448

ADP, Adenosine diphosphate; MaxA, Maximal aggregation; AUC, area under the curve; Superscript * shows a significant difference between groups at $p<0.05$.

TableS4: Participants' 24 hour recall dietary intake categorized by intervention

Variable	Zweigelt 2015		Zweigelt 2018		White grape juice		p-value
	Mean	SD	Mean	SD	Mean	SD	
Energy (kcal)	1483.86	539.45	1550.29	642.43	1800.55	832.06	NS
Protein (g)	74.99	48.76	75.72	40.01	93.29	63.41	NS
Total fat (g)	76.42	30.41	72.79	33.68	87.31	50.44	NS
Carbohydrate (g)	120.64	46.75	151.64	79.69	148.45	62.31	NS
Water (g)	1918.32	1289.45	2162.00	1361.15	2223.73	1461.73	NS
Alcohol (g)	3.44	10.89	0.00	0.00	8.71	19.18	NS
Caffeine (mg)	44.84	73.73	47.38	97.22	35.10	71.89	NS
Theobromine (mg)	2.73	7.83	0.36	1.14	21.49	51.43	NS
Total sugar (g)	33.62	32.23	49.12	42.86	53.76	34.95	NS
Total dietary fiber (g)	11.08	7.46	12.45	6.41	11.73	6.72	NS
Calcium (mg)	745.20	451.17	948.71	717.69	845.15	520.59	NS
Iron (mg)	8.55	2.40	11.30	5.52	10.90	5.18	NS
Sodium (mg)	3014.02	1788.79	3122.07	1275.25	3498.10	2282.26	NS
Total saturated fatty acids (g)	25.78	14.72	28.43	16.99	30.28	20.23	NS
Total monounsaturated fatty acids (g)	28.02	11.67	24.30	9.84	32.41	21.01	NS
Total polyunsaturated fatty acids (g)	15.65	5.66	14.34	6.37	17.19	11.83	NS

TableS5: Participants' 24 hour recall dietary intake categorized by study visit

Variable	Visit 1 (V1)		Visit 2 (V2)		Visit 3 (V3)		P-value (One-way ANOVA)
	Mean	SD	Mean	SD	Mean	SD	
Energy (kcal)	1953.39	575.73	1548.33	659.38	1332.98	686.49	NS
Protein (g)	94.56	58.57	81.24	51.42	68.20	42.15	NS
Total fat (g)	103.37	38.84	71.18	32.71	61.97	33.19	0.0337*
Carbohydrate (g)	158.55	49.97	143.41	70.85	118.76	68.20	NS
Water (g)	2117.74	1416.66	2025.47	1305.92	2160.85	1409.62	NS
Alcohol (g)	3.18	10.06	3.44	10.89	5.53	17.49	NS
Caffeine (mg)	44.38	98.19	30.49	61.34	52.45	80.45	NS
Theobromine (mg)	0.00	0.00	16.27	50.20	8.31	18.41	NS
Total sugar (g)	50.46	29.15	51.82	41.03	34.23	39.99	NS
Total dietary fiber (g)	12.72	5.75	12.21	8.17	10.34	6.30	NS
Calcium (mg)	912.95	416.12	747.32	518.42	878.80	744.14	NS
Iron (mg)	13.54	4.61	9.24	3.81	7.98	3.65	0.0124*
Sodium (mg)	4018.21	1902.05	3265.74	1867.17	2350.25	1234.34	NS
Total folate (ug)	358.83	151.42	250.56	124.23	209.62	110.06	0.0434*
Folate (ug_DFE)	461.07	227.55	305.40	159.91	256.19	145.59	NS
Total saturated fatty acids (g)	39.70	17.93	24.24	12.35	20.56	14.94	0.0216*
Total monounsaturated fatty acids (g)	36.43	17.20	26.48	13.52	21.83	10.42	NS
Solid fats (g)	54.67	28.11	29.24	17.97	25.19	22.54	0.0434*

Superscript * represents a significant difference at V1 compared to V2 and V3 at $p<0.05$. Solid fats are fats that are naturally present in animal products or hydrogenated/partially hydrogenated vegetable oils including lard, tallow, butter,

shortening, palm kernel, coconut oils, cocoa butter, margarines; DFE, dietary folate equivalents

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Chapter IV: Red wine and vascular function: A review of factors that contribute to variable results

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Abstract

Moderate alcohol consumption has been associated with lower cardiovascular disease mortality and morbidities compared to abstinence and heavy intake. Clinical studies utilizing red wine have reported a significant improvement in vascular function. However, the responses across different studies are variable, which could be due to factors related to study designs and type of wine utilized. The purpose of this review is to address factors that might contribute to the discrepancies in the results. Thirty-four original articles (22 acute studies, nine short-term studies, and three single-arm studies) were included. Seventy percent of acute studies (single intake between 15 minutes to eight hours) reported statistically significant improvement in vascular function compared to baseline values, while about 40% noted benefits from red wine intake relative to comparison arms. In short-term studies (longer than 24 hours and less than four months), 40% and 20% reported significant improvements in vascular function from baseline or when compared to other groups, respectively. To reduce variability and allow for comparisons across multiple studies, factors such as duration, volume and frequency of wine intake, participant characteristics, vascular function measurements and meal effects are important. Detailed polyphenolic profiles should be provided to elucidate the effects of red wine on vascular function.

Introduction

Cardiovascular disease (CVD) is the major cause of death worldwide[1]. The disease is multifactorial, and is thought to develop from atherosclerosis, the progression of plaque blockage in the arteries[2]. One of the initial events that promotes atherosclerosis is a reduction in nitric oxide, an endogenous vasodilating compound involved in vascular endothelial function[3]. Vasculature homeostasis involves but is not limited to vascular endothelial and smooth muscle cells, and related outcomes[4]. Physiological assessment of vascular function is assessed by a number of measures, including flow-mediated dilation and arterial stiffness[5]. Under the homeostatic state, the vascular system has protective mechanisms to maintain blood flow and protect the vascular endothelium that is exposed to the blood stream, which regulates fluidity, vascular tone, platelet aggregation, immune responses, and inflammation[6,7]. Several factors influence vascular endothelial dysfunction, including aging, genetics, and lifestyle behaviors[8] such as smoking, lack of physical activity, and poor diet[9]. While many dietary factors can influence vascular function, this review focuses on the effects of red wine consumption. Other health outcomes associated with red wine intake, including changed in lipid profiles and inflammatory markers, and risk of cancer, are beyond the scope of this review.

A Mediterranean diet pattern is associated with improved endothelial function in at-risk individuals[10]. This diet is rich in fruits, vegetables, and plant-based fats, and often includes modest amounts of red wine[11]. While fruits, vegetables and plant-based fats are widely accepted as essential elements of a health-promoting diet, the inclusion of red wine is controversial. The lack of consensus about red wine may be due, in part, to

differences in epidemiological and clinical study designs and variability in the content of different red wines used in both acute and short-term clinical trials.

Several epidemiological studies have reported that moderate red wine consumption is associated with improved markers of cardiovascular health markers[12]. These benefits may be due to the bioactive compounds shown to modulate endothelial function, including polyphenols such as anthocyanins, (-)-epicatechin, and quercetin[13]. Alcohol may also be involved, as well as benefits often arising from the stress-reducing and social aspect of red wine consumption[14].

The Dietary Guideline for Americans (DGA) 2020-2025[15] addresses alcoholic beverage intake. Recognizing that some groups should abstain from alcohol intake (e.g., pregnant women) and noting that those who do not drink alcohol should continue that practice, the DGA notes that for those who choose to drink alcoholic beverages, one serving per day for adult females and two portions per day for adult males is appropriate. For all types of wine, 148 mL; 5 fluid ounces of 12% alcohol is considered as one portion. The Guidelines do not distinguish between red and white wine[15]. Although the World Health Organization does not have specific recommendations for alcohol intake, most countries have their own guidelines based on the grams of ethanol consumed[16]. One serving of alcoholic beverages in each country contains different amounts of ethanol, ranging from 10-18 g, and the daily limit in men is generally higher than in women[16]. The recommended daily intake can range from 10-42 g of ethanol for females and 20-56 g ethanol for males, which is approximately 1.33-2 times higher than for females[16].

The purpose of this review is to summarize and discuss the results of clinical research studies on red wine and vascular health. Discrepancies in study design, different outcome measures, variability in the volume and content of the red wine, and lack of details in the publications are addressed. Suggestions are offered for future research.

Methods

Original articles published between 1966 and 2019 indexed in scientific literature bases (PubMed, Biological Abstracts, CAB Abstracts, Food Science and Technology Abstracts, LILCAS, Scielo, MED-LINE, and Science Direct) were identified. Relevant reports published prior to 1966 were included if cited in the references of the primary papers.

The literature search was performed by matching the terms related to red wine, alcoholic beverages, and vascular endothelial function assessment in humans. The terms for red wines and alcoholic beverages included “grape wine”, “red wine”, “champagne”, “spirits”, “vodka”, “beer”, “sake”, “rice wine”, “shochu”, “whisky”, “wine”, and “alcoholic beverage”. The terms used for vascular endothelial function were developed from a review paper[17] and were identified as “vascular stiffness”, “vasodilation”, “vascular function*”, “endothelial function*”, “vascular reactivity”, “blood vessel reactivity”, “pulse wave velocity”, “augmentation index”, “flow-mediated dilation”, “FMD”, “vasodilatation”, “vascular endothelium-dependent relaxation”, “blood vessel dilatation”, “peripheral resistance”, “peripheral arterial tonometry”, “reactive hyperemia index”, and “RHI”. An advanced function was used in PubMed database queries to

confine the searches to “human” and “clinical studies”. The articles found in each database were consolidated, duplicates were removed, and the remaining articles were selected based on the title and abstract as the primary screening step. Full-text articles were screened based on the inclusion and exclusion criteria. The inclusion criteria required that human clinical studies be conducted in randomized controlled designs, and that provided a defined amount of red wine consumed. Exclusion criteria included review papers, animal studies, epidemiological studies (such as cohort and cross-sectional), reports that provided extracts, and if red wine was administered via any routes other than oral consumption. Information from the resulting papers was organized into tables showing study designs, number and type of participants, beverages used as comparisons, and red wine intervention details (volume, production location, varietal/type of grape, polyphenolic content, and type and amount of food served, if applicable). The outcome measures, study duration, location where the study was conducted (an indirect index of genetic and background diet), and origin of the red wine were also noted.

The studies were divided into acute and short-term interventions. Acute studies assessed the effects of red wine after consumption between 15 minutes to eight hours later. Short-term studies followed vascular function greater than 24 hours after intake.

Results

Thirty-four original articles were included in the review[11,18-48] (**Table 1**). No serious adverse effects were reported in any study. Overall, among 34 studies 64% of the acute studies reported statistically significant improvements in vascular function compared to baseline values. Thirty-six percent of the acute studies noted improved vascular function from red wine intake compared to other beverages or comparison arms (such as smoking only, or no food or beverage provided). Forty percent of the short-term studies reported significant improvements in vascular function from baseline, while and 20 percent noted significant benefits when compared to other beverages.

Three studies did not provide data on the change from baseline (time effect) within the red wine intervention (Vukovic et al.[32], Leighton et al.[46], and Zilken et al.[48]), which left 31 studies included in the discussion of factors including study duration, design, participant health conditions, age, sex, given amount of red wine, vascular function assessment methods, location, and origin of provided red wine. As for the discussion of “comparison beverages”, 31 studies were included while three[34,44,49] single-arm interventions (Botden et al., Hamed et al., and Andrade et al.) were excluded as they only compared pre-post red wine consumption and had no comparison arms.

Variability in vascular outcome measures

Study duration (acute vs. short-term)

Among the 31 studies, 21 were acute[18-33,35-39] and 10 were short-term [11,40-48] (**Table 2**). Eleven of the 21 acute studies (52%) and six of the 10 short-term studies (60%) significantly improved vascular function from baseline.

Discussion

The acute studies that showed significant improvements in vascular function either when compared to baseline ranged from 30 minutes to eight hours (480 minutes), which were mixed between the body metabolism and gut microbiome metabolism.

Within the first two hours, vascular responses to red wine intake could be to digestion, absorption, and metabolism in the liver[50-52]. Alcohol can be metabolized faster with no food in the stomach compared with food present. Nonetheless, blood alcohol concentration typically peaks approximately one hour after consumption[53]. Certain types of polyphenols (e.g., anthocyanins[54]) could be absorbed in the forms of aglycones or conjugates, metabolized through phase I and II pathways in the liver, and a low concentration of aglycones and conjugates might appear in the plasma within a few hours after ingestion[55]. However, at six to eight hours after intake, the vascular responses would also be influenced by the metabolism of polyphenols by the gut microbiome. Polyphenols with larger molecular weights (e.g., ellagitannins[56]) would not be absorbed in the small intestine and continue to the large intestine for gut microbiome metabolism and would then appear in the systemic circulation[55].

The short-term studies that reported significant improvements in vascular function when compared to baseline were conducted between 14 and 60 days of duration. Regular consumption for this duration would involve microbiome adjustments as noted above. While not with red wine, a systematic review reported that short-term diet interventions related to the gut microbiome could take up to three months (90 days) in humans to observe changes in vascular function at the physiological level[57,58]. However, one short-term red wine study[11] with the same duration (90 days) did not show a significant improvement in vascular function response either when compared to baseline. The possible explanation may be due to gut microbiota resilience. Studies have demonstrated that healthy microbiota change within 24 to 48 hours after a dietary challenge but returned to baseline profiles as the intervention condinued[59-61]. Gut microbiota has been shown to influence cardiovascular function in both animal models and clinical studies, so the return of gut microbiota profiles to baseline in longer interventions may explain the lack of significant improvements in vascular function[62]. Overall, the vascular function responses in the acute red wine studies could be a combination of postprandial effects and gut microbiome metabolism, while chronic consumption may involve the adjustment of the gut microbiome, including the abrupt shift in gut microbiota composition and a return of the microbiota to its pre-disturbance state[57].

Acute studies with the measurements within two hours after red wine consumption may be appropriate to assess the vascular function responses from the alcohol and low-molecular weight polyphenol metabolism but may not be able to assess the effects from the metabolites produced by the gut microbiome. The acute studies

over six hours may cover both the postprandial effects of the vascular function response and gut microbiome metabolism of polyphenols. However, the influence of alcohol on vascular outcomes might be reduced as the blood alcohol concentration is highest within one to two hours after intake and then typically declines rapidly.

Short-term studies with a duration of 14 to 60 days may show some significant changes from baseline and/or when compared to other beverages, but the factors influencing long-term effects are difficult assess due to gut microbiome adjustment to the new behavior (daily red wine drinking for several days). A study with red wine consumption longer than 60 days may theoretically contribute to the changes in the physiological level[57,58], but only one study was conducted of this duration, and no significant changes either from baseline[11].

A systematic review assessed the bioavailability of anthocyanins on different tissues from experiments in animal models (i.e., rat, mice, and pig) and the associations with health outcomes[63]. The authors observed that acute (0 to 24 hours) exposure to polyphenols resulted in the presence of parent compounds in the organs. In contrast, a longer exposure showed the predominant presence of metabolites which could be explained by the saturation of bilitranslocase, an anthocyanin transporter in the stomach, inducing the parent anthocyanin to be degraded into metabolites earlier than the breaking down process in the small intestine and large intestine[63], suggesting that the bioavailability of anthocyanins can change when the digestive tract is exposed to anthocyanin-rich foods for a certain period of time.

Another reason that the acute and short-term studies cannot be compared directly is that the acute studies assessed the postprandial response while the short-term

interventions evaluated outcomes at the fasting state. A meta-analysis reviewed studies exploring the effects of an intervention (red wine is included) provided with a high-fat meal on endothelial function as assessed by flow-mediation (FMD). When the FMD responses at two, three, and four hours post-consumption were compared with the measurement at baseline, the percent change of FMD was slightly lower after the high-fat meal consumption. The FMD% at fasting and four hours after eating were significantly lower in those at risk compared to healthy participants[64].

Type of study design

Among the 31 studies reviewed, three used a single-arm design, 21 used crossover designs, and seven were parallel-armed **(Table 3**)**.** A significant improvement in vascular function when measured from baseline were noted for 11 of the 21 crossover studies (52%), three of the seven parallel studies (43%), and all three single-arm studies (100%).

Discussion

Overall, significant improvements in vascular function were more common in the crossover designs than in parallel studies). The advantages of a crossover design are that each individual serves as their own control, which minimizes the variability between participants since they receive all interventions in a randomized order[65] and also adds statistical power since one person can be considered to have two separate responses, depending on data obtained when they were in each treatment arm. However, one of the limitations of the crossover design is the variation in the wash-out period where an inadequate wash-out duration can result in a carryover effect whereby baseline

parameters are not reestablished[67]. This might help explain why approximately half of the red wine studies reported no significant changes in vascular function.

Parallel-arm study designs have several strengths. No carryover effects exist as in a crossover study. However, this study design requires a larger recruitment and retention effort than a crossover design and may produce some unbalanced baseline characteristics[66], even when age, sex, health conditions, and BMI are within the inclusion and exclusion criteria. Some parallel studies that assess microbiota as one of the outcomes may be unable to account for differences in individual microbiome profiles or the metabolism of large molecular weight polyphenols. Parallel-arm studies often do not allow analysis of individualized responses across different treatment arms[67].

A single-arm trial design is the easiest for recruitment and retention and least costly to conduct. The main limitation is that while pre- to post-change data is available for each individual, no comparison group exists. Therefore, if changes in vascular function outcomes are noted, difficulty exists to determine if the changes were due to the red wine intake, or simply to the “treatment” of being involved in a study (the “placebo effect”)[68].

Participant health status

Among 31 studies, 22 were conducted with healthy participants[18-21,24-31,35-39,41,42,44,46,48] , five assessed people with coronary artery disease (CAD)[11,22,23,32,33,43], three were conducted in people with hypercholesterolemia[40,47], and one among people with type 2 diabetes(T2DB; **Table 4**)[45]. Of the 22 studies with healthy participants[18-21,24-31,35-39,41,42,44,46,48], twelve[19,24,25,29-31,36,38,39,42,44] (55%) reported significant improvement in

vascular function compared to baseline values. Among the five studies with CAD participants[11,22,23,32,33,43], three[23,33,43] (60%) showed significant improvements in vascular function.

Among results from the three studies with hypercholesterolemia participants[40,47,49], two[40,47] (67%) showed significant improvement in vascular function from baseline. A study with participants with T2DB did not show a significant change in vascular function from baseline[45].

Discussion

People with many chronic health conditions generally have impaired vascular function compared to healthy individuals. Impaired vascular function among those with chronic health challenges can be due to several factors, including higher oxidative stress and inflammation[69], both of which can disrupt the vasodilating factor, nitric oxide, and harden the blood vessels, resulting in dysfunction of the vasculature in response to shear stress[70]. One study compared the vascular function responses following 15 days of 250 mL/d red wine consumption among people with different health conditions, including healthy (n=7), arterial hypertension (n=9), and hypercholesterolemia (n=10)[49]. At baseline, vascular function (assessed by FMD) was not significantly higher in healthy people than in those with arterial hypertension or hypercholesterolemia[49]. After 15 days of red wine consumption, only the hypercholesterolemia group showed a significant improvement in vascular function compared to baseline, while values in the hypertensive group did not change[49]. The other two studies with participant with hypercholesterolemia showed opposite results, with one reporting a significant improvement from baseline[71] while the other did not[47].

The participants' health characteristics are important factors to consider when designing a study, as the intervention may be influenced by certain conditions but have no effects on others. As the majority of the studies were conducted in healthy individuals, extrapolation of results is only appropriate to other healthy people. Unfortunately, nutrition research is limited among populations with chronic conditions since most foods are not designed or intended to treat disease.

Sex

Among 31 studies, eight enrolled only males[19,20,22-24,26,32,33,38,41,46,48], one admitted only premenopausal women, one studied only postmenopausal females[34,47] while 14 included both sexes[18,21,25,27-30,37,39,40,42,44,45] and seven did not identify the sexes[11,31,35,36,43]. Four[19,24,33,38] of the eight male-only studies (50%) showed significant improvement in vascular function compared to baseline values. Results with premenopausal women[34] showed a significant improvement in vascular function compared to their baseline values, while the postmenopausal women study reported no changes[47]. Among the 14 studies that included both sexes, seven[25,29,30,39,40,42,44] (50%) showed significant improvements in vascular function compared to baseline values (**Table 5**).

Discussion

Participants from both sexes[18,21,25,27-30,37,39,40,42,44,45] were provided with the same amount of red wine, whereas the DGA recommendation is different for each sex[15]. Most studies exceeded the DGA recommended intake level for women (150 mL). Providing the same amount of red wine to males and females did not consider differences in

weight or metabolic body size, which can produce variability of results. Future studies should either analyze female data separately from males or conduct a study specifically with females and provide them with red wine at or below the DGA recommendation for their sex. Within the group of females, women at child-bearing age and postmenopausal women should be considered in separate groups as premenopausal women have cyclical hormonal changes, with estrogen providing a vasodilating effect that helps to maintain vascular function[72]. A review paper assessed the changes of cardiometabolic outcomes (e.g., serum cholesterol, oxidative stress, and C-reactive protein) in different phases of their menstrual cycles and found the values were significantly higher during the luteal phase compared to other phases[73]. Studies that include premenopausal women should standardize the start of the intervention based on a similar period of the female's cycle[74]. The methods used to monitor the menstrual cycle and standardize the period/day of outcome measurements include a daily diary, phase designation, phasic standardization, and continuous standardization. The techniques were used separately or together, depending on the study design[75]. Since postmenopausal women have ceased the production of estrogen, their vascular responses to red wine intake must be considered separately from their premenopausal counterparts[47]. To understand more about the relationship between red wine consumption and vascular function in women, future studies focusing on pre- and post-menopausal women with an appropriate serving size would help elucidate the discrepancies of vascular function responses due to sex and hormonal changes in adults.

Age

Twenty-two studies enrolled adults between the ages of 19 and 49, [18-21,24-31,35-39,41,42,44,48] while nine were conducted in adults 50 to 70 years old[11,22,23,33,40,43,45-47] (**Table 6.1**). Thirteen of the 22 studies (59%) with the younger participants showed significant improvements in vascular function compared to baseline, while four of nine studies (44%) with the older population showed significant improvements of vascular function compared to baseline (Table 6.1). When the age parameters are subdivided by sex, results from studies with the younger male participants showed three[19,24,38] of six studies (50%) with significant improvement of vascular function compared to their baseline levels (**Table 6.2**). For the older male-only population, one[33] of two studies (50%) showed a significant improvement in vascular function compared to baseline.

Discussion

The tendency of significant vascular function improvements from baseline became slightly less in studies with older participants, but the trends were relatively consistent among male-only studies. The aging process decreases elasticity and increases stiffness of vasculature, promotes atherosclerosis, and causes DNA damage, resulting in altered vascular physiology and hemodynamics, such as high blood pressure. As part of senescence, autophagy, the destruction of damaged proteins and organelles, can be disrupted, leading to circulating expired cell components that could promote oxidation and inflammation. Excessive oxidants could bind to nitric oxide, reducing its availability to help regulate vasodilation. Prolonged low-grade inflammation can promote atherosclerosis, increasing blood flow resistance and stiffening the

vasculature[69], which might worsen vascular function in older adults compared to younger populations.

With aging in men, testosterone declines with age and this physiological change may impact the integrity of vasculature as lower testosterone concentration is associated with impaired endothelial function and increased arterial stiffness, although testosterone's effects on vascular function are not as potent as estradiol in women[76].

Measurement of vascular function

Endothelial function is a complex dynamic that can be assessed through different measures which are not interchangeable. Favorable results of red wine intake on vascular function observed from multiple methods can strengthen the evidence that red wine may impact cardiovascular health through multiple mechanisms, but each measurement technique has both strengths and limitations.

Flow-mediated dilation is a non-invasive technique that assesses blood flow in the brachial artery using ultrasound or magnetic resonance imaging[70,77]. Compared to baseline values, a significant increase in FMD indicates an improvement in endothelial function, while a decrease represents the opposite. One study included in this review assessed endothelial function with FMD, but the results were normalized to an endothelium-independent value in response to 0.4 mg of sublingual nitroglycerin and presented as the ratio of endothelial-dependent dilatation to endothelial-independent dilatation (EDD/EID)[43], which creates difficulty in comparing these results to other data presented by FMD.

Endothelial Peripheral Arterial Tonometry (PAT) is another non-invasive assessment of microvascular function that measures the pulse wave amplitude of the index finger after a 5-minute vascular occlusion in the test arm, and compares the changes to the pattern noted in the non-occluded arm[20]. Algorithms are used to calculate a reactive hyperemia index (RHI), with values less than 1.69 correlated with endothelial dysfunction. Other vascular function outcome measures calculated by PAT include the augmentation index (AI), a measure of arterial stiffness represented as the percentage of the ratio between augmentation pressure (peak of systolic blood pressure subtracted from the inflection point) and pulse pressure. When compared to baseline values, a decrease in AI represents an improvement in endothelial function.[78]. In addition to PAT, the AI can be assessed by several methods including pulse-wave velocity (PWV)[32,39], strain-gauge plethysmography (SGP)[27,31,34], wave reflection[23], a sphygmocor system[26], aortic AI[29], pulse-wave analysis (PWA)[47] and forearm arterial blood flow (FBF)[44].

Among the 31 studies, 18 (63%) used FMD[11,18,19,21,22,24,25,28,33,35,37,38,40-43,46], three followed SGP[27,31,45] and two each employed PWV[32,39] and FBF[44] (**Table 7**). One study each used PAT[20], Laser Doppler Imager (LDI) with iontophoresis[36], wave reflection[23], aortic AI[29], and the sphygmocor system[26].

Discussion

Overall, the results varied depending on the methods used and the outcomes reported. The studies with FMD and AI had similar trends, with approximately 50% of the studies showing significant improvements in vascular function from baseline.

One possible explanation for the discrepancies in the results across different measurements of vascular function could be the difference in techniques and operator skills in obtaining the data. Although the terms vascular function, endothelial function, and arterial stiffness can represent similar outcomes, their definitions are dissimilar and each of them can be assessed by different methods[79]. For example, the “gold standard” for arterial stiffness is assessed by non-invasive PWV and the main outcome is AI[80], while endothelial function is often determined using either invasive quantitative angiography[81] or non-invasive FMD[82]. The outcome of AI can also be measured by PAT and the outcomes are generated from the same dataset used to calculate RHI, another endothelial function measure[79]. A study that addressed the limitations of comparing results across different measurement techniques and outcomes by measuring endothelial function and arterial stiffness using PWV, a vascular profile device (VP-1000), a Sphygmocor, and PAT found correlations between PWV and AI measured by the VP-1000 but not by the Sphygmocor[79]. Another study compared the endothelial function between FMD and PAT in healthy participants and patients with peripheral arterial disease and concluded that FMD and PAT produced different results[83]. One possible explanation of the discrepancies in results could be that FMD and PAT measure different parts of the vascular beds[84,85]. Flow-mediated dilation measures function of the conduit arteries while PAT measures responses in the peripheral vessels, which involves macro- and micro-circulation[85]. The vasodilation mechanism in FMD is predominantly mediated by nitric oxide[84], while the response measured by PAT is the combination dynamics mediated by nitric oxide and the sympathetic nervous system[85]. Different levels of operator skill are may also account for differences in

measurements between FMD and PAT[84]. An accurate FMD measurement depends on a skilled and experienced technician, whereas the PAT is primarily self-automated, requiring minimal training of the operator[84]. Thus, the vascular function outcomes in the red wine studies reviewed here present challenges in making interchangeable conclusions.

Volume of red wine intake

The standard portion size of wine for an adult varies by country. For example, the United States (US) DGA 2020-2025 defines one serving as 150 mL (5 fluid ounces) and suggests that women should not exceed one and men should not exceed two servings per day[15]. While the World Health Organization (WHO) does not have a universal standard for alcohol consumption, the recommended daily intake in most countries is based on grams of ethanol/day, ranging from 10g to 56g, regardless of sex[16]. Based on the US standard, one portion of red wine would contain 12 to 14 g ethanol[86], assuming 12% alcohol by volume. For the purposes of this review, the portion size is divided into three groups: 1) below the US DGA standard (<150mL); 2) within the DGA standard (150-300 mL); above the standard (>300mL).

Some studies apportioned the red wine by the amount of alcohol or volume, based on body weight[19,21,26,32,33], while others used a fixed amount for all participants[11,18,20,22-25,27-31,34-42,44-48] (**Table 8**). In this review, the average weight in kilogram (kg) of participants was used to calculate the estimated volume of red wine used in the study. If the weight information was unavailable, 55-kg and 70-kg body weights were used as the standards for adult females and males, respectively.

Discussion

Two of the 31 studies both provided red wine below the standard, serving 100 mL of red wine and comparing its vascular function effects to several other beverages[20,42]. One was an acute study providing 100 mL of red wine mixed with 100 mL of sparking water, consumed with a high-fat meal (150 g French fries, 200 g pork sausage, 30 mL curry sauce)[20]. The intervention compared the wine intake to 200 mL of sparkling water, 200 mL of a soft drink, and 200 mL of distilled spirits containing a similar amount of alcohol as in the wine[20]. Compared to baseline, the results showed that endothelial function, assessed by PAT and reported as the RHI, was maintained with red wine and spirit intake, while the sparkling water and soft drink showed significant decreases two hours postprandially [20]. The change in the RHI one and two hours following the soft drink intake was significantly lower compared to water, while the RHI results following red wine and spirit intakes were not significantly different compared to sparking water[20].

The second study was a short-term study that provided 100 mL of red wine and compared FMD responses to participants who consumed 250 mL of beer, 30 mL of vodka, or 200 mL of water[42]. Only the red wine group showed significantly improved endothelial function when compared to either their baseline values or to the responses elicited by any of the other three beverages[42].

Twenty studies provided red wine volumes in a standard amount (150-300 mL), of which 15 were acute [18,19,21-33], five were short-term studies[11,40,41,43,44]. When compared to baseline values, 12 studies reported improvement in endothelial function following red wine consumption[19,23-25,28-33,40,43,44].

Eight studies provided red wine in a volume above the standard amount (>300 mL). Five[34-39] were acute studies and three[45-47] were short-term. When compared to baseline values, four of the acute studies reported a significant improvement in endothelial function[36,38,39]. A one-arm study by Botden et al. showed a significant improvement in endothelial function after three weeks of daily red wine consumption, although no acute effect was observed within 16 hours following the first intake[34].

Overall, most clinical studies assessing the effects of red wine on vascular function were within the alcohol intake recommendation of the DGA, and this level appeared to have the highest rate of vascular function improvement following red wine intake both from baseline. When the wine volume increased above the standard level, fewer studies reported improvements in vascular function. The higher volume of red wine intake produces an increase in alcohol content and potentially higher polyphenol concentrations than a lower volume.

As the relationship between alcohol intake and all-cause mortality is commonly reported as a J-shaped curve, meaning only light to moderate level (approximately 1-2 servings) was associated with lower risk and mortality compared to abstinence and higher consumption[87]. One meta-analysis reported a mean alcohol intake of 30g/day (~2 servings of 150 mL each, 14% red wine) was associated with favorable parameters related to coronary artery disease (i.e., increase in HDL and decrease in coagulation protein, fibrinogen)[88]. Certain studies provided red wine containing alcohol that exceeds the optimal level for health benefits, which might result in impaired vascular function or no significant differences compared to comparison arms.

Within the same red wine, the polyphenolic content increases relative to the volume increase. However, when compared different red wines to each other, some red wines with the higher volume may have less polyphenolic content. For example, the study by Whelan et al. provided red wine at 280 mL containing 328 mg GAE, while the study by Boban et al. provided less red wine volume at 210 mL, but the polyphenol content was 588 mg GAE[19,33].

The definition of drinking patterns, such as mild, moderate, and heavy, vary depending on the studies. A systemic review assessed self-reported methods used to measure alcohol consumption in older adults aged 65 and over, and the results showed a lack of standardization with various definitions of alcohol abstinence, usual alcohol intake, and at-risk drinking behaviors. of alcohol intake in older adults. The diverse definitions of drinking patterns might affect the conclusion of the relationship between alcohol intake and health outcomes or mortality in epidemiological studies[89]. The comparison of alcohol intake across countries in Europe, North America, and Australia was challenging due to various factors, including different alcohol standards and survey questionnaire use[90].

Comparison beverage or interventions

The types of comparisons can be divided into five groups: 1) red wine with other active ingredients, 2) dealcoholized red wine, 3) other alcoholic beverages (vodka and beer), 4) water, and 5) other non-alcoholic beverages (cola and grape juice). The summary and description of the comparison are provided in Table 9.1 and 9.2, respectively.

Dealcoholized red wine

Eleven studies compared vascular function effects following red wine consumption to dealcoholized red wine[18,19,22-24,28,29,38,39,46,47]. Only two of these showed significantly improved vascular function in the red wine group[23,29] (Table 9.2).

One possible explanation for the events that dealcoholized red wine showed significantly better vascular function response than original red wine could be due to participants' alcohol metabolism. People do not respond to alcohol the same way due to their genetics and habitual alcohol consumption. Some people are sensitive to alcohol-triggering allergy-like symptoms, including flushing, itching, and headache[91,92], which might impair vascular function resulting in a worse response in some studies. Individuals who regularly consume alcohol may increase their capacity to metabolize alcohol with decreased chances of intoxication[93], which might explain some cases of better vascular function responses following red wine consumption compared to dealcoholized red wine. The tolerance development could be explained by modifying gene expressions related to metabolic enzymes and proteases[93].

Another explanation for better vascular function outcomes in red wine consumption compared to dealcoholized red wine was that the dealcoholizing process might remove some alcohol soluble polyphenols that have vascular function effects[94], resulting in the worse vascular function response in some cases with dealcoholized red wine.

Three studies compared the effects of red wine along with other active ingredients[25,30,41]. One study showed that one had significantly different vascular function effects than the other[25].

Other alcoholic beverages

Eight studies[19,26,33,36-38,42,46] compared vascular function effects of red wine intake to other alcoholic beverages. Two of these reported the red wine intervention showed significantly better vascular function responses relative to the comparison groups, which were vodka and beer in one study[42] and an alcohol-matched control in the other[36] (Table 9.2). Other than the alcohol effects, each beverage has its own unique polyphenolic profile. Red wine is a rich source of anthocyanins, catechins, proanthocyanidins, stilbenes, and other phenolics[95], while white wine is predominant in hydroxycinnamic acids[96]. Beer is a rich source of tannins, phenolic acids, and flavonols[97]. Distilled spirits contain predominantly ellagic acid, likely from wood aging[98], but the contents vary depending on the production method[99].

Ellagitannins are hydrolyzable tannins with anti-inflammatory and anti-hypertensive properties[100]. The source of ellagitannins in red wines, mainly castalagin and vescalagin, is primarily derived from the oak barrel during aging. The concentration and specific subtypes of ellagitannins found in each red wine depend on the type and origin of the wood barrel, whether it had been used for winemaking previously, and the aging time in the barrel and the bottle[101]. One study compared the concentration of ellagitannins in red wines produced in different areas of Bordeaux, France, or Rioja, Spain, aged in different oak wood barrels (French oak or American oak, respectively), and stored for different periods in the barrel (Rioja: 12, 24, and 36 months) or in the bottle (Bordeaux: 1994-2010 and Rioja: 2000-2013)[101]. The results showed that the concentration of ellagitannins in the Bordeaux red wines decreased over time in the bottle, while the ellagitannin profiles of Rioja wines were consistent throughout the four

to 12 years of aging[101]. Although anthocyanins and ellagitannins both have a low level of absorption in the small intestine, derivatives or the parent compound were present in small amounts in the urine and plasma within one to five hours after consumption of anthocyanin-rich[102] and ellagitannin-rich food[103]. Oak ellagitannins also delayed malvidin-3-glucoside degradation[104], suggesting a positive and potential synergistic relationship between these two polyphenols.

In studies where the vascular function outcomes showed similar responses between red wine, beer, white wines, or spirits, either similarities in alcohol content or the combined effects of alcohol and polyphenols in the beverages were the likely factors. Unfortunately, most of these studies do not provide a detailed polyphenol profile, hindering comparisons at the chemical level.

Water

Eight studies[20,26,31,32,37,38,42,47] compared vascular function effects of red wine to water, with four of them reporting significant improvement in vascular function following red wine consumption[26,32,37,105] (Table 9.2). One potential explanation could be due to the side effects of alcohol, since it induces diuresis, resulting in dehydration and activating a thirst response and oral dryness[106]. Dehydration affects the hormone angiotensin and the mechanoreceptors that are involved in regulation of the cardiovascular system, which could result in increased blood pressure and impaired endothelial function[107]. The hydration status of participants prior to the intervention is rarely addressed, and this may be a confounding factor when assessing vascular function. In a crossover trial that compared the vascular function responses between

hydration and hypo-hydration induced by vigorous exercise and water restriction, the results showed that the vascular function (as assessed by FMD) was significantly decreased by 27% when 2% of body mass was lost due to dehydration[108]. Non-alcoholic drinks such as cola[21] and grape juice[40] were used as one of the comparisons arms in clinical studies that assessed the effects of red wine on vascular function, but the responses following SSBs in these studies were not significantly different from the red wine intervention group [21,40].

Non-alcoholic beverages

Sugar-sweetened beverages such as cola increase blood glucose, which alters inflammatory markers and immune cells involved in atherosclerosis, eventually developing plaque obstructing the blood flow and impairing vascular function responses[109] (Table 9.2). Two clinical trials using purple grape juice showed improvements in vascular function effects from baseline[110] or when compared to sugar-matched control[111], while no study appears to have assessed the effects of white grape juice on vascular function. One study that used purple grape juice[40], and compared to red wine, the vascular function response, as assessed by FMD, was not significantly different. Some studies with white grape juice showed significant improvements in markers related to cardiovascular health, including platelet [112] and HDL cholesterol [113].

One study reported no significant differences in vascular function following the intake of either red wine, water (as a control), orange juice, or green tea[114]. As red wine, green tea, and orange juice have all been reported to improve vascular function, a significant difference in these responses may have been difficult to distinguish. Orange

juice contains hesperidin as a major polyphenol, and intake of this flavanone has been associated with lower CVD mortality[115]. A four-week crossover study comparing the effects of commercial and fresh orange juice on FMD and related vascular outcomes reported that both juices decreased inflammatory markers related to vascular dysfunction, such as VCAM, hs-CRP, and E-selectin although no significant change in FMD was observed. A decline in LDL was only observed after the fresh orange juice intake[116]. Red (blood) orange is a unique type of orange rich in anthocyanins[117] other than hesperidin and a number of clinical studies have reported that its consumption significantly improved vascular function as assessed by FMD[118-120].

A crossover randomized clinical trial (RCT) assessed the effects of hot water, green tea, green tea extract, or epigallocatechin gallate (EGCG) on vascular function as assessed by FMD at baseline and two hours after consumption. Only the green tea intervention produced a significant improvement in FMD from baseline[121]. The authors suggested that other compounds in green tea, or their metabolites produced after processing by the gut microbiome, may explain their results[121]. A meta-analysis of nine clinical studies assessing the effect of green or black tea intake noted a median intake of 500 mL (approximately two to three cups) and found an overall increase in FMD of around 2.6% compared to controls[122].

Alcohol abstinence (with/without other vascular dysfunction manipulation)

Nine studies used alcohol abstinence (or no alcohol) as the comparison arm[11,24,27-30,43,45,46,48], of which five noted that red wine consumption significantly improved vascular function compared to consuming no alcohol[24,28-30,48] (Table 9.2). This

event is similar to several observational studies that have repeatedly found a J- or U-shaped curve of associations between alcohol consumption and risk of CVD, suggesting that those who are light to moderate alcohol drinkers had a lower risk of CVD when compared to either abstinence or heavy consumption[123].

Other manipulations, such as smoking or hypoxia, were used in some studies as the comparison arms to first provoke a decline in endothelial function, after which red wine was ingested and assessment of vascular function was conducted[24,26,28,29,32]. Cigarettes contain nicotine, compound that can induce vascular dysfunction by inhibiting programmed cell death and promoting atherosclerosis, which hardens the vasculature and impairs vascular function[124]. Hypoxia is a state in which oxygen is unavailable in sufficient amounts at the tissue level to maintain adequate homeostasis and is due to a low blood supply or low oxygen content in the blood (hypoxemia)[125]. The intensity of hypoxia can vary from mild to severe and can present in acute, chronic, or acute and chronic forms, which can affect vasculature homeostasis, including vascular permeability and growth, inflammation, and repair of vascular injury[126]. The effects of hypoxia on the vasculature are inconsistent, possibly due to variable levels of hypoxia and whether the exposure is acute or chronic. Some researchers have suggested that hypoxia causes vasodilation in systemic arteries and vasoconstriction in pulmonary arteries[127] while others reported vasoconstriction in systemic arteries[128]. One study examined the effects of hypoxia on microvascular and large blood vessels as assessed by LDL and found that acute exposure to normobaric hypoxia significantly reduced endothelium-dependent vasodilatory capacity in small and large vessels[129]. Although hypoxia is generally considered to have deleterious effects for vascular function, people

who are adjusted to hypoxic environments such as highlanders in Tibet, Peru, and Ethiopia appear to preserve their vascular function[130].

Discussion

Overall, the studies that compared vascular function results with other beverages, smoking or hypoxia reported less significant differences between the groups than the when the data was analyzed relative to baseline values. When the comparison groups did not contain bioactive compounds (i.e., water[26,32,37]) or participants refrained from red wine intake in a comparison group (such as smoking only[24,28,29]), the effects of red wine on vascular function tended to be significantly better than those arms.

Meal effects

Among 34 studies, 20 studies reported meals given or instructed along with the red wine consumption, while 14 studies did not mention whether the food was provided. In 20 studies that participants consumed red wine with food, ten[24,25,29,30,33,36,43,44] showed significant improvement in vascular function from baseline, while five[24,25,28-30,48] of 20 studies showed significantly better vascular function outcomes when compared to other beverages (Table 10). The studies that showed significant improvement in vascular function responses from baseline included short-term studies that instructed participants to maintain their usual dietary intake and exercise habits[44,49] and acute studies that provided participants with meals, including three studies provided 30 g of white bread and 30 g of 4% fat cottage cheese[22,24,28,29], one study with 60 g of white bread plus vegetable soup (736 kcal)[25,30]. One study gave participants a baguette with five grams of margarine, two lettuce leaves, a half-slice of tomato, one slice of lean

cheese, along with 150-200 g of low-fat yogurt and a banana[18,33].. Another study gave a "standard breakfast and lunch" (no details of the meals were provided) served at 15 and 200 minutes after beverage consumption[36].

Other acute studies that provided meals but did not show a significant improvement in vascular function from baseline were studies that noted their meals as high fat, including a study with 150 g French fries and 200 g hot pork sausage with 30 mL curry sauce[20], a study providing a hamburger with a bun, cheese, sauce and French fries[21], and a study provided participants with choices of omnivore and vegetarian meals containing 900 kcal, 50 g fat[114]. Another study did not consider the meal given as high fat, but provided 120 g pasta with tomato sauce, 25 g olive oil, 50 g bresaola (salted meat), 60 g bread, and an apple[27].

In short-term studies, participants were free-living and no standardized foods outside of the intervention were given. Rather, participants were instructed to follow certain dietary patterns throughout the intervention[11,41,43-45]. A one-arm study asked participants to maintain their regular diet[44]. Other studies specified the types of diet, such as a Mediterranean diet vs. a low-fat diet[11], a high-fat diet vs. a control diet[41], or a diet for people with diabetes[45]. One study instructed red wine to be consumed with a meal but did not give the details of the meal[43].

Discussion

The type and amount of food given with red wine will produce different absorption patterns of polyphenols, alcohol as well as altering the postprandial dynamics related to the vascular function. When alcohol is consumed with solid food, gastric emptying is prolonged with moderate amounts of alcohol (4-10% by volume)[131] as well as with high

amounts (~15%)[132]. However, the consumption of alcoholic beverages alone accelerates gastric emptying, which could result in more pronounced vascular function responses[133,134].

The vascular function responses following different alcohol levels vary depending on the concentration. Low to moderate alcohol consumption induces vasodilation[135], while high alcohol levels have been shown to induce vascular endothelial inflammatory markers and reduce nitric oxide available, resulting in vasoconstriction[136].

Different food components will also influence vascular function responses[137]. A meal with added sugar could induce hyperglycemia, which would increase oxidative stress and reduce nitric oxide bioavailability, resulting in impaired vascular function[138]. A meta-analysis compared the vascular function responses (both in macro- and micro vasculatures) between acute hyperglycemia and normoglycemia noted a significant reduction in macrovascular function following acute hyperglycemia from both an oral glucose tolerance test or after consumption of food containing a high amount of simple sugars (i.e., cookie and rice milk)) but not microvascular endothelial dysfunction during acute hyperglycemia[138]. The glycemic index (GI) is a measure of how much 50 g of specific items could raise the blood glucose level. The higher GI indicates more refined and sugary foods, which is associated with an increased risk of cardiovascular disease morbidity and mortality[139].

A high-fat meal is known to impair endothelial function and is a long-term risk factor that can eventually lead to atherosclerosis[140]. Postprandial effects alone affect vascular changes[141]. The type of fat in a food or meal affects vascular function

differently, as saturated fat tends to impair vascular function by inducing inflammatory responses and downstream pathways, while unsaturated fats, especially monounsaturated and long-chain polyunsaturated fatty acids, are involved in producing anti-inflammatory mediators that modulate favorable vascular function responses[142].

A high protein diet did not improve cardiometabolic health and vascular function[143] while moderate animal protein intake has shown favorable vascular function effects[144]. However, the vascular function trends may not always follow the patterns as different studies utilized various kinds of food and amounts. For example, one study compared the postprandial vascular function effects following isocaloric meals consisted predominantly of carbohydrates, protein, and fat in healthy overweight and slightly obese men but did not see significant differences in FMD[145].

Factors not considered in study designs

Polyphenol quantification

Of the 34 studies reviewed, 12[18,19,22,23,25,26,30,33,34,44,46,47] identified some polyphenolic compounds and/or reported the concentration of the compounds. This information varied considerably, using different units and focusing on different compounds such as gallic acid equivalents (GAE) and caffeic acid (Table 11). In this review, the concentrations of the polyphenolic compounds were calculated based on the volume of red wine provided in each study.

Studies reporting concentration as total polyphenols (mg GAE)

Eight studies reported the polyphenolic compounds in red wine as total polyphenol or "mg GAE"[19,33]. One study compared the effects of red wine on endothelial function with dealcoholized red wine and red wine devoid of polyphenols (588 vs. 586 vs. 20 mg GAE, respectively)[19]. A second study compared the effects of red wine on endothelial function with white wine (328 vs. 59 mg GAE)[33]. A three-arm parallel RCT study that provided participants with either 400 mL of red wine (containing 1,000 mg GAE), 400 ml of dealcoholized red wine (containing 1,000 mg GAE) or 400 mL water daily for six weeks and assessed vascular endothelial function by pulse wave analysis (augmentation index) reported no significant changes from baseline or when compared to the dealcoholized red wine or water[47]. The study by Agewall et al. compared the effects of red wine on endothelial function, assessed by FMD, with dealcoholized red wine (487 vs. 277 mg phenol/250 mL) as the comparison[18] while the study by Krnic et al. noted the phenol content of red wine at 1,274 mg phenol/ 210 mL red wine[26]. The study by Agewall noted that the dealcoholized red wine with the lower phenol content significantly improved endothelial function, while the red wine with the slightly higher phenol content did not[18]. The study by Krnic et al. reported that red wine improved endothelial function (assessed by sphygmocor and calculated as the AI) to the same extent as vodka and beer, and all three beverages produced a response significantly better than water[26]. A single-arm study by Botden et al. provided 672 mg total polyphenols/ 336 mL of red wine and did not show a significant improvement in endothelial function within 16 hours (as assessed by strain-gauge plethysmography), but showed a significant improvement in vascular function from baseline after three

weeks of daily consumption[34]. Another study provided 250 mL of red wine containing 725 mg of total phenols daily for 21 days and showed a significant improvement in endothelial function (as assessed by strain-gauge forearm blood flow) compared to baseline values[44]. One study provided red wine containing 759 mg polyphenols daily for four weeks and compared the results to dealcoholized red wine containing 786 mg polyphenols[46]. No significant changes in endothelial function occurred, whether calculated as changes from baseline or when compared to the dealcoholized red wine.

Studies reported polyphenols in mg caffeic acid

Four studies provided the polyphenolic content as "mg caffeic acid"[22,23,25,30]. Two of these compared the effects of red wine on endothelial function (assessed by FMD and wave reflection, used to calculate the AI) with dealcoholized red wine (red wine vs. dealcoholized red wine with 1,645 vs. 1,625 mg caffeic acid, respectively)[22,23]. The other two studies compared the combination effects of red or white wine and green or refined olive oil on endothelial function (red wine 163 vs. white wine 32.5 mg caffeic acid)[25,30]. Three of the four studies noted significant improvements in endothelial function compared to baseline values, as well as to the control (dealcoholized red wine[23] or white wine with refined olive oil[25,30]). A fourth study reported no change from baseline for a red wine group (1,645 mg caffeic acid) but noted a significant decline in endothelial function (as assessed by FMD over 90 minutes) 60 minutes after intake compared to the dealcoholized red wine control (1,625 mg caffeic acid)[22].

Discussion

A major challenge in comparing results from different studies is the lack of standardized compositional information about the red wines, since different units of measurement are reported as phenols, polyphenols, and GAE. In this review, 12 studies provided information about phenolics but used different assays[18,19,22,23,25,26,30,33,34,44,46,47]. Differences in measurement units might be due in part to the advancement of technology for the measurement of polyphenolic compounds since older studies that report GAE may not have had contemporary equipment, standards and methodology to accurately quantify polyphenols[96]. The quantification techniques for polyphenol assessment have evolved over time and new methods are developing to uncover previously unidentified compounds that may contribute to critical sensory attributes and health benefits[146]. Providing the total polyphenol content (TPC) using the Folin-Ciocalteu assay can give a broad idea about the amount polyphenols are in the food but does not differentiate between the major polyphenol subtypes.

The polyphenol content of the red wines considered in this review can also be compared to studies that provided red wine extracts. Three studies that utilized red wine polyphenol extracts (280 and 560 mg over four weeks[147] or 550 mg for four weeks[50] or 550 mg for two weeks[148,149]) reported no significant change from baseline in vascular function compared to a control (microcrystalline cellulose)[147-149]. Two additional studies assessed the effects of resveratrol extracted from red wine in both an acute study (30 mg[150] trans-resveratrol vs. placebo or water over 45 minutes) and a short-term study (10 mg trans-resveratrol vs. placebo[151] over three months) on FMD and noted a significant improvement in endothelial function compared to baseline values and to a

microcrystalline cellulose control[149,150]. The acute study also tested the effects of extracts with higher concentrations of trans-resveratrol (90 and 270 mg) but not showed any significant improvement in vascular function compared to the control[150]. Although the resveratrol in red wine has been suggested to be one of the key compounds that contributes to the protective effects against metabolic diseases, including CVD[152], the amount of resveratrol in 150 mL of red wine is estimated to be only 0.05-1.07 mg[13], far below the amounts provided in the extracts.

Baseline diet of participants and geographic location

The population tested could affect the red wine study results, based on differences in genetics, background diet and gut microbiome profiles, among other factors. In this review, eighteen studies were conducted in Europe, with eight from Greece[22-25,28-31], three in Croatia[19,26,32], two each in Italy[27,45] and the Netherlands[34,35], and one each from the United Kingdom[36], Ireland[39], and Germany[20]. Two studies were conducted in North America, with one from the United States of America[21] and another from Canada[37]. Four studies were conducted in South America, two studies in Brazil[11,40] and the other two in Chile[41,43]. Two studies each were conducted in Australia[46,47] and New Zealand[18,33]. Three studies were conducted in Asia, with one study from Japan[38], one from Taiwan[42], and one from Israel[44] (Table 12). Among the 18 studies conducted in Europe, ten[23-25,29-31,36,39] (56%) showed significant improvement of vascular function from baseline. Among the four studies conducted in New Zealand and Australia, one study (25%) showed a significant improvement in vascular function compared to

baseline values[33]. All three studies[38,42,44] (100%) in Asia showed significant improvements in vascular function compared to their baseline measures.

Discussion

The majority of the studies were conducted in Europe, with significant improvements in vascular function in response to red wine intake reported in approximately 50% of the trials. Variability in the background diets from different regions in Europe may influence some of the results. For example, the mean daily polyphenol intake in Europe was estimated to range from 584 to 1,786 mg, with the lowest and highest consumption in Greece and Denmark, respectively[153]. In Poland, the mean polyphenol intake was 1,757 mg/day, approximately half of which were from flavonoids (897 mg/day) and the remainder from phenolic acids (800 mg/day)[154]. In Japan, a cross-sectional study of older adults reported high variability of polyphenol intake, ranging from 183 to 4,853 mg/day with an average of 1,493 mg/day, mainly coming from coffee and green tea[155]. In the US, the estimated polyphenol intake in a typical diet is approximately 1,768 mg/2,000 kcal/day[156].

Although no dietary reference intake for polyphenols is currently established in the US or elsewhere, the US Academy of Nutrition and Dietetics recently proposed a recommendation of 400-600 mg/day of flavan-3-ol intake to support parameters of cardiometabolic health, including blood pressure, cholesterol, and glucose control[157]. Abundant polyphenol-rich food intake is encouraged, as it is associated with more favorable health outcomes in general[156]. Incorporating red wine into the diet may be a good source of these polyphenols. The study by Botden et al.[34] provided 360 mL red wine containing 672 mg total polyphenols, and the 375 mL red wine in the study by

Zilken et al.[46] contained 759 mg polyphenols. However, the vasculoprotective and other benefits from polyphenol intake can vary depending on individuals[158,159], the type and concentration[160] of polyphenols being consumed, and the interaction between polyphenols and other food matrices[161], among other factors.

An individual's ability to metabolize ethanol in red wine may also contribute to the discrepancies in the research literature. Ethanol metabolism regulated by genes that encode two main alcohol metabolic enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)[162], which influence the whose rate kinetics can determine of a person is a slow or fast alcohol metabolizer[133]. Adaptation may occur in individuals who habitually consume alcohol, which increases the rate of alcohol clearance from the body[134]. When compared with Caucasians[163], east Asians (e.g., Chinese and Japanese) have a high prevalence of genetic variation with slow ADH and ALDH kinetics, resulting in the accumulation of acetaldehyde and discomforts such as flushed skin, runny nose, abdominal pain, and headache[164]. Caucasians tend to metabolize alcohol relatively quickly compared to Hispanics/Latinos, African Americans, and East Asians[165]. Variability and discrepancies in results from red wine studies may therefore be due, in part, to the ADH and ADLH activities in individuals.

In addition to genetics related to alcohol metabolism, gut microbiome profiles vary between individuals, and the differences could influence the physiological responses related to cardiovascular diseases[58,166]. External factors, including lifestyle and diet, also affect gut microbiome, as observed in the lower diversity in gut microbiome in individuals living in urban areas compared to rural areas[167].

Drinking cultures and habits that people do when people consume alcohol may affect the interpretation of the results. The cultures can be categorized into two types, wet and dry cultures. The wet culture is predominant in European countries where the Mediterranean diet is widely practiced and alcoholic beverages, mainly wine, are consumed as a part of the meal. The dry culture is common in Scandinavian countries and North America (the United States and Canada), where alcohol consumption is not included in daily life but rather for socializing and celebration, where intoxication usually occurs. The drinking cultures among Asian cultures such as East Asian (Japanese, Chinese, and Korean) immigrants in the US and Northeastern Thailand were more for socializing, which is close to the dry culture[168,169].

Red wine origin, viticultural practices, and processing

The red wines used in clinical studies varied in their origins. Eighteen studies utilized local red wines[18-30,32,33,40,45-47], five used imported red wines[34,35,37,38,42], while eleven did not identify the origin or specific type of red wines used[11,31,36,39,41,43,44]. Among 31 studies considered in the discussion, 53% of studies in Europe, 100% in South American studies, and 25% in Australia/New Zealand reported significantly improved vascular function from baseline values (Table 13).

Discussion

The environment and geography can influence grape phenotype expression, resulting in different amounts and types of polyphenolics[170]. A study from Italy showed significant differences in flavonols, anthocyanins, phenolic acids, and resveratrol among red wines from 10 different geographic regions[171]. While most red wines included in this

review were produced from grapes grown in warm regions of Europe, the fruit is also grown in a variety of other climates worldwide. Differences in sensory attributes of wine vinted from warm- vs. cool-weather grapes have been reported, which can also be reflected in the polyphenolic profiles[172-174]. Indeed, investigators from Japan have reported differences in chemical composition between imported and locally produced wine[175,176].

The polyphenol content and type, as well as the alcohol content, likely contribute to the beneficial vascular effects noted by many researchers. As a complex food containing hundreds of compounds, the identification of a single bioactive molecule or group of compounds is challenging. Both the type and amount of polyphenols in red wine can vary, depending on environmental factors (terroir) and variety of grape[177,178], with some of these differences noted by sensory evaluations[179-181].

Although the majority of the studies in this review reported the general region of the red wines, more details regarding the location and type of red wines used in future studies can help researchers understand if variations in these qualities significantly influence vascular function. The effects of red wine with graded anthocyanin (or other polyphenols) concentrations on vascular function have not been evaluated in clinical trials, but an *in vitro* study that compared the effects of red wine polyphenols from different locations on the activation of endothelial nitric oxide synthase, an enzyme that regulates vasodilation and generally represents an improvement in vascular function, observed varying responses. Although the study concluded that the polyphenolic contents of red wines were not different worldwide, select red wine from France

contained the highest amount of resveratrol and had the highest *in vitro* activity of endothelial nitric oxide synthase, the enzyme involved in vascular vasodilation[182].

Anthocyanins are among the most abundant polyphenols in red wine, which provide much of the color and can also influence vascular function[183]. Red wines from different regions of Spain and Portugal were found to contain varying amounts of anthocyanins and catechin[184]. The anthocyanin concentration in red wine is generally lower in regions where the grapes grow in warm temperatures and are exposed to a high intensity of sunlight[96,185,186].

The vascular effects of wine produced from grapes growing in different geographies are largely unexplored. When the thrombotic effects of 45 red and white wines were assessed using *in vitro* and mouse models, one Cabernet Sauvignon wine showed anti-thrombotic effects, while the remainder of the wines produced primarily prothrombotic responses[187]. Since differences in polyphenol profiles exist when the same test food is given to rats, mice and humans[188], the thrombotic data mentioned above must be interpreted cautiously. Human studies following vascular responses to wine vinted from warm- vs. cool/cold-weather climates would be a useful area for future study.

Conclusion

Although the majority of the acute and short-term studies on red wine intake show significant improvements in endothelial function compared to baseline and controls, significant discrepancies in outcomes exist. Some factors that may account for the variable results are the study design and duration, and age and sex of the test population. Other factors that affect vascular outcome measures, including the volume of red wine tested, complementary foods or assessments after fasting, and background diet should also be considered. Detailed information about the grape growing region, the grape varietal, and polyphenolic concentrations would be helpful in evaluating and comparing health outcomes.

Red wine contains considerable amounts of polyphenolic compounds that can contribute to daily polyphenol intake Following the US DGA guideline is reasonable, with the portion size and frequency of red wine intake appropriate for those already consuming alcohol. A well-balanced diet is encouraged to consume together with red wine to promote health benefits.

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Tables

Table 1: Summary of clinical studies exploring the effects of red wine on vascular function

Type of studies	Number of intervention studies found	Total number of participants randomized	Total numbers of participants consuming red wine	Percentage of trials showing statistical improvement in vascular function (%)		Vascular function measures that were significant
				From baseline	vs others beverages or controls	
Acute	22	353	277	64	36	FMD, PWA, FBF, Plethysmography, PWV, endoPAT, Laser doppler, Aortic AI, Sphygmocor, Wave reflection
Short-term	9	314	170	57	22	FMD, PWA, Plethysmography
Single - arm	3	*	60	100	-	Plethysmography, FBF, FMD**

* = not randomized; ** = only the group with hypercholesterolemia showed significant improvement of vascular function from baseline; FMD, flow-mediated dilation; PWA, pulse wave analysis; FBF, forearm blood flow; PWV, pulse wave velocity; endoPAT, endothelial peripheral arterial tonometry; AI, augmentation index; % values rounded to whole numbers

Table 2: Summary of clinical studies exploring the effects of red wine on vascular function categorized by study duration

Study duration	Total sample size (number of participants)	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	638	31	17	55
Acute	340	21	11	52
Short-term	298	10	6	60

% values rounded to whole numbers; acute study defines as a single intake between 15 minutes to eight hours; short-term study is an intervention longer than 24 hours and less than four months

Table 3: Summary of clinical studies exploring the effects of red wine on vascular function categorized by study design; RCT= randomized clinical trial

Study design	Sample size	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	638	31	17	55
RCT crossover	278	21	11	52
RCT parallel	300	7	3	43
One-arm	60	3	3	100

% values rounded to whole numbers

Table 4: Summary of clinical studies exploring the effects of red wine on vascular function categorized by participant health condition

Health status	Sample size	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	638	31	17	55
Healthy	425	22	12	55
CAD	101	5	3	60
Hypercholesterolemia	79	3	2	67
T2DB	17	1	0	0

CAD, coronary artery disease; T2DB, Type II diabetes; % values rounded to whole numbers

Table 5: Summary of clinical studies exploring the effects of red wine on vascular function categorized by sex

Sex	Sample size	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	638	31	17	55
Male only	88	8	4	50
Female only	64	2	1	50
Mixed	268	14	7	50
NM	218	7	5	71

NM, not mentioned; % values rounded to whole numbers

Table 6.1: Summary of clinical studies exploring the effects of red wine on vascular function categorized by age; % values rounded to whole numbers

Age	Sample size	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	638	31	17	55
<50	444	22	13	59
50+	194	9	4	44

Table 6.2: Summary of clinical studies exploring the effects of red wine on vascular function categorized by age among male-only studies

Sex/age	Sample size	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Male only	88	8	4	50
<50	63	6	3	50
50+	25	2	1	50

Table 7: Summary of clinical studies exploring the effects of red wine on vascular function categorized by vascular function assessment methods

Vascular function measurements	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	31	17	55
FMD	18	9	50
PAT	1	0	-
LDI	1	1	-
PWV	1	1	-
SGP	3	2	-
wave reflection	1	1	-
aortic AI	1	1	-
Sphygmocor	2	1	-
PWA	1	0	-
FBF	2	1	-

% values rounded to whole numbers; FMD, flow mediated dilation; PAT, peripheral arterial tonometry; LDI, laser doppler imager;SGP, strain-gauge plethysmography; AI, augmentation index; PWA, pulse wave analysis; FBF, forearm arterial blood flow; - means the data is not available

Table 8: Summary of clinical studies exploring the effects of red wine on vascular function categorized by range of red wine volume

Volume	Sample size	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	638	31	17	55
<150 ml	90	2	1	50
151-300 ml	393	21	12	57
>300 ml	155	8	4	50

% values rounded to whole numbers

Table 9.1: Summary of clinical studies exploring the effects of red wine on vascular function that compared between two or more intervention arms; % values rounded to whole numbers

Comparison beverages	Number of intervention studies found	numbers of studies that assessments were available		Numbers of studies that showed statistical improvement in vascular function (%)		Percentage of trials showing statistical improvement in vascular function (%)	
		From baseline	vs Others	From baseline	vs Others	From baseline	vs Others
Total	31	28	31	14	11	50	35

Table 9.2: Detail summary of clinical studies exploring the effects of red wine on vascular function that compared between two or more intervention arms

No.	Authors	Sample size	Comparison arm	From baseline			Comparison		
				+	↔	-	+	↔	-
1	Bulut	10	water		o			vs water	
2	Napoli	10	abstinence		o			vs fasting only	
3	Boban	9	water/DRW/ PSRW/Eth	at 60m				vs water/DRW/ PSRW/Eth	
4	Krnic	10	water,vodka,beer		o		vs water	vs vodka/beer	
5	Djousse	13	non-alcohol (cola)		o			vs cola	
6	Vukovic	10	water	NA			vs water		
7	Agewall	12	DRW		o				vs DRW
8	Karatzi 2004	15	DRW		o				vs DRW
9	Karatzi 2005	15	DRW	at 30,60, 90m			vs DRW		
10	Karatzi 2007	12	DRW,abstinence	at 30m			vs smoking only	vs DRW	
11	Karatzi 2008	15	RW+others	at 1,2h			RW+GOO vs RW+ROO/WW+GOO/WW+ROO		
12	Papamichael 2004	16	DRW,abstinence		o		vs smoking only	vs DRW	
13	Papamichael 2006	20	DRW,abstinence	at 60, 90 m			vs smoking only / vs DRW		

14	Papamichael 2008	15	RW+others	at 1,2,3 h				RW+GOO vs RW+ROO/WW+GOO/WW+ROO	
15	Tousoulis	83	water	at 1,4 h				vs water	
16	Whelan	14	Other alcohol (WW)	at 360m				vs WW	
17	Hijmering	20	water			o		vs water	
18	Vauzour	15	other alcohol (alcohol-matched control)	at 4,8h			vs alcohol/flavor-matched control		
19	Spaak	13	water, other alcohol (alcohol-matched control)		o		vs water	vs mock alcohol drink	
20	Hashimoto	11	water, DRW, other alcohol (JP vodka)	at 120m				vs DRW, water, JP vodka	
21	Mahmud	8	DRW	at 30,60 ,90m				vs DRW	
22	Muggeridge	7	water		o			vs water, orange juice, green tea	
23	Huang	80	water, other alcohol (beer/vodka)	o			vs water/beer/vodka		
24	Leighton	42	RW+others	NA	HFD+RW > HFD	Med + RW ↔ Med; HFD+RW ↔ Med +RW			
25	Cuevas	11	RW+others				o		

26	Guarda	19	abstinence	o				vs no alcohol	
27	Thomazella	42	abstinence		o			vs no wine	
28	Coimbra	24	non-alcohol (grape juice)	o				vs grape juice	
29	Napoli	17	abstinence		o			vs no alcohol	
30	Zilken	28	DRW, other alcohol (beer), abstinence	NA		vs no alcohol/beer/DRW			
31	Naissides	45	water, DRW				o		

Table 10: Summary of clinical studies exploring the effects of red wine on vascular function that participants consumed a meal (s) along with the wine or were instructed to follow certain diet during the intervention

No.	First author/Year published/Country	Meal	Kcal;prot;carb;fat	Effects/Outcomes	
				From baseline	VS other(s)
1	Bulut/2013/Germany	Yes (HFD) 150 g French fries, 200 g hot pork sausage, 30 ml curry sauce	energy 873.0 kcal water 240.8 g protein (14%) 30.6 g fat (77%) 75.9 g carbohydr. (9%) 18.4 g	RW:↔,↔	RW vs water:↔,↔
				Water ↔,↓	NA
				Cola ↔,↓	Cola vs water ↓,↓
				alc:↔,↔	alc vs water:↔,↔
2	Agewall/2000/New Zealand	Yes [1 baguette filled with 5 of magerine, 2 leaves of lettuce, half of sliced tomato, a slice of lean cheese and a little pepper, low fat yogurt (150 g), and banana]	energy 350.0 kcal water 282.9 g protein (18%) 15.5 g fat (28%) 10.7 g carbohydr. (54%) 45.6 g	RW:↔	DRW> RW
				DRW:↑↑	
3	Djousse/1999/USA	Yes [Whopper with cheese and french fries [0.8 g fat; 15 kcal/kg; 20% protein, 48% fat, 19% carbs]]	energy 746.5 kcal water 204.8 g protein (18%) 32.0 g fat (55%) 45.7 g carbohydr. (28%) 51.0 g	RW↔	RW vs cola:↔
				Cola↔	
4	Karatzi/2004/Greece	Yes [1 slice of white bread (30 g) and 30 g of cottage cheese (4% fat)]	energy 106.7 kcal water 34.6 g protein (23%) 6.0 g fat (19%) 2.3 g	RW ↔	DRW > RW at 60 m
				DRW ↔	

			carbohydr. (58%) 15.1 g		
5	Karatzi/2007/Greece	Yes [1 slice of white bread (30 g) and 30 g of cottage cheese (4% fat)]	energy 106.7 kcal water 34.6 g protein (23%) 6.0 g fat (19%) 2.3 g carbohydr. (58%) 15.1 g	RW+smoking:↔	RW +smoking:↔ DRW+ smokimg
				Smoking:↓ at 30, 60m	Smoking > RW+ smoking; Smoking > DRW+smoking
				DRW+smoking:↔	
6	Karatzi/2008/Greece	Yes [1 slice of white bread (30 g) and 30 g of cottage	energy 106.7 kcal water 34.6 g protein (23%) 6.0 g fat (19%) 2.3 g carbohydr. (58%) 15.1 g	RW+GOO ↑ 1,2 h	RW + green olive oil > others
				RW+ ROO ↔	
				WW+GOO ↔	
				WW+ ROO ↔	
7	Napoli/2004/Italy	Yes [120 g spaghetti with tomato sauce, 25 g olive oil, 50 g bresaola, 60 g bread, 1 apple~ 1050 kcal]	energy 877.8 kcal water 221.4 g protein (13%) 27.8 g fat (59%) 58.3 g carbohydr. (28%) 61.2 g	RW:↔	RW↔Fasting ↔meal only
				fasting:↔	
				meal only ↔	
8	Papamichael/2004/Greece	Yes [1 slice of white bread (30 g) and 30 g of cottage cheese (4% fat)]	energy 106.7 kcal water 34.6 g protein (23%) 6.0 g fat (19%) 2.3 g carbohydr. (58%) 15.1 g	RW+smoking:↔	RW+smoking ↔ DRW+smoking
				smoking:↓ at 15, 30, 60m	Smoking < RW+ smoking; Smoking< DRW+smoking
				DRW+ smoking: ↔	
9	Papamichael/2006/Greece	Yes [1 slice of white bread (30 g) and 30 g of cottage cheese (4% fat)]	energy 106.7 kcal water 34.6 g protein (23%) 6.0 g fat (19%) 2.3 g	RW+smoking:↓ at 60, 90m	DRW+ smoking > RW+smoking @ 90m
				smoking ↔	Smoking > RW+ smoking at 60 m

			carbohydr. (58%) 15.1 g	DRW+smoking:↓ at 30, 60 m	
10	Papamichael/2008/Greece	Yes [vegetable soup; 2 slices of white bread]	energy 233.3 kcal water 344.0 g protein (16%) 8.9 g fat (10%) 2.5 g carbohydr. (75%) 42.5 g	RW+ green olive oil:↓ RW+ refined olive oil:↓ WW+green olive oil: ↔ WW+ refined olive oil: ↔	Control time > all 4 groups
11	Whelan/2004/New Zealand	Yes [2 slices of toast with jam; baguette filled with 5g magerine, 2 leaves of lettuce, 1/2 sliced tomato, low-fat cheese, 200g low-fat yogurt, 1 banana]	energy 519.4 kcal water 370.7 g protein (16%) 20.2 g fat (22%) 12.5 g carbohydr. (62%) 78.4 g	RW:↑ control ↔ WW:↑ at 360m	RW ↔WW
12	Vauzour/2009/UK	Yes [Standardized breakfast at 15 mins after the beverage and standardized lunch at 200 mins after the beverage]		Champagne:↑ at 480 mins control:↑ at 480 mins	Champagne vs control↔
13	Muggeridge/2019/UK	Yes (>900 kcal, 50 g fat, have choices of savory or total vegetarian meals)		↔ ↔ ↔ ↔	RW ↔ others
14	Cuevas/2000/Chile	Yes 39.3%kcalfat ~113 g/d total fat [36 g/d SFA, 35g/d MUFA, 32 g/d PUFA, 0.12 CLCn-3, 0.61g/d cholesterol] +17.6%kcal protein ~ 2,565 kcal		RW+HFD vs HFD: ↑	RW+HFD ↔ RW+CD; CD>HFD
		27.3%kcal fat~ 77 g/d total fat [23 g/d SFA, 38 g/d MUFA, 10 g/d PUFA, 0.38 g/d VLCn-3, 0.3g/d cholesterol] + 17.6%kcal protein ~ 2,565 kcal		RW+CD vs CD :↔	
15	Guarda/2005/Chile	Yes		RW ↑ Control ↑	RW vs no alcohol: ↔

16	Leighton/1999/Chile	2535 kcal; 27.3% kcal fat; 55.1% carb; 17.6% prot; 675g fruits/veg; 157g fish/chicken; 59.3g beef/pork; 44g fiber		NA	Med + RW ↔ Med
					Med > HFD
		2600 kcal;39.7% fat; 42.8% carb; 17.5% prot; 246 g fruits/veg; 75 g fish/chicken; 209 g beef/pork; 12 g fiber			HFD+RW ↔ Med +RW
					HFD+RW > HFD
17	Thomazella/2011/Brazil	Mediterranean diet (rich in whole grains, vegetables, fruits, nuts, and olive oil, plus red wine)		RW: ↔	RW ↔ no wine
		low-fat Therapeutic lifestyle changes diet (low/fat-free food, high intake of fruits/veg/whole grains/ moderate lean meat, no alcohol)		no wine:↔	
18	Napoli/2005/Italy	Diabetic food		RW: ↔	RW vs no alcohol↔
				no alc ↔	
One-arm study					
19	Hamed/2010/Israel	Maintain stable diet and no physical activity		RW:↑	NA
20	Andrade/2009/Brazil	participants were encouraged not to change their daily dietary habits and maintain prescription medications		HCHOL (n=5): ↑	NS (but non-sig lower in HCHOL and AH groups)
				AH (n=7): ↔	
				healthy (n=2): ↔	

Table 11: Summary of clinical studies exploring the effects of red wine on vascular function that provided red wine polyphenol content

Units of polyphenols	Number of intervention studies found	numbers of studies that assessments were available		Numbers of studies that showed statistical improvement in vascular function (%)		Percentage of trials showing statistical improvement in vascular function (%)	
		From baseline	vs Others	From baseline	vs Others	From baseline	vs Others
Total	12	11	10	7	3	63.64	30
total polyphenol (mg GAE)	8	7	6	4	1	57.14	17
mg caffeic acid	4	4	4	3	2	75	50

% values rounded to whole numbers; GAE, gallic acid equivalent

Table 12: Summary of clinical studies exploring the effects of red wine on vascular function categorized by countries where the studies were conducted

Continents (study conducted)	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	31	17	55
Europe	18	10	55
North America	2	0	0
South America	5	3	60
Australia/Oceania	3	1	33
Asia	3	3	100

% values rounded to whole numbers

Table 13: Summary of clinical studies exploring the effects of red wine on vascular function categorized by the original country of red wine

Continents (Wine origin)	Total number of studies	Studies with significant improvement from baseline (time effect)	Percentage of trials showing statistical improvement in vascular function from baseline (time effect) (%)
Total	31	17	55
Europe	15	8	53
North America	1	0	0
South America	2	2	100
Australia/Oceania	4	1	25
NM	9	6	67

; % values rounded to whole numbers; NM, not mention

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Chapter V: Perspectives and Conclusions

Plant-based functional food has developed from traditional uses for essential nutrition or medicinal purposes to disease prevention and health enhancement. Demand for functional food will continue to increase as more nutrition research characterizes the potential health benefits of certain foods. When combined with emerging technologies such as precision nutrition and artificial intelligence, clinical studies will aid in the development of new models for health promotion on both the individual as well as population levels.

In this dissertation, the history of food as medicine was discussed in Chapter 1, followed by an overview of the evolution of nutrition from providing essential nourishment to a focus on functional health benefits. Building on discoveries from *in vitro* and animal studies, epidemiologists have clarified potential relationships between food consumption and health outcomes, as illustrated by two topics of focus for this dissertation. One is the potential protection of sorghum intake against esophageal cancer[1]. A second topic is the “French paradox” where lower coronary heart disease risks were identified among French adults who regularly consumed wine, even in combination with a high saturated fat diet[2]. Results from epidemiological studies can be used to propose hypotheses for further exploration using preclinical and clinical studies.

The advancement of nutrition research also allows scientists to improve the quality of food products for safety[3], sensory attributes[4,5], and consumer satisfaction[6]. Optimizing conditions for producing and processing foods can enhance the bioactivity of nutrients and other functional compounds in plants. Although results from *in vitro* and

animal studies may be suggestive of health effects in humans, clarification of the role of food on biological outcomes is needed through human trials. Accordingly, for this dissertation, human studies were conducted with two functional foods, sorghum and red wine, and are presented in Chapters II and III, respectively.

Sorghum is one of the most widely used grains in the world. In Chapter II, three probe studies utilizing conventional and extruded sorghum flour were conducted to explore their effects on plasma amino acids and glucose responses. Healthy men aged 21 to 34 years participated in the studies using a randomized crossover experimental design. The results showed that consumption of 68 g of extruded sorghum flour significantly increased postprandial plasma glucose 90 minutes after intake compared to sorghum flour prepared by the conventional methods, while no significant differences in plasma amino acids were observed after eating 34 or 68 g of either type of flour. Although the results of this study were inconsistent with the results from other studies that assessed the glucose responses following extruded sorghum (whole grain or proanthocyanidin extract), differences in extrusion conditions might play a role in modifying the properties of the starch along with individual variability resulting in discrepancies in the observed outcomes. Perhaps more importantly, since sorghum is a common grain in the food supply of many underdeveloped countries where protein-calorie malnutrition is a challenge, the results presented here suggest that incurring the costs of extrusion under the conditions tested may not be of value to increase protein availability.

Wine is an ancient food, and in current times, is considered by many to be heart-healthy. However, the suggestion that wine, particularly red wine, can be

vasculoprotective is based on a compelling epidemiological data, but with only a limited number of clinical trials, of which most are conducted with wine from grapes grown in warm climates. However, wine grapes grow in cold climates as well, and little to no information about the vascular effects of wines vinted from cold-climate grapes appears to exist. Chapter III details the results of a pilot study on two vintages of red wine produced in Hokkaido, Japan, where the vines are covered in snow during the winter and have a relatively short summer growing season. As described in this chapter, the vascular responses to a single intake (240 mL) of Hokkaido Zweigelt red wines produced in either 2015 or 2018 was compared with a white grape juice control beverage in middle-aged men. Measurements of vascular responses include the augmentation index (AI), AI at 75 beats/minute (AI75), reactive hyperemia index, platelet aggregation, and systolic and diastolic blood pressure (SBP and DBP, respectively), which were measured at baseline and two- and four-hours post-consumption. The study was conducted in a randomized three-arm crossover design with a washout period of seven days between each intervention. When measured in 2019, prior to the anticipated start of the study, the anthocyanin concentration of Zweigelt produced in 2018 was considerably higher than that of the 2015 vintage. However, due to the covid pandemic, clinical trials at UC Davis were suspended for 17 months, and when the intervention was conducted during 2021-2022, the analyses in 2022 showed a similar content of anthocyanins and other polyphenols between the two vintages, both of which were higher than the white grape juice control. Interestingly, the results showed a significantly lower SBP and DBP following 2018 Zweigelt red wine consumption compared to the 2015 vintage or the control. When further compositional

analyses were conducted on the two wines, the concentration of hydroxytyrosol of 2018 Zweigelt was approximately two times greater than the 2015 vintage, which may help explain the differences in blood pressure. However, wine is a complex mixture of many compounds that likely function both individually and synergistically to produce bioactivity, and further research is needed to more clearly define which compounds or mixtures of them are responsible for the vasculoprotective effects.

When comparing vascular function outcomes among the existing clinical studies on red wine, considerable discrepancies exist. Chapter IV provides a review of factors contributing to these variable results. Differences in study design, duration, participant characteristics, volume and frequency of wine intake[7], whether the wine was consumed with food, and the methods of measurements are identified. To aid in future research and enable better comparison between studies on red wine and vascular function, these factors need to be carefully considered, along with providing more information on the history and composition of the wine, such as detailed polyphenolic profiles, origin of the wine grape, and processing methods.

Plant-based foods have many beneficial properties that benefit human health. Two additional chapters, Appendix A and B, provide evidence to support the role of strawberries in vascular function, and the role of fruits and nuts to support skin health, respectively.

Sorghum and red wine are consumed worldwide and can be adaptable to many diets under the recommendations of dietary guidelines from several countries. Dietary guidelines are a good resource to guide the amount and frequency of consumption. However, guidelines are not a guarantee that results from preclinical and clinical studies

will directly translate to any individual. The results from chapters II and III provide promising evidence that food produced in different years and processed differently can significantly change the responses in human health outcomes. These acute feeding studies provide snapshots of potential events that may happen over time and support the planning of larger clinical studies with more robust study designs. Caution is urged, however, since the results from studies in chapters II and III are based on highly controlled conditions and likely do not account for a number of other potentially confounding factors existing in free-living populations where background diet, beverage intake and other lifestyle and biological dynamics exist.

In summary, the concept of functional food serving a role beyond basic nutrition is rooted in traditional use history and advanced through scientific research and innovative public health programs[8]. Functional foods, particularly those that are plant-based, are complex mixtures of nutrients and other bioactive compounds that likely work synergistically to produce health benefits. Chapter III illustrates the complexity and interplay of known and possibly unknown compounds the resulted in differences in blood pressure after red wine intake. Although some key compounds in the wine were similar (polyphenols and anthocyanins), the variable blood pressure results helped identify hydroxytyrosol, not commonly associated with red wine, as a potential vascular mediator. Resveratrol is also present in red wine and while the amount in a single portion is far below the threshold of bioactivity shown from *in vitro* studies[9], the potential synergy of this polyphenol with other beneficial compounds in red wine should nonetheless be considered. Finally, functional foods must be viewed in a real-life context, where availability, taste preferences, and economics should be considered.

One goal of nutrition research is to apply the knowledge and advanced understanding stemming from our research efforts to free-living settings for maximum personal and public health benefits.

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Appendix A

Food & Function

ROYAL SOCIETY OF CHEMISTRY

REVIEW

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Effects of short-term consumption of strawberry powder on select parameters of vascular health in adolescent males

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Cardiovascular disease is a leading cause of death in the United States and much of the developed world, costing billions of dollars in lost work time, lower productivity and high health care expenditures. Research on foods and bioactive food components that have cardioprotective benefits may provide new insights as to how modest changes in one's diet may result in a reduced risk of vascular disease. In intervention trials, the consumption of strawberries, either fresh or freeze-dried, has been reported to improve select markers of cardiovascular health, including improved lipid profiles, microvascular function, and platelet reactivity. Consistent with the above, epidemiological studies suggest beneficial effects of strawberries on vascular function. Preliminary studies on the effects of freeze-dried strawberry powder on vascular health are reviewed in the current paper.

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

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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[62]
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.

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

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.

Peripheral arterial tonometry was assessed after an overnight fast and one hour after the intake of the assigned

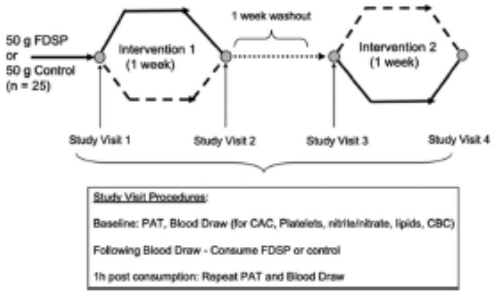

Fig. 1 Study design and visit procedures.[71]

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

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.

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

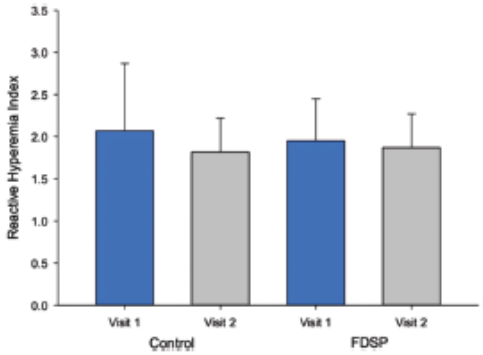

Fig. 2 Reactive hyperemia index with short-term consumption of FDSP *vs.* control powder.

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]

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]

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

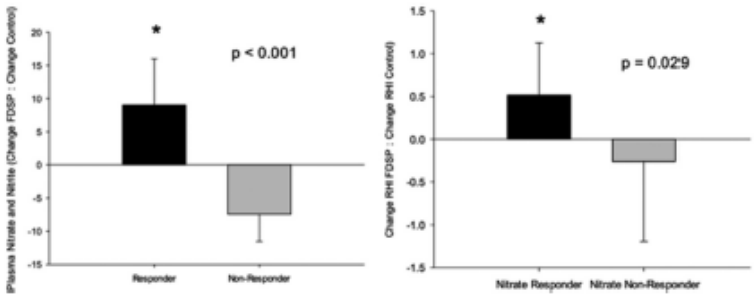

Fig. 3 Individuals who had an increase in fasting plasma nitrate and nitrite levels with one week of FDSP intake relative to control powder intake (left panel) also had an improved vascular function response as measured by the reactive hyperemia index (RHI; right panel).

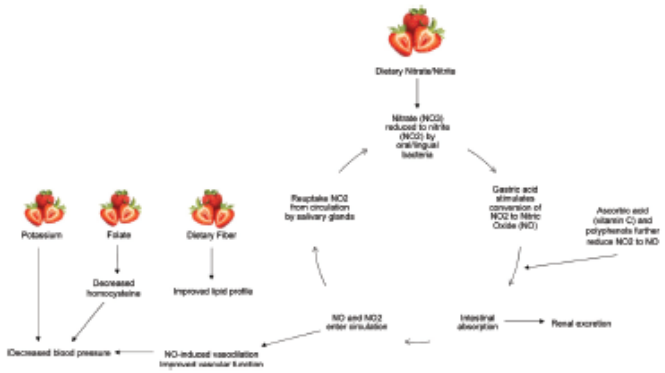

Fig. 4 Potential mechanisms modulating the vascular effects of strawberry consumption. NO3: nitrate; NO2: nitrite; NO: nitric oxide.

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.

Conflicts of interest

There are no conflicts to declare.

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Appendix B

RESEARCH
Narrative Review

Plant-Based Foods for Skin Health: A Narrative Review

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ABSTRACT
The potential role of plant-based foods in the promotion of skin health is an emerging area of nutrition research. Plant-based foods are rich in bioactive compounds, including vitamin C, vitamin E, beta carotene, polyphenols, and phenolic acids, which can contribute to oxidant defense, lower inflammation, and promote structural support of the skin. Epidemiological studies have associated higher intakes of select fruits and vegetables with positive skin health. Beneficial effects of certain fruits, vegetables, nuts, legumes, and polyphenolic-rich beverages on the skin have been reported, with each of these providing a unique phytochemical composition. Although most studies use extracts, this review will focus on data from whole foods and minimally processed products. Collectively, the evidence to date suggests a promising future for plant-based dietary interventions that promote skin barrier health and function. However, additional research is required to address issues such as the optimal quality and duration of intake as well as potential mechanisms. Studies in the above areas will help formulate specific targeted dietary recommendations.
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SKIN, THE LARGEST ORGAN IN THE HUMAN BODY, acts as a barrier to protect internal organs and cells from external elements. It also helps regulate body temperature, mediates sensations of touch, and produces vitamin D, a key regulator of bone, immune, and vascular health.[3] Both intrinsic and extrinsic factors affect skin health and aging.[4] An individual's genetic background influences intrinsic factors such as skin pigmentation, skin thickness, microvasculature structure, and sex hormones.[4] Extrinsic factors such as smoking, diet, sleep, exercise, chronic diseases, and environmental factors including temperature, pollution, humidity, and UV radiation (UVR) can increase inflammation and oxidative stress that accelerate skin aging.[4-6] Indeed, repeated UVR exposure can increase pro-inflammatory cytokines that contribute to the development of wrinkles and adverse pigmentation of the skin.[7] Moreover, age- or obesity-related induction of protein glycation and inflammation can increase skin rigidity and impair skin repair.[8]

Suboptimal nutrition can adversely affect skin health, as evidenced in studies of micronutrient deficiencies. For example, deficiencies of vitamin A and vitamin C (VitC) can lead to thickening of the skin.[9] Poor wound healing has been observed with deficiencies of VitC and essential fatty acids,[9-11] and petechiae can be a result of vitamin E and vitamin K deficiencies.[9] Inadequate intakes of riboflavin, niacin, pyridoxine, biotin, zinc, and essential fatty acids can lead to various forms of dermatitis.[9,12] Classic studies on pellagra[13] and acrodermatitis enteropathica[14] identified niacin and zinc deficiencies, respectively, as causative factors. Although the data on micronutrient deficiencies are extensive, data on how specific foods or diets can influence skin health in well-nourished populations are limited.

Epidemiological studies suggest that abundant dietary intakes of specific plant-based foods are key in the maintenance of skin barrier health and function. A robust intake of vegetables, olive oil, and legumes was correlated with lower actinic skin damage caused by long-term UVR exposure among 2000 people aged 70 and older in Australia, Greece, China, Japan, and Sweden.[1] Better adherence to the Dutch Healthy Diet Index guidelines that promote a diet rich in fruits and include yogurt, milk, and vegetables was significantly associated with fewer wrinkles in women.[2] Among Japanese women, a significant inverse association has been observed between wrinkling and green and yellow vegetable intake.[15] In contrast, diets consisting mainly of meat, refined grains, snacks, soft drinks, coffee, and alcoholic beverages were associated with more wrinkling in women.[2]

Plant-based foods are rich in polyphenols, carotenoids, and select vitamins typically not found in appreciable amounts in other food categories. However, each food has a unique nutrient profile that provides an array of bioactive compounds that either alone or synergistically may afford protection for the skin.

Given this information, we conducted a preliminary survey of recent literature on the potential effects of plant foods on

skin barrier health and function. A majority of the trials discussed used study designs of dietary components and foods individually, at times, above dietary recommendations. However, the goal of this review is to spark interest in this field, as well as provide an overview of the available data for both the public and nutrition professionals who have an interest in the role of diet for the maintenance of skin barrier health and function. This review aims to provide more specificity in terms of the fruits and vegetables that may improve skin barrier function and meet the recommendations of the 2020-2025 Dietary Guidelines for Americans (DGAs).

METHODS

Articles were identified on PubMed and Google Scholar using the following key terms (or combinations of them): "fruit," "vegetable," "nut," "legume," "bean," "food," "skin," "wrinkle," "erythema," "hydration," "elasticity," "aging," "photoaging." All studies available in English were reviewed. Eligibility criteria for clinical trials included dietary interventions and skin parameter measurements relevant to wrinkles, erythema, hydration, and elasticity. Studies focused on plant-based foods and beverages that were whole or processed into extracts were considered. Extracts were equated to equivalent quantities as whole foods or beverages to deduce feasibility of consumption. Isolated compounds were not considered. Dermatological skin diseases such as acne and psoriasis were not considered. Animal or in vitro studies were included for select plant-based foods or relevant bioactive compounds that supported potential mechanisms of action for the clinical trials.

RESULTS

Twenty studies involving 13 plant-based foods were identified, including 8 fruits and vegetables, 2 nuts and legumes, and 3 polyphenolic-rich beverages. Products used in dietary interventions included whole foods, nutritional pastes, beverages, juice, and extracts (Table). For the included trials, study participants were adults between 18 and 86 years old. Most studies reported the Fitzpatrick skin phototype (FSPT), a standard tool used to categorize an individual's skin type based on melanin pigmentation and factors and potential skin reaction to UVR exposure. Individuals with FSPT I and II have less melanin pigmentation and increased sensitivity to harmful effects of UVR such as sunburn and premature aging, and individuals with FSPT III and IV tend to tan.[16]

Fruits and Vegetables

Fruits and vegetables are rich in bioactive compounds including carotenoids,[17] vitamins, and polyphenols.[18] These are distributed to the skin and promote oxidant defense and structural integrity and reduce inflammation to help protect against UVR-induced damage.[18,19] Many fruits and vegetables are excellent sources of VitC, with reduced intake of VitC associated with dry or wrinkled skin in women.[20] VitC is a cofactor for prolyl and lysyl hydroxylases that are important for collagen synthesis. It also functions as a major circulating antioxidant that can quench reactive oxygen species (ROS) derived from UVR.[21] A number of fruits and vegetables are also substantial sources of carotenoids. Supplementation of carotenoids in the range of 24 to 25 mg per day for 12 weeks in healthy men and women aged 20 to 57 was observed to significantly reduced UV-induced erythema.[22,23] In another study, carotenoid supplementation also inhibited an increase in UVR-induced CD45+ inflammatory cells.[24] In addition, the intake of 13.1 mg of carotenoids daily for 26 days significantly reduced oxidative stress-induced lymphocyte DNA damage in young adults.[25]

RESEARCH SNAPSHOT

Research Question: What is the evidence for plant-based food intake for skin health in humans?

Key Findings: Consumption of select fruits and vegetables rich in vitamins and polyphenols, nuts and legumes abundant in mono- and poly-unsaturated fats, as well as polyphenolic-rich beverages, can improve skin health. Beneficial skin effects vary but include an improvement in hydration, hyperpigmentation, wrinkles, erythema, collagen, and elasticity. More clinical studies are needed to determine the amount and duration of consumption for each food to elicit beneficial effects and to understand the underlying mechanisms.

Mangos. Mangos (*Mangifera indica* L) are rich in carotenoids (especially beta carotene) as well as VitC and the phenolic gallic acid.[26] Ataulfo mangos have the highest VitC content among the mango varieties commonly found in the United States,[27] with 85 g providing an estimated 107 mg of VitC (143% of the recommended dietary allowance [RDA] for adult women).[28] This mango variety is also rich in beta carotene, with 85 g providing 2219 μg (16% of the RDA for adult women). Ataulfo mangos are 4 times higher in beta carotene than the reference level reported by the US Department of Agriculture (USDA) Food Data Central (FDC) #169910 (640 μg/100 g of raw mango).[28] A significant decrease in deep facial wrinkles was seen in postmenopausal women aged 50 to 70 with FSPT II or III after consuming 85 g (0.5 cup) of fresh-frozen Ataulfo mangos 4 times a week for 16 weeks.[29] Curiously, those who consumed 250 g (1.5 cups) in the same intake pattern had an increase in wrinkles. This may have been due to the amount of sugar present in the fruit. Glucose and fructose are known to increase glycation of collagen and elastin fibers, which disrupt the integrity of the subcutaneous tissue supporting the skin.[30]

Mangos are also the primary dietary source of the polyphenol mangiferin. Ataulfo mangos have been found to contain 183 to 996 μg/g of mangiferin depending on harvest date, which is higher than 4 other mango varieties tested.[27] UV-irradiated mice fed a mango extract containing 13.5% of mangiferin for 12 weeks showed a significant decrease in wrinkle length, along with an increase in collagen bundles and inhibition of collagen fiber damage through a reduction in inflammation.[31] Supplementation of mangiferin to mice also resulted in a decrease in the pro-inflammatory indices of inducible nitric oxide synthase, interleukin-1β, and interleukin-6 and inhibition of nuclear factor-kappa B subunit 2 and IkB phosphorylation in the skin.[32]

Table 1. Overview of dietary clinical studies examining the effects of plant-based foods and extracts on skin parameters

Plant-based item	Country / first author (year)	Study design	Subjects	Smokers	BMI[b]	Age (y)	Intervention	Form and intake of product	Location of skin	Fitzpatrick skin phototype	Effects[f]
Fruits and vegetables											
Mango	USA / Fam (2020)[29]	Randomized parallel arm—16 wk	32 postmen opausal women	Excluded	(1) 26.4 ± 4.0; (2) 22.9 ± 2.6	All range: 50-70; (1) 61 ± 5.1; (2) 60 ± 5.3	(1) 85 g (0.5 cup) fresh-frozen mangos; (2) 250 g (1.5 cups) fresh-frozen mangos	Fresh-frozen mangos, 4 times/wk	Lateral canthi, cheeks	II and III	*Lateral canthi 85 g:* **Deep wrinkle severity:** ↓[g] *Lateral canthi 250 g:* **Emerging wrinkle severity:** ↑[h] **Average wrinkle severity:** **Average wrinkle length:** ↑ **Fine wrinkle severity:** ↑ *Cheeks:* **Erythema:** ↑ 85 g, ↔[i] 250g
Melon	France / Egoum enides (2018)[34]	RCT[a], double-blind, parallel arm—34 d	44 White adult men (159%) and women (84.1%)	Not specified	Not specified	All range: 18-50 (mean 37.2)	(1) 20 mg dried melon concentrate; (2) control pill	Extract in a capsule, 20 mg/d	Buttock, back, or arms	II and III	**MED**[j]: ↑
Orange	Italy / Puglia (2014)[39]	Crossover—15 d	20 White adults	Excluded	Not specified	UV irradiation: 26-47; sunlamp exposure: 45-70	Blood orange (Moro, Tarocco, and Sanguinello) extract, per capsule: ANC[c] 2.8%-3.2%, hydroxycinnamic acids (caffeic, cumaric, ferulic, sinapic acid) 1.8%-2.2%, flavone glycosides (narirutin, hesperidin) 8.5%-9.5%, ascorbic acid 5.5%-6.5%	Extract in a capsule, 100 mg/d	Forearm and dorsal hand	UV irradiation: II and III; sunlamp exposure: II and IV	*Forearm:* **Skin erythema index:** ↓ *Dorsal hand:* **Melanin index:** ↓

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Table 1. Overview of dietary clinical studies examining the effects of plant-based foods and extracts on skin parameters (*continued*)

Plant-based item	Country / first author (year)	Study design	Subjects	Smokers	BMI[b]	Age (y)	Intervention	Form and intake of product	Location of skin	Fitzpatrick skin phototype	Effects[f]
Tomato	Germany / Groten (2019)[52]	RCT, double-blind, parallel arm, multicenter—12 wk	145 adult men (23%) and women (77%)	8 smokers (5.5%)	≤30	All range: 20-50; (1) mean 40.9; (2) mean 40.9	(1) Tomato nutrient complex, per capsule: tomato—7.5 mg lycopene, 2.9 mg phytoene and phytofluene, 0.4 mg beta-carotene, 2.8 mg tocopherols; rosemary—2 mg carnosic acid; (2) control: medium-chain triglycerides	Extract, 2 capsules/d	Buttock	I and II	MED: ↔ a*[h]: ↓ L*[h]: ↔
	UK / Rizwan (2011)[51]	RCT, single-blind, parallel arm—12 wk	17 White women	Excluded	Not specified	All range: 21-47 (median 33)	(1) Tomato paste with olive oil, 55 g; per serving: 16 mg lycopene; (2) control: olive oil, 10g	Paste, daily	Upper buttock	I and II	MED: ↑ **Erythema index: ↑** **Procollagen I: ↑** **Fibrillin-1: ↓**
	Germany / Stahl (2001)[50]	RCT, parallel arm—10 wk	22 adult men (36%) and women (64%)	Included with a limit of ≤3 cigarettes/d	Not specified	All range: 26-67	(1) Tomato paste (40 g) with olive oil (10 g); per serving: 16 mg lycopene, 0.5 mg β-carotene, 0.1 mg lutein; (2) control: olive oil, 10g	Paste, daily	Scapular region	II	a*: ↓
Kale	Germany / Meinke (2017)[56]	RCT, parallel arm—10 mo	29 women	10 smokers (34.5%)	Not specified	All range: 40-56 (mean 49.2)	(1) Curly kale extract, per capsule: total 550 μg carotenoids: 430 μg lutein, 70 μg beta-carotene, 30 μg lycopene, 20 μg zeaxanthin; (2) control: olive oil	Extract, 3 capsules/d	Inner forearm, and cheeks	II	**Collagen I/elastin ratio: ↑**

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Table 1. Overview of dietary clinical studies examining the effects of plant-based foods and extracts on skin parameters (*continued*)

Plant-based item	Country / first author (year)	Study design	Subjects	Smokers	BMI[b]	Age (y)	Intervention	Form and intake of product	Location of skin	Fitzpatrick skin phototype	Effects[f]
Pomegranate	USA / Henning (2019)[60]	RCT, parallel, 3-arm, open-label—12 wk	74 women	Not specified	(1) 26.6 ± 5.0; (2) 27.1 ± 5.1; (3) 29.9 ± 6.7	All range: (30-40); (1) 35.1 ± 4.3; (2) 35.9 ± 4.1 37.9 ± 4.2	(1) Pomegranate juice, per cup: 100 mg punicalagin, 23 mg ellagic acid; (1) pomegranate extract, per capsule: 100 mg punicalagin, 44 mg ellagic acid; (3) control: dextran	Juice, 8 oz/d or extract in a capsule, 1,000 mg/d	Inner arm	II, III, and IV	**MED: ↑** Melanin index: ↓
Passion fruit	Japan / Maruki-Uchida (2018)[64]	RCT, double-blind, parallel arm—8 wk	32 women with dry skin	Not specified	Not specified	All range: 35-54	(1) Passion fruit seed extract containing per capsule: 5 mg piceatannol; (2) control: dextrin	Extract, 2 capsules/d	Cheeks	Not specified	**Moisture content: ↑** TEWL[m]: ↓ Skin elasticity: ↓ L* a* b*[n]: ↓
Grape	Japan / Yamakoshi (2004)[71]	Open-label—Total 11 mo: 1st period, 6 mo; 1-mo break; 2nd period, 5 mo	12 Japanese women with chloasma	Not specified	Not specified	All range: 34-58 (45.4 ± 6.1)	Grape seed extract, per capsule: 81%, 54 mg PAC[d]	Extract in a capsule, 67 mg, 3 times/d	Cheeks	Not specified	**L*: ↑ Diameter of chloasma: ↓ Melanin index: ↓**
	Japan /Tsuchiya (2020)[73]	RCT, double-blind, parallel arm—12 wk	97 women with lentigo spots on cheeks	Not specified	(1) 21.07 ± 1.78; (2) 21.08 ± 2.59	All range: 30-60; (1) 44.28 ± 6.50; (2) 44.66 ± 5.93	(1) 200 mg dealcoholized red wine oligomeric PACs, per bottle: 208 mg	Extract in beverage, 200 mL/ d	Cheeks	Not specified	**Lentigo scores: ↓ SC[o] water content: ↑**
Nuts and legumes											
Almond	USA / Foolad (2019)[83]	RCT, parallel arm—16 wk	28 postmenopausal women	Excluded	(1) 30.7 ± 7.31; (2) 29.7 ± 7.66	All range: 53-80, (1) 63.63 ± 7.09; (2) 58.93 ± 6.10	(1) 20% of daily kcals consumed as almonds; average 2.1 oz/d; (2) control: 20% of daily kcal consumed as calorie-matched nut-free snack	Whole raw almonds, daily	Lateral canthi	I and II	**Overall wrinkle severity: ↓ Overall wrinkle width: ↓** TEWL: ↔ Sebum production: ↔

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Table 1. Overview of dietary clinical studies examining the effects of plant-based foods and extracts on skin parameters (*continued*)

Plant-based item	Country / first author (year)	Study design	Subjects	Smokers	BMI[b]	Age (y)	Intervention	Form and intake of product	Location of skin	Fitzpatrick skin phototype	Effects[f]
	USA / Rybak (2021)[84]	RCT, single-blind, parallel arm—24 wk	49 postmenopausal women	Excluded	Not specified	(1) Range: 51-77; 61.72 ± 8.76; (2) range: 47-84; 65.14 ± 8.14	(1) 20% of daily kcals consumed as almonds; (2) control: 20% of daily kcal consumed as calorie-matched nut-free snack	Whole raw almonds, daily	Lateral canthi, cheeks, forehead	I and II	*Lateral canthi:* **Average wrinkle severity:** ↓ *Cheeks and forehead:* **Average pigment intensity:** ↓ Hydration: ↑ TEWL: ↔ Sebum: ↔
Soybean	Japan / Izumi (2006)[94]	RCT, double-blind, parallel arm—12 wk	26 women	Not specified	Not specified	All range: 35-48; (1) 40.1 ± 1; (2) 40.5 ± 0.95	(1) 25 mg fermented soybean extract containing 10 mg (40%) isoflavone aglycones; (2) control: color-matched, no extract	Extract in a 250-mg capsule, 4 capsules/d	Lateral canthi, cheeks	Not specified	*Lateral canthi:* **Fine wrinkles:** ↓ Linear wrinkles: ↔ **Skin microrelief:** ↑ *Cheeks:* **Skin elasticity:** ↑
	Korea / Lee (2015)[95]	RCT, parallel arm—8 wk	65 women with dry and dark skin	Not specified	(1) 21.99 ± 1.7; (2) 21.43 ± 1.95	All range: 25-60; (1) 42.58 ± 4.60; (2) 43.41 ± 4.68	(1) Barley and soybean formula, per 100 mL: 3 g; (2) control: no formula	Extract in beverage, 100 mL/d	Forearm and front cheeks	Not specified	**Skin hydration:** ↑ **SC thickness:** ↓
Polyphenol-rich beverages											
Cocoa	South Korea / Yoon (2015)[101]	RCT, double-blind, parallel arm—24 wk	64 women with visible wrinkles ≥ grade 2	Not specified	Not specified	All range: 48-86; (1) 63.3 ± 13.9; (2) 60.0 ± 12.6	(1) Cocoa powder, per day: 320 mg total cocoa flavanols; (2) control: nutrient-matched cocoa-flavored beverage without flavanols	Cocoa powder dissolved in 150-200 mL hot water, 4 g/d	Lateral canthi, cheeks, buttock	Not specified	*Lateral canthi:* **Wrinkle depth:** ↓ *Cheek:* **Skin elasticity:** ↑ Skin hydration: ↔ *Buttock:* **MED:** ↑

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Table 1. Overview of dietary clinical studies examining the effects of plant-based foods and extracts on skin parameters (*continued*)

Plant-based item	Country / first author (year)	Study design	Subjects	Smokers	BMI[b]	Age (y)	Intervention	Form and intake of product	Location of skin	Fitzpatrick skin phototype	Effects[f]
	Germany / Heinrich (2006)[102]	RCT, double-blind, parallel arm—12 wk	24 women	Excluded	Not specified	All range: 18-65	(1) High flavanol, per day: 329 mg total cocoa flavanols (61.1 mg epicatechin, 20.4 mg catechin); (2) low flavanol, per day: 27 mg total cocoa flavanols (6.6 mg epicatechin, 1.6 mg catechin)	Cocoa powder dissolved in 100 mL hot water, 18 g/d	Dorsal skin (back and scapular region)	II	a*: ↓ Cutaneous blood flow: ↑ Skin density: ↑ Skin thickness: ↑ Skin roughness: ↓ Scaling: ↓ Skin hydration: ↑ TEWL: ↓
Coffee	Japan / Ueda (2017)[107]	RCT, double-blind, parallel arm—4 wk	31 women with reported skin dryness	Excluded	(1) 20.7 ± 2.1; (2) 21.3 ± 1.7	All range: 25-35; (1) 31.3 ± 3.7; (2) 29.9 ± 3.4	(1) CPPs[e], caffeine-free, per 100 mL: 297.8 mg CPP; (2) control: taste-matched, no CPP	Extract in beverage. 100 mL/d	Cheeks, periorbital	Not specified	Skin scaliness: ↓
	Japan / Fukagawa (2017)[108]	RCT, double-blind, parallel arm—8 wk	49 women with xerotic skin	Excluded	Range: 18.5-25.0	All range: 25-40	(1) CPP, caffeine-free, per 100mL: 270 mg CPP; (2) control: taste-matched, no CPP	Extract in beverage, 100 mL/ d	Lower cheeks, hands	Not specified	*Lower cheek:* Skin dryness: ↓ TEWL: ↓ SC hydration: ↑ Skin surface pH: ↓ SC lipids: ↑ SC lactic acid: ↑ *Hands:* Skin dryness: ↓ TEWL: ↓ SC hydration: ↑ Skin surface pH: ↓

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Table 1. Overview of dietary clinical studies examining the effects of plant-based foods and extracts on skin parameters (*continued*)

Plant-based item	Country / first author (year)	Study design	Subjects	Smokers	BMI[b]	Age (y)	Intervention	Form and intake of product	Location of skin	Fitzpatrick skin phototype	Effects[f]
Green tea	Germany / Heinrich (2011)[115]	RCT, double-blind, parallel arm—12 wk	60 women	Excluded	Range: (18-25)	All range: 40-65	(1) Green tea beverage, per 1L: 1402 mg total tea catechins; (2) control: taste-matched, no catechins	Extract in beverage, 1 L/d	Back scapular region, inner forearm	II	*Back and scapular region:* a*: ↓ *Inner forearm:* **Viscoelasticity: ↓** **Biological elasticity: ↑** **Skin density: ↑** Skin thickness: ↔ **Skin roughness: ↓** **TEWL: ↓** **Skin volume: ↓** **Skin scaling: ↓** **Skin hydration: ↑**

[a]RCT = randomized controlled trial.
[b]BMI = body mass index (measured as kg/m^2).
[c]ANC = anthocyanin.
[d]PAC =
[e]CPP = coffee polyphenol.
[f]Significant changes ($P < .05$) are bolded.
[g]↓ = decrease.
[h]↑ = increase.
[i]↔ = no changes.
[j]MED = minimal erythema dose.
[k]a* = skin redness.
[l]L* = skin lightness.
[m]TEWL = transepidermal water loss.
[n]b* = skin pigment.
[o]SC = stratum corneum.

Melons. Melons (*Cucumis melo* L) are rich in VitC and beta carotene, with cantaloupes containing higher amounts than honeydews.[33] In 44 White subjects aged 18 to 50 with FSPT II or III, consumption of dried melon pulp juice concentrate (MPJC) was associated with decreased UVR-induced damage. Participants consumed the MPJC or a color-matched control capsule daily along with a control topical cream for 30 days.[34] Participants' buttocks were irradiated at baseline and after supplementation, and a significant increase in the minimal erythema dose (MED) was observed in the MPJC group compared with controls. The MED represents the lowest amount of UVR required to produce mild sunburn or redness. A higher MED is thought to correlate with decreased susceptibility to UVR damage.[35] In another study, examining women aged 40 to 70 with FSPT II to IV, participants consumed a capsule containing the MPJC along with grape seed extract, VitC, and zinc for 8 weeks. Significant improvements in skin color, luminosity, dark circles under the eyes, erythema, and overall subjective satisfaction was noted.[36] The authors of both studies suggested that superoxide dismutase was a primary driver contributing to the observed effects of MPJC; however, this mechanism seems unlikely since superoxide dismutase is a protein that is catabolized during digestion and not absorbed intact.[37] Regrettably, the studies did not provide information to estimate the equivalent amount of melons consumed. One cup of cantaloupe melon cubes (USDA FDC #169092) provides about 58.7 mg of VitC (65%-90% of RDA for adults) and 3230 μg beta carotene.[28]

Oranges. Oranges (*Citrus sinensis*) are widely recognized as being rich in VitC. The nutrient and polyphenolic profiles differ depending on the variety and environmental factors during the growing season.[38] The most common flavonoids found in citrus include quercetin, hesperidin, and narirutin. Another flavonoid subclass, the anthocyanins, are found in blood oranges, which give the pulp and juice its red color. Inhibition of UV-induced erythema was observed in 20 White participants aged 26 to 47 years with FSPT II and III when supplemented with 100 mg/d of a powdered extract, derived from a combination of blood orange varieties containing 2.8% to 3.2% anthocyanins, 1.8% to 2.2% hydroxycinnamic acids, 8.5% to 9.5% flavone glycosides, and 5.5% to 6.5% VitC, all on a weight-to-weight basis.[39] The same study found a significant inhibition of UV-induced melanogenesis in 25 participants with FSPT II and IV, aged 40 to 70 years. Melanogenesis was assessed in 3 areas containing solar lentigos as well as in 1 lentigo-free area on each hand. Melanin is an important essential molecule for skin photoprotection, absorbing a broad range of UV rays, dissipating energy as heat to protect the skin from DNA damage.[40] However, chronic UV exposure, particularly to UVB radiation, could exceed the melanin absorbance threshold, resulting in increased ROS generation. Increased melanogenesis can disrupt the equilibrium within melanocytes and may be observed as darkening of the skin or hyperpigmentation.[41]

Blood orange juice provides about 6.65 mg of total anthocyanins per 100 mL serving (USDA flavonoid database 3.3, 2018).[42] A reduction in DNA damage and an increase in the concentrations of plasma anthocyanin cyanidin 3-glucoside, VitC, and carotenoids were noted in adults aged 20 to 27 years old who consumed 600 mL of blood orange juice for 21 days.[43] In mice, the intake of a citrus extract rich in hesperidin and narirutin for 7 weeks resulted in a significant inhibition of UV-induced transepidermal water loss (TEWL). This is an important measure of skin barrier function that assesses water diffused from the dermis and epidermis through the stratum corneum to the skin surface.[44] The study also reported improved skin hydration and reduced epidermal thickening.[45] Consumption of unripened Jeju mandarin orange extract (50, 100, and 200 mg/kg body weight) for 10 weeks in mice reduced UV-induced TEWL, wrinkle depth, epidermal thickness, and collagen degradation.[46] The amount of anthocyanins and VitC in the extract in the human study mentioned earlier[39] can be met through 300 mL or 1.3 cups of blood orange juice, containing 225 mg VitC and 10.5 mg cyanidin-3-glucoside.[43]

Tomatoes. Tomatoes (*Lycopersicon esculentum*) are rich in lycopene, a carotenoid with strong oxidant defense capabilities.[47,48] Human skin and plasma contain the highest amounts of lycopene compared with other body tissues.[49] Among men and women aged 26 to 67 with FSPT II, a significant decrease in UV-induced erythema, along with an increase in serum lycopene levels, was observed after 10 weeks of daily intake of 40 g of tomato paste, providing approximately 16 mg lycopene, compared with an olive oil control group.[50] In women aged 21 to 74 with FSPT I or II, consumption of 55 g of tomato paste containing 16 mg of lycopene along with olive oil daily for 12 weeks significantly increased the erythemal threshold compared with the intake of olive oil alone.[51] Additionally, those consuming the tomato paste showed a significant increase in procollagen I and inhibition of collagenase metalloproteinase-1 expression, mitochondrial DNA damage, and a reduction in fibrillin-1 in response to UVR-induced tissue injury. Twelve-week supplementation with a carotenoid-rich tomato nutrient complex that included rosemary extract significantly decreased UV-induced erythema in adults aged 20 to 50 with FSPT I or II compared with a control containing medium-chain triglycerides.[52] A significant increase in serum lycopene was also observed in the tomato nutrient complex group, but not the control group. In the studies mentioned here, 40 to 55 g of tomato paste provided 16 mg of lycopene. Raw tomatoes contain less bioavailable form of lycopene compared with processed tomatoes.[53] Therefore, an estimate of 390 g or 2.5 cups of raw tomatoes (USDA FDC #321360) may be needed to provide the same level of lycopene as the studies.[28] Due to seasonal availability of local tomatoes, processed tomato products may be a suitable alternative due to consistent availability, longer shelf life, and concentration of bioavailable bioactive compounds.

Kale. Kale (*Brassica oleracea*) is rich in carotenoids, VitC, and glucoraphanin, a glucosinolate that is converted to sulforaphane, which can decrease inflammation and oxidative stress mediated by the Nrf2 signaling pathway.[54,55] Consumption of carotenoid-rich curly kale extract (2200 μg lutein, 1000 μg beta carotene, 50 μg alpha carotene, 400 μg lycopene, 700 μg zeaxanthin, 100 μg cryptoxanthin) daily for 10 months improved collagen I and elastin levels in 29 women aged 40 to 56 with FSPT II compared with an olive oil control.[56] In addition, a significant increase in epidermal and

dermal thickness was observed when mice prone to accelerated skin aging consumed spray-dried kale or a glucoraphanin-enriched kale extract daily for 43 weeks, compared with controls.[57] The beneficial effects were prominent in the glucoraphanin-enriched group compared with those who consumed spray-dried kale. The amount of carotenoids in the extract closely matches 1 cup (118 g) of boiled kale (USDA FDC #169238), which contains about 5880 μg lutein and zeaxanthin, 2040 μg beta carotene, 11.8 μg alpha carotene, and 30.7 μg cryptoxanthin.[28] Stir-frying and steaming were reported to preserve more glucosinolates compared with boiling.[58]

Pomegranates. Pomegranates (*Punica granatum* L) are rich in anthocyanins, the ellagitannin punicalagin, and ellagic acid.[59] A significant increase in MED was observed with daily intake of 8 oz (237 mL) of pomegranate juice or its extract for 12 weeks in 74 women aged 30 to 40 with FSPT II to IV.[60] The pomegranate juice and extract provided similar amounts of punicalagin and ellagic acid. The intake of pomegranate juice concentrate powder significantly decreased wrinkle formation, inhibited reduction in collagen type I and hyaluronan concentrations, and increased skin water content in UVB-treated mice compared with the group not receiving the powder.[61] Supplementation also inhibited pro-inflammatory cytokine interleukin-1β and myeloperoxidase activity that promotes the formation of ROS, while increasing the anti-inflammatory cytokine interleukin-10. One cup of pomegranate juice is equivalent to a serving of fruit and counts toward the daily recommendation.

Passion Fruits. Passion fruits (*Passiflora edulis*) contain edible seeds that provide polyphenols that can benefit the skin.[62] The seeds have more polyphenols than the pulp or rind, with piceatannol only present in the seeds.[63] Improved skin barrier function, as evidenced by a significantly increased moisture content and decreased TEWL, was observed in 32 Japanese women aged 35 to 55 with dry skin complaints who consumed passion fruit seed extract containing 5 mg piceatannol for 8 weeks compared with controls.[64] Increased facial water content and viscoelasticity were also observed in adults who consumed piceatannol-rich beverages for 8 weeks.[65] Piceatannol, a polyphenolic compound, has been shown to increase oxidant defense, as evidenced by a reduction in amine-induced hydrogen peroxide generation in rats after 6 weeks of daily supplementation.[66] Approximately 2.3 g of raw passion fruit seeds are needed to obtain 5 mg (2.2 mg/g) piceatannol,[63] an amount that may be obtained from 1 fruit.

Grapes. Grapes (*Vitis vinifera*) contain significant amounts of polyphenols, including anthocyanins,[67] flavan-3-ols, resveratrol,[68] and proanthocyanidins[69] as well as VitC.[70] Six months of daily supplementation with a grape seed extract providing 162 mg of proanthocyanidins to 12 Japanese women who had melasma (brown skin patches) significantly reduced the melanin index and melasma size and improved skin lightening.[71] The same research group had previously supplemented guinea pigs with the grape seed extract for 8 weeks and observed an inhibition in melanin synthesis and increased lightening of the skin.[72] Another study observed a significant decrease in lentigos on the cheeks of women aged 30 to 60 years who consumed 200 mL of a beverage containing 200 mg of dealcoholized red wine oligomeric proanthocyanidins compared with a calorie-matched control drink for 12 weeks.[73] Stratum corneum water content was significantly increased in the test group and significantly decreased in the controls. Taken together, the studies suggest reduced melanogenesis and skin lightening from the proanthocyanidins-rich grape extracts. Improved cellular oxidant and anti-inflammatory defenses, as seen with an inhibition of Nrf2-dependent antioxidant enzymes in the skin, along with a reduction in epidermal thickness, were observed with 14 days of grape seed extract supplementation (2 mg/kg body weight) in UV-irradiated mice.[74] The previously mentioned studies provided about 3 to 4 times higher proanthocyanidins than the estimated mean daily intake of 57.7 mg in the United States.[75] Generally, grapes are a good dietary source of proanthocyanidins, and their juice contains about 524 mg/L.[75] To obtain 162 to 200 mg of proanthocyanidins provided in the studies, approximately 300 to 382 mL or 1.3 to 1.6 cups of grape juice should be consumed.

The DGAs recommend 2 cups of fruits, especially whole, and 2.5 cups of vegetables with specific suggested amounts for dark-green, red, and orange ones.[76] Although the studies mentioned illustrate that the estimated amount of select fruits and vegetables generally aligns with the guidelines, different plant foods have different phytochemical profiles, so specificity is needed when making dietary recommendations for different skin conditions.

Nuts and Legumes

Nuts and legumes have an abundance of beneficial fats and are a good source of plant-based protein as well as other micronutrients. A lower risk of severe photoaging, assessed by a physician using a 6-grade ordinal scale, has been associated with a higher intake of n-3 polyunsaturated fatty acids (n-3 PUFA) from plant-based sources[77] and monounsaturated fatty acids (MUFA) from vegetable oils but not dairy products and meats.[78] Furthermore, women with the highest intake of n-3 PUFA, especially eicosapentaenoic acid, were less prone to severe photoaging.[77] Although plant-based foods are not significant sources of eicosapentaenoic acid, it can be metabolized from alpha linolenic acid that is commonly found in vegetable and flax seeds, walnuts, and oils. The 2020-2025 DGAs recommend an intake of 5 oz per week of nuts, seeds, and soy products.[76] However, higher amounts may be required to promote specific skin health benefits.

Almonds. Almonds (*Prunus dulcis*) are rich in alpha tocopherol, MUFA, and polyphenols, all of which provide oxidant defense.[79,80] One ounce of almonds (USDA FDC #170567) contains 8.94 g of MUFA, 3.5 g of PUFA, 6 g of protein, and 7.27 mg of alpha tocopherol.[28] Alpha tocopherol is the most abundant form of vitamin E in human tissues and functions in part by quenching lipid peroxidation[81] and increasing the levels of plasma glutathione (GSH), an endogenous antioxidant.[82] A significant decrease in overall wrinkle severity and width was observed in postmenopausal women aged 55 to 80 with FSPT I or II who consumed almonds providing 20% of total calories for 16 weeks, compared with energy-matched nut-free snacks.[83] A follow-up study using the same dietary intervention found a significant

decrease in average wrinkle severity in postmenopausal women aged 47 to 84 with FSPT I or II after 24 weeks of daily nut intake. In addition, facial pigment intensity decreased significantly with almond intake, and no changes were observed in the nut-free snack group.[84]

The beneficial effects of almonds on skin esthetics are thought to be due in part to their oxidant defense capability. At 10% or 20% of total daily calories, almonds have been found to increase both plasma and red blood cell alpha tocopherol[85] and decrease pro-inflammatory high-sensitivity C-reactive protein.[86] An increase in glutathione peroxidase activity has also been noted from daily intake of 0.7 oz (20 g) of almonds for 8 weeks in overweight and obese women.[87] Additionally, the consumption of almond skin powder decreased oxidized glutathione, increased plasma glutathione and the plasma glutathione-to-oxidized glutathione ratio, and upregulated glutathione peroxidase activity in healthy adults.[88] Glutathione neutralizes free radicals and is a cosubstrate for glutathione peroxidase, an important enzyme that quenches hydrogen peroxide and lipid hydroperoxides.[89] Based on a 2000 kcal diet, 20% of calories from almonds estimates to 400 kcal, which can be obtained from approximately 2.4 oz (68 g), providing 21.5 g of MUFA (9.7% of 2000 kcal), 8.4 g of PUFA (3.8% of 2000 kcal), and 17.5 mg of alpha tocopherol (117% of RDA). However, it is likely that the amount of calories metabolized and absorbed from almonds is less than the estimated amount. A study has shown that the energy content of almonds determined primarily by Atwater factors overestimates the actual amount of metabolizable energy.[90] The only current PUFA and MUFA recommendations are from the American Heart Association, which are based on heart health outcomes and recommend a daily intake of 10% and 15%, respectively from total calories.[91] Although daily intake of 2.4 oz of almonds is higher than the current American Heart Association guidelines of 1.75 oz of nuts 4 times per week, the previously mentioned study focused on the benefits on skin. The results are of interest and should be repeated at lower intakes, possibly for longer periods of time.

Soybeans. Soybeans (*Glycine max*) are rich in the isoflavones genistein and daidzein that have structures similar to estrogen and may interact with this hormone's receptors.[92] The reduction in estrogen during menopause has been associated with changes in the dermal layers that increase skin conditions such as wrinkling, dryness, and poor wound healing.[93] One ounce of mature raw soybeans (USDA FDC #174270) contains 1.2 g of MUFA, 3.2 g of PUFA, and 10.3 g protein. A significant decrease in fine wrinkles and increased skin microrelief (a network of furrows and ridges) at the lateral canthi and elasticity in the cheeks was observed in Japanese women aged 35 to 48 who consumed an isoflavone-rich soybean extract (40 mg soy isoflavone aglycones) for 12 weeks compared with a control.[94] Additionally, a significant increase in hydration and a decrease in stratum corneum thickness was observed in adults aged 25 to 60 who consumed a soybean and barley beverage for 8 weeks compared with controls.[95] An increase in hyaluronan levels in dermal fibroblasts and a decrease in hyaluronidase-2 (an enzyme that degrades hyaluronan) mRNA and protein levels were seen only in the soy/barley group, further supporting the improvement seen in skin hydration. The amount of 40 mg of isoflavones is similar to data from current epidemiological and clinical studies suggesting an intake of 50 to 90 mg of isoflavones or 15 to 25 g of soy protein per day for women for general health.[96]

Polyphenol-Rich Beverages

Coffee, green tea, and cocoa are widely consumed beverages rich in polyphenols. These beverages typically contain the methylxanthines caffeine and theobromine, with caffeine a possible concern for some consumers. The US Food and Drug Administration has cited 400 mg of caffeine a day as an amount not typically associated with negative effects.[97] Importantly, decaffeination has little to no effect on polyphenol content,[98,99] thus it may be a suitable alternative form of coffee and tea.

Cocoa. Cocoa (*Theobroma cacao*) is a rich source of flavan-3-ols (flavanols), a flavonoid-subclass that can inhibit lipid peroxidation, neutralize ROS, and chelate metals that enhance the production of ROS.[100] Furthermore, cocoa flavanols can inhibit enzymes involved in ROS production and upregulate protective genes involved in cellular stress responses. Cocoa is particularly rich in theobromine and is relatively lower in caffeine (~10 mg) per serving compared with tea and coffee. Among Korean women aged 43 to 86, daily consumption of a cocoa beverage containing 320 mg of flavanols for 24 weeks significantly improved measures of elasticity and the MED, as well as skin roughness, suggesting an improvement in wrinkle depth compared with a nutrient-matched control drink.[101] In addition, intake of a cocoa beverage containing 329 mg of flavanols for 12 weeks significantly decreased UV-induced erythema, skin roughness, scaling, and TEWL and increased skin density, thickness, hydration, and blood flow to the cutaneous and subcutaneous tissues in women aged 18 to 65 with FSPT II compared with a drink containing low flavanol at 27 mg.[102] The cocoa beverages used in the studies were powders mixed with water. It is important to note that most processing of natural cocoa powders reduces the amount of flavanols.[103] Therefore, depending on the source of cocoa powder, the amount needed to obtain ~ 300 mg of flavanol can vary substantially.

Coffee. Coffee (*Coffea* L) is rich in polyphenols, particularly chlorogenic acid.[104,105] An observational study assessed the amount of chlorogenic acid consumed from coffee by 131 Japanese women aged 30 to 60 and noted a significant association between higher consumption of coffee (≥450 mL/d) or coffee polyphenols (≥900 mg/d) with lower hyperpigmentation.[106] Daily consumption of a decaffeinated beverage containing 297 mg of coffee polyphenols for 4 weeks significantly improved scaly skin in the cheeks and around the mouth in Japanese women aged 25 to 35 compared with a control drink.[107] In another study, the daily intake for 8 weeks of a 100 mL decaffeinated beverage with 270 mg coffee polyphenols containing mainly chlorogenic acid significantly improved skin permeability barrier function, as evidenced by a decrease in dryness, TEWL, and pH, as well as an increase in stratum corneum lipids, lactic acid, and hydration, in 49 women with dry, itchy, and cracked skin compared with controls.[108] Roasted coffee beans contain 7.95 to 8.75 mg/g of total polyphenols that could decrease to 1.17 to 1.58 mg/g after 12 months of storage.[109] The Specialty

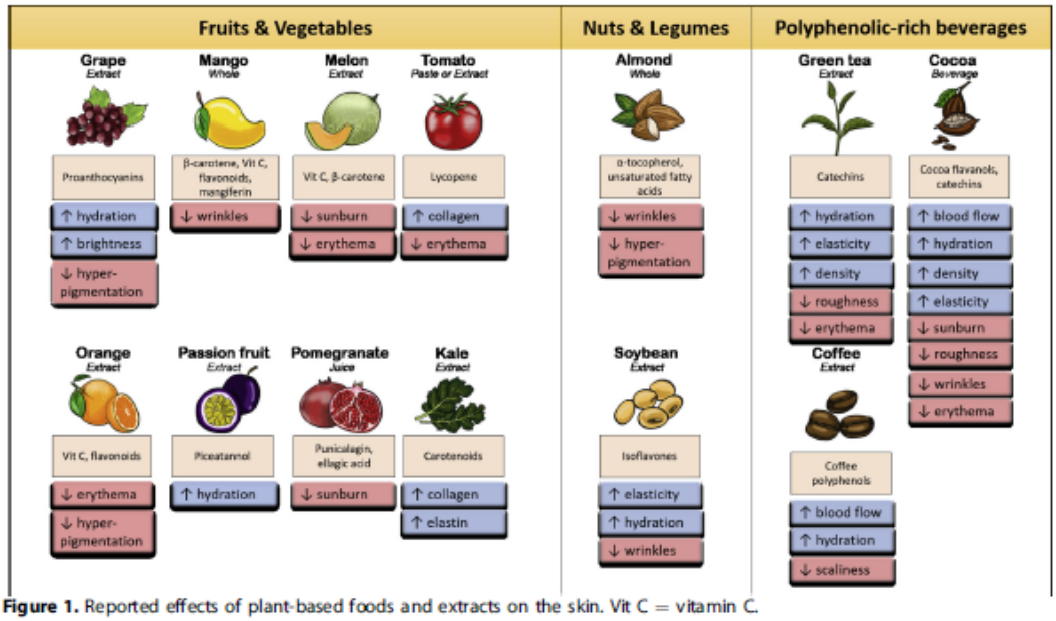

Figure 1. Reported effects of plant-based foods and extracts on the skin. Vit C = vitamin C.

Coffee Association recommends brewing coffee at a ratio of 1.63 g of beans per fluid ounce of water.[110] Considering that 29% and 36% of American adults drink 2 and 3 or more cups per day, respectively, this would equate to a daily intake of 270 to 300 mg coffee polyphenols when one uses an estimate of 8 mg total polyphenols per gram of beans.[111]

Green Tea. Green tea (GT; *Camellia sinensis*) provides a number of flavanols,[112] particularly epigallocatechin gallate (EGCG).[113] A review of studies on tea flavanols reports protection against UVR and anti-allergenic properties that may be beneficial to the skin.[114] Daily consumption of 1 L of a GT drink providing 1402 mg total of tea flavanols (980 mg EGCG) for 12 weeks in 60 women aged 40 to 60 with FSPT II significantly decreased UV-induced erythema, roughness, TEWL, and viscoelasticity (resistance toward an applied vacuum) compared with a control beverage devoid of polyphenols.[115] Increased serum flavanol levels, skin density, and biological elasticity (ability to return to original position) were also observed. In both groups, skin volume, scaling, and hydration significantly increased, which likely reflected the large amount of fluid consumed. However, the GT group showed a greater increase in hydration compared with the controls. In another study, supplementation with GT extracts containing 540 mg total flavanols for 12 weeks significantly decreased UV-induced erythema in 14 adults with FSPT I or II when given the maximum UVR dose.[116] However, no significant changes were seen when the same adults were given lower levels of radiation. In humans, flavanol metabolites have been found in the skin after daily intake of GT extract containing 450 to 540 mg of tea flavanols for 12 weeks.[116,117] An increase in the expression of genes related to skin moisturizing factors in response to EGCG was also observed in vitro.[118] Typically, 1.8 to 3 g of leaves are used to brew a cup of tea, but the amount of EGCG varies drastically from 0.07 to 6.1 mg/3 g of leaves.[119] Therefore, depending on the quality and processing of the tea leaves, as little as 1 or more than 10 cups of green tea may be needed to achieve an intake of at least 500 mg of tea flavanols. The European Food Safety Authority has found that modest amounts of green tea infusions and similar beverages are safe, but caution against taking more than 800 mg/d EGCG through extracts, because rare cases of liver injury have been reported.[119]

CONCLUSION

The studies mentioned here collectively provide evidence of the potential benefits of plant-based foods for skin health and esthetics (Figure). In an era of personalized nutrition, this review can help dietitians provide better dietary recommendations of certain plant-based foods to compliment a well-balanced diet. Many of the foods and extracts discussed are rich in bioactive compounds such as VitC, alpha tocopherol, beta carotene, polyphenols, and phenolic acids that provide oxidant defense, support mechanisms to lower inflammation, or promote structural support and UV protection in the skin.[120] Some of the clinical studies above explored the role of whole and minimally processed foods (juice, beverage, paste), and others used extracts. When possible, we equated the intake of bioactives from extracts to the amounts present in whole food and beverages to evaluate the feasibility of bioactivity from dietary intake; however, further studies are clearly warranted to confirm these

speculations. The food items discussed were added to the participants' habitual diet, mostly on a daily basis, with some at higher amounts than the currently recommended daily amounts. Overall, we found that the consumption of colorful fruits and vegetables abundant in vitamins, carotenoids, anthocyanins, and polyphenols is indicated for skin health and esthetics. In general, yellow, orange, and red fruits such as mangos, melons, citrus, tomatoes, and vegetables such as red bell peppers and dark-green leafy kale are good sources of carotenoids. Fruits with deep red or purple colors such as grapes, pomegranate, and passion fruit are rich in anthocyanins and polyphenols. Nuts and legumes are also encouraged, along with cocoa, coffee, and tea that are rich in polyphenols. Decaffeinated options do not appear to dilute benefits and should be considered for caffeine-sensitive individuals. Although intake of an abundance of plant-based foods is desirable, overconsumption of a single food or extract can be of concern, as illustrated previously by the intake of a large amount of mangos.[29]

In conclusion, clinical studies in the field of nutrition and skin research support growing evidence to help dietitians make targeted dietary recommendations. More investigations on whole foods and beverages, as well as those fortified with plant-based extracts, are needed to extend current findings.

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STATEMENT OF POTENTIAL CONFLICT OF INTEREST

K. Sivamani serves as a scientific advisor for LearnHealth and Arbonne and as a consultant to Burt's Bees, Novozymes, Nutrafol, Abbvie, Leo, UCB, Sun and Regeneron Pharmaceuticals.

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V. W. Fam and P. Charoenwoodhipong collected the data. V. W. Fam created the table and figure. V. W. Fam wrote the first draft with contributions from P. Charoenwoodhipong and R. M. Hackman. V. W. Fam, R. R. Holt, C. L. Keen, R. M. Hackman, P. Charoenwoodhipong, and R. K. Sivamani reviewed, commented on, and provided further contributions on subsequent drafts of the manuscript.