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Clinical Approaches to Traditional Uses of Select Fruits for Human Health and Performance:
Focus on Goji Berry and Mango

By

XIANG LI

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

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DAVIS

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Clinical approaches to traditional uses of select fruits for human health and performance: Focus on goji berry and mango

Abstract

Fruits play a significant role in human nutrition, since they are good to excellent sources of vitamins, dietary fibers, and phytonutrients such as polyphenols and carotenoids. While many fruits have a long history of use as traditional remedies in cultures worldwide, scientific evidence to help explain the biochemistry, physiology and nutrition affected by these foods has only started to emerge in the last few decades. Although phytonutrients currently have no specific dietary intake recommendations, the U.S. Dietary Guidelines 2020-2025 recommend at least two daily servings for adults.

Epidemiological studies have shown that the regular consumption of fruits is associated with decreased risk for many age-related chronic conditions including cardiovascular and certain eye diseases. However, epidemiological data is observational and does not inform about cause-and-effect relationships. Cellular, animal and a limited number of human studies provide intriguing evidence regarding the potential health benefits of certain classes of phytonutrients, for instance, the protective effects of carotenoids on age-related macular degeneration and flavanols on vascular diseases. However, more clinical studies are needed to better clarify these relationships.

This dissertation explores the role of select phytonutrient-rich fruits on two aspects of the aging process. Mango is a tropical fruit that has been used in traditional remedies in multiple tropical regions worldwide. Based on its use history and unique polyphenol profile, the fruit may be useful to support cardiovascular health. Goji berry has been used in traditional Chinese medicine for more than two thousand years for its "eye-brightening". Chapter I summarizes the evidence about mango and goji berry, as well as blueberry and cocoa, as phytonutrient-rich fruits with potential cardiovascular and eye health benefits. Chapter II is a published clinical trial that investigated the effects of dried goji berry intake on macular pigment optical density and skin carotenoids in healthy middle-aged individuals. Chapter III explores the role of mango intake on markers related to cardiovascular function in postmenopausal women. Chapter IV is a detailed review about the role of lutein and zeaxanthin in age-related macular degeneration, the importance of these two carotenoids in maternal and infant health and concludes with a focus on goji berries as a potential dietary source to benefit eye health. Finally, a summary of this work and a discussion about future research directions is presented in Chapter V.

CHAPTER I

LITERATURE REVIEW

Introduction

In plants, a fruit is the seed-containing section, which is formed from the ovary after flowering. Fruits have their vivid colors due to the presence of phytochemicals as pigments, which are natural compounds that protect against threats and insults such as insects and ultraviolet sunlight. Bright colors of fruits also attract animals and human beings for seed dispersing purposes.[1] The belief of using fruits as traditional medicine exist in many cultures worldwide. For instance, cocoa beans and blueberries have been used traditionally as therapies among indigenous people in North America. The fruit, leaves, seed, and bark of the mango plant have been used as traditional medicine in Southeast Asia, Oceania, Africa, and Central America. Goji berries have been used as traditional Chinese medicine (TCM) for two thousand years.

Phytochemicals can be classified primarily as terpenoids, phenolics, alkaloids, nitrogen-containing plant constituents, and organosulfur compounds.[2,3] Examples of major phytochemical groups that are abundant from dietary sources and related to human health include carotenoids and polyphenols. Carotenoids are a type of terpenoid. Carotenoids can be classified as carotenes and xanthophylls.[4] Phenolics can be classified as phenolic acids and polyphenols. Two primary subclasses of phenolic acids are hydroxybenzoic acid and hydroxycinnamic acid.[5] Polyphenols include flavonoids, tannins, stilbenes, lignans, and xanthones. As one of the most studied categories of polyphenols, subclasses of flavonoids can be categorized to flavanones, flavones, anthocyanins, flavanols (flavan-3-ols), chalcones, flavonols, and isoflavonoids.[6]

Among the thousands of phytochemicals that have been identified in plants, both health promoting and toxic compounds exist. For instance, some tannins decreased the activity of digestive enzymes or the bioavailability of protein or minerals and have been considered as anti-nutrients.[7] Phytochemicals that exist in plant-based dietary sources and have value in human health maintenance and prevention of diseases are defined as phytonutrients.[8]

Fruits, vegetables, legumes, spices, nuts, wine, cocoa, tea, and olive oil are examples of foods rich in bioactive phytonutrients.[9,10] The consumption of these dietary components has been related to decreased risk of developing chronic diseases, including cardiovascular diseases (CVD), age-related eye diseases, type II diabetes, cancers, and all-cause mortality.[11-15] Observational studies also have reported that the total dietary polyphenol intake was inversely associated with the risk of hypertension, hypercholesterolemia, and cardiovascular events.[16,17]

Polyphenols under different categories may play various roles in reducing CVD risk. In the United States, the estimated flavonoid intake is 345 mg/day, with flavanols as the most abundant source.[18] The three most consumed flavanols are catechin, epicatechin, and their polymers. Sub-analyses of a cohort study indicated that dietary intakes of flavanols along with lignans, dihydrochalcones, and hydroxybenzoic acids showed a stronger inverse association with the risk of overall CVD events than other phenolic compounds.[16] Another cohort study reported that the dietary intakes of anthocyanins, dihydrochalcones, dihydroflavonols, proanthocyanidins, catechins, flavonols, hydroxybenzoic acids, and stilbenes were significantly associated with decreased risks of total CVD.[19]

Blueberries and cranberries contain high amount of anthocyanin and proanthocyanidin, respectively, with moderate concentration of flavonoids.[20,21] Cocoa is rich in flavanols,

especially epicatechin and catechin.[22] Mango, as the fourth leading fruit crop worldwide, is high in carotenoids, phenolic acids, and mangiferin, a polyphenol classified as a xanthonoid.[23]

Carotenes exist in dietary sources primarily as α-carotene, β-carotene, and lycopene.[24] Major xanthophylls that exist in dietary sources include lutein (L), zeaxanthin (Z), and β-cryptoxanthin. Epidemiological studies report inconsistent results on the relationship between dietary L and Z intakes and the risk of age-related macular degeneration.[25,26] However, clinical studies have shown that the supplementation of L and Z was able to increase the level of these compounds in the retina, suggesting their protection against age-related macular degeneration (AMD).[27] A major dietary source of L and particularly of Z is goji berry, which also have other in carotenoids, as well as phenolic acids, and flavonoids.[28,29]

While many examples of fruits used traditionally for health promotion exist, this literature review focuses on the evidence of mango, cocoa, blueberries, and cranberries in cardiovascular health, and goji berries in eye health. The application of modern scientific methods to assess traditional remedies is important because evidence-based data is necessary to transfer historical stories and ancient wisdom to contemporary life and advancement of health and human performance.

Traditional uses and contemporary evidence of select fruits in cardiovascular health

Cocoa

Cocoa is the dried and fully fermented product obtained from the seeds of *Theobroma cacao L.* and is the main ingredient in chocolate products.[30] While used traditionally in a number of cultures, one of the best examples of its medicinal use is from the Kuna Indians who have lived for centuries on remote islands off of the Caribbean coast of Panama. This group of indigenous

people is famous the lack of hypertension, an infrequent prevalence of CVD, diabetes, and cancer, and a longer lifespan, compared to Panamanians living on the mainland.[31,32] However, when these people migrate to an urban environment, the incidence of hypertension and vascular diseases increased significantly.[22,33] Nutritional assessments showed that the consumption of total fruit, fish, and cocoa-containing beverages were significantly higher among Kuna Indians living on the island compared to those residing in Panama City, even though the overall dietary intake of added sugars and salt was higher in the indigenous group.[22] Scientists hypothesized that cocoa may be an influencing factor in the low prevalence of CVD in this population, due to its high concentration of flavonoids. The primary flavonoids in cocoa are flavanols, including monomeric catechins, epicatechin, and polymeric procyanidins.[34] Cocoa also contains methylxanthines, i.e., theobromine and caffeine, which remain in flavanol-poor cocoa butter and cocoa solids (also called cocoa cake) after pressing.[30]

The effect of cocoa flavanols on vascular function was first reported more than two decades ago.[35] Extending the observations about cocoa flavanols to broader dietary patters, studies exploring the association between the intake of flavonoids and the risk of CVD have yielded inconsistent results, suggesting that certain sub-classes may be more effective than others in terms of cardio-protection. Some observational studies have shown that the dietary intake of flavanols was associated with a decreased risk of CVD and ischemic heart diseases.[36,37] Epidemiological studies have reported that a moderate amount (<100g /week) of chocolate consumption was associated with a decreased risk of CVD.[38,39] A Cochrane meta-analysis concluded that the consumption of flavanol-rich chocolate and cocoa products had a small but statistically significant effect on reducing systolic and diastolic blood pressure, both by 1.76 mmHg.[40] Similarly, another systematic review and meta-analysis included 42 acute and short-

term (<18 weeks) randomized controlled trials concluded that chocolate, cocoa, and flavanol intake significantly improved vasodilation function as measured by flow-mediated dilatation (FMD), reduced diastolic blood pressure by 1.6 mmHg, and marginally improved the serum cholesterol profile.[41] However, these results were strongest among individuals with moderately elevated blood pressure and untreated hypertension.[42]

Postprandial vascular effects after cocoa flavanol consumption have been well studied. The vasodilation function in healthy males, measured by FMD, was significantly increased one to four hours after taking a cocoa drink containing 917 mg of cocoa flavanols, compared to baseline values and to those consuming a control beverage with 37 mg of flavanols.[43] The pattern of improvement in FMD from the flavanol-rich cocoa beverage was closely mirrored when participants also consumed 1 or 2 mg/kg of body weight of (–)-epicatechin dissolved in water, suggesting (–)-epicatechin and its metabolites were the main contributors of the vascular effects. Additional research from the same study found that while (–)-epicatechin is a primary contributor to the vasodilation function, dimeric and oligomeric procyanidins that are metabolized by the gut microbiome may also contribute to the vasculo-protection.

In healthy young men, daily intake of a cocoa extract containing 130 mg of (–)-epicatechin and 560 mg of procyanidins for 30 days significantly improved FMD and reduced blood pressure and arterial stiffness as measured by pulse wave velocity (PWV), while 20 mg of (–)-epicatechin and 540 mg procyanidins, or a control capsule, did not.[44] However, total cholesterol was decreased after in both groups consuming cocoa, suggesting synergistic effects of (–)-epicatechin and procyanidins, possibly through gut microbiome-mediated catabolism. Cocoa also contains methylxanthines, which are biologically active. While the intake of theobromine and caffeine alone did not result in a significant change in vascular endothelial function measures,

interestingly, the combination of methylxanthines (122.4 mg) plus a high cocoa flavanol (820 mg) drink induced a significant improvement in FMD response than the beverage only.[45] In addition, plasma metabolites of (–)-epicatechin were higher after consuming the flavanol-rich cocoa drink with methylxanthines than when the flavanols were consumed alone, indicating a likely interaction between theobromine, caffeine, and cocoa flavanols on vascular function.

Several molecular mechanisms regarding the effects of flavanols on blood pressure and vasodilation have been proposed. Flavanols such as (–)-epicatechin can increase nitric oxide (NO) production directly by increasing endothelial nitric oxide synthase (eNOS) expression.[46] The release of NO consequently increases intracellular cGMP which then induces a relaxation of vascular smooth muscle cells. The increased eNOS activation may also be modulated by flavanols through a calcium/calmodulin pathway by increasing the intracellular calcium concentration, or by phosphatidylinositol 3-kinase/protein kinase B (Akt)-dependent eNOS phosphorylation.[47] In addition, (–)-epicatechin has been shown to down-regulate the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by inhibiting the synthesis of vasoconstrictors such as endothelin-1, and therefore increase the utilization of NO.[48] Flavanols may also directly inhibit angiotensin-converting enzyme activity, which increases NO production.[49] Apart from modulating NO production, flavanols may also induce the release of endothelium-derived relaxing factors such as hydrogen peroxide and prostacyclin.[50]

Cocoa flavanols also benefit cardiovascular health by inhibiting platelet activation and adhesion. In healthy individuals, platelet aggregation induced by collagen and adenosine diphosphate, and the expression of P-selectin, was significantly decreased compared to a placebo group after the daily intake of 234 mg of cocoa flavanols and procyanidins for 28 days.[51] Some studies have reported that the reduction in platelet aggregation was not different between flavanol-rich dark

chocolate or low-flavanol dark chocolate mixed with white chocolate, suggesting potential anti-adhesive effects from methylxanthines.[52,53] While the exact mechanisms to explain the interaction between flavanols and platelets are still under investigation, proposed mechanisms from *in vitro* and *ex vivo* models include an inhibition in the expression of endothelial adhesion molecules (e.g., vascular cell adhesive molecule-1, intercellular adhesive molecule-1, E-selectin), and the down-regulation of pro-inflammatory factors such as interleukin (IL)-6 and tumor necrosis factor-α, which also decrease the recruitment of other proinflammatory compounds.[47]

Mango

Mango (*Mangifera indica L.*) originated from the Indian subcontinent and has been cultivated for thousands of years. The bark, leaves, roots, and flowers of the tree, and the peel, kernel, and pulp of the fruit, have been used in traditional medicine in tropical and sub-tropical regions throughout the world. The bark and leaves have been used for treating diarrhea and diabetes in Bangladesh, and Ghana, with the pulp and kernel used for hemorrhaging in the lungs and intestines in India.[54]

Mango fruit is a rich source of fiber, vitamins C and E, folate, potassium, β-carotene, and phenolic compounds. Dietary intake of vitamins C and E, and β-carotene, are associated with reduced risks for CVD.[55,56] Major phenolic compounds reported in mango pulp include mangiferin, quercetin, kaempferol, myricetin, catechin, gallic acid, ferulic acid, protocatechuic acid, and chlorogenic acid.[23,57] One study observed that a higher mango intake was associated with improved nutrient intakes, diet quality, and body mass index (BMI), factors known to reduce the risk of CVD.[58] Clinical trials also suggest that mango fruit may have protective effects against the development of CVD. Daily intake of 200g of fresh-cut Ataulfo mango for 30 days

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decreased blood lipids and increased the plasma antioxidant capacity in healthy adults.[59] In obese men and women aged 20-50 years, supplementation of 10 g/d freeze-dried mango pulp for 12 weeks decreased blood glucose levels but not inflammatory or any cholesterol markers.[60,61] Another study reported that the daily intake of 400 g of fresh frozen mango pulp significantly decreased systolic blood pressure (SBP) only in individuals with a BMI of 18-26.2 kg/m^2.[62] In contrast, plasminogen activator inhibitor 1, IL-8, and mitochondrial pyruvate carrier-1 were significantly reduced in individuals when the BMI was partitioned as > 28.9 kg/m^2. In addition, in participants with impaired glucose sensitivity, the supplementation of 100 or 300 mg/d of mango fruit powder with 250 ml water daily for four weeks significantly increased the vasodilation of arteries as measured by the reactive hyperemia index compared to a placebo group.[63]

Mangiferin is a unique compound in mango that has been studied for its vasculoprotective effects (Figure 1). A mango bark extract (Vimang®) with a high concentration of mangiferin decreased cholesterol in plasma and liver, and reduced oxidative stress in mice.[64,65] A subsequent human study showed that the daily intake of 900 mg of Vimang® for 90 days reduced a measure of serum oxidative stress compared to a control group among older individuals.[65] In both the animal and human studies, the marker of oxidative stress, while considered valid at the time of the study, is now viewed with limitations. In overweight hyperlipidemic individuals, the daily intake of 150 mg of mangiferin for 12 weeks significantly improved lipid profiles and glucose homeostasis.[66] In hyperuricemic rats, mangiferin intake significantly reduced SBP, serum uric acid and inflammatory markers, and increased the expression of eNOS.[67] One potential mechanism to help explain the vasodilatory effect of mangiferin may be due to the increased expression of eNOS, and therefore enhanced the production of NO.[68]

Reports have shown that the composition of phenolic compounds in mango varied significantly among different varieties.[69] Mango varieties with high polyphenol content, such as Ataulfo, may play a more prominent role in cardiovascular health, but the interaction between the polyphenols, carotenoids and other bioactive compounds in mango must be considered. Further studies may also focus on the potential effects of mango by-products on metabolic health, since the total concentration of phenolic compounds is higher in the kernel, peel, leaves, and bark compared to the edible fruit.[67,70] Such explorations may be useful in processing what is considered as agricultural waste into useful extracts.
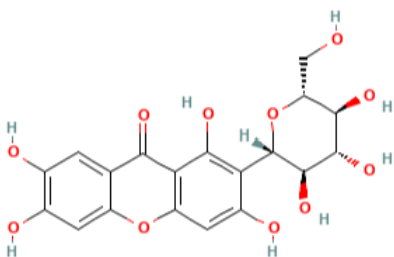
Figure 1. Chemical structure of mangiferin.[71]

Blueberries and cranberries

Low-bush blueberry (*Vaccinium angustifolium* A.) and high-bush blueberry (*Vaccinium corymbosum* L.) are two common species originally grown in North America. Native Americans have a long folklore history of using both types of blueberry plants to treat rheumatism and infection.[21]

Anthocyanins are responsible for the red, blue, and purple color in ripe berries. Blueberries are one of the most abundant sources of anthocyanins in commonly consumed fruits.[72] The total anthocyanin level in fresh blueberries is significant, reaching up to 487 mg/100g. Blueberries also contain appreciable amounts of proanthocyanidins and hydroxycinnamic acids (mainly

chlorogenic acid), along with vitamins and minerals, fiber, and small quantities of flavonols and flavanols.[73] Epidemiological studies suggest that a higher dietary anthocyanin intake is associated with a lower risk of hypertension,[74,75] and reduced arterial stiffness in women, though these studies do not specify blueberries as the sole source of these bioactives.[76] A meta-analysis of 19 cohort studies reported that the dietary intake of anthocyanins was associated with a decreased risk of coronary heart disease and CVD mortality, but not myocardial infarction, stroke, or total CVD risk.[77]

The effects of blueberries on markers of CVD risk have been studied. In obese postmenopausal women with pre- and stage I-hypertension, daily consumption of 22 g of freeze-dried blueberry powder containing 469 mg of anthocyanins for eight weeks significantly reduced systolic and diastolic blood pressure by 7 mmHg and 5 mmHg, respectively, and arterial stiffness measured by brachial-ankle PWV, compared to their baseline values or to a placebo group.[78] In healthy males, FMD was significantly increased one, two, and six hours after the intake of 34, 57, and 80 g of blueberry powder mixed in water (containing 766, 1,278, and 1,791 mg of polyphenols, respectively), compared to a control drink.[79] However, no changes were seen in arterial stiffness measures. In addition, the increase in polyphenol metabolites and decrease in neutrophil NADPH oxidase in plasma were correlated to FMD, suggesting that the phenolic metabolites after blueberry powder consumption effectively improved vasodilation functions by elevating the bioavailability of NO through inhibition of NADPH oxidase. Later the research group identified that the FMD improvements were mainly due to anthocyanin metabolites.[80] The blood pressures of overweight and obese smokers who consumed 250 g of blueberries for three weeks showed no significant changes from baseline values .[81] Among mid-aged women who were at risk for type II diabetes, daily consumption of 240 ml of wild blueberry juice with 314 mg of anthocyanins for

seven days significantly improved serum nitrates and nitrites, but no change were noted for in glucose metabolism parameters, cholesterols, inflammatory markers, platelet adhesion molecules, vasodilation, or blood pressure, compared to baseline and the placebo group.[78] Taken together, the above results suggest that clinical trials with blueberries may need require a few weeks or longer of regular intake in order to observe clinically significant changes.

Similar to blueberries, the American-cranberry (*Vaccinium macrocarpon* A.) is also a plant that is native to North America and has a long history of botanical uses by indigenous people, such as for urinary tract disorders and diarrhea.[82] Cranberries are rich in numerous phenolic compounds, including A-type procyanidins, anthocyanins, flavanols, benzoic acid, and ursolic acid.[21] Due to the extremely low sugar and high tart and astringency nature, cranberry products often contain added sugar or are blended with other fruits to improve palatability. To date, no systematic reviews or meta-analysis has noted an efficacy of cranberries in reducing CVD risks. However, potential cardio-protective effects may exist due to mechanisms discussed above.

Traditional uses and modern evidence of goji berries in eye health

Goji berry (*Lycium barbarium* and its closely related species *Lycium chinese*), also termed wolfberry or Gou Chi Zi, has been used in TCM for its "eye-brightening" effects for millennia.[83] In Chinese culture, goji berries can be consumed as a snack, an ingredient in soup, or as a tea alternative. Goji berries have the highest known content of Z of any commonly consumed food.[84] In addition, goji berries also contain modest amounts of β-cryptoxanthin, β-carotene, neoxanthin and L.[83] The Z and L content among dried goji berries ranges from 25 to 152 mg/100g, and 0.3 to 1.9 mg/100g, respectively, based on different cultivars.[85] Additionally, the predominant form of Z in goji berries is a dipalmitate (Figure 2), connecting with a diester linkage, which has

shown a significantly higher intestinal absorption than monoester and free Z due to the high efficacy of hydrolysis, mainly by carboxyl ester lipase.[86]

According to the National Health and Nutrition Examination Survey, the average L and Z intake among adults in the United States was 1.58 – 1.76 mg/d. Therefore, to increase dietary intake of these two xanthophylls, goji berry is an excellent source, which can complement other food sources such as green leafy vegetables, egg yolk, yellow corn products, and orange bell peppers.[87]

Recent clinical research on goji berry shows promising effects in protecting against AMD. Healthy older individuals who consumed 10 mg of Z extract from goji berries daily for 90 days showed no change in macular pigmentation or soft drusen, and significantly higher circulating Z levels, compared to a control group, which presented an increase in soft drusen.[88] In an uncontrolled trial, individuals with early stage AMD who consume a beverage daily for five months that contained 12 mg of L and 2 mg of Z derived from marigold flower and goji berry, respectively, showed lower interocular pressures, better best-corrected visual acuity (BCVA) scores and higher circulating levels of L and Z compared to their baseline levels.[89] Unfortunately, the study lacked a control group, did not test the effect of Z separately, and did not clarify whether the form of Z extracted from goji berry was the dipalmitate. Another study investigating the effects of an herbal formula among healthy adults with dry eyes noted that those chewing tablets containing L (6, 10, or 14 mg), Z (1.2, 2.2, or 2.8 mg), combined with extracts from blackcurrant, chrysanthemum, and goji berry showed dose-dependent reductions in eye fatigue symptoms and tear secretion, as well as improved macular pigment optical density (MPOD), a common non-invasive method to measure the total L and Z in the center of the macula, compared to those in a placebo group.[90] The basis of this formula was derived from

TCM, so the multicomponent formulation could not directly assess the role of any single ingredient. Another study in patients with early AMD reported that the MPOD was significantly higher in those consuming 25 g/day of goji berries (containing approximately 15 mg of Z and 2.5 mg of L) for 90 days, compared to their baseline levels and to a habitual diet control group. The BCVA was also significantly improved in the goji berry group compared to their baseline values.[91] We recently reported that MPOD and skin carotenoid scores were significantly increased in healthy middle-aged individuals consuming 28 g/day of goji berries (containing approximately 28.8 mg of Z and an estimated 0.15 mg of L) five times a week for 90 days, compared to a group taking a dietary supplement with 6 mg of L and 4 mg of Z.[92] These results illustrate that MPOD levels can increase in healthy individuals even without early signs of AMD. While these results are encouraging, longer intervention periods with a larger number of participants would be helpful to replicate and extend these initial observations.

In addition to AMD, goji berries have also been studied as a therapy for retinitis pigmentosa, an inherited retinal disease. Patients who consume 0.35 g/d of Lycium barbarum polysaccharides (LBP) for 12 months showed a significant improvement in visual acuity and macular thickness, compared to control subjects who did not consume L or Z.[93]

Other bioactive compounds found in goji berries include flavonoids, vitamins, minerals, betaine, cerebrosides, phenolic acids, and certain amino acids which may also support the overall health of the eye, particularly when working synergistically.[28,29,94] Based on preclinical evidence, potential benefits of goji berry intake on glaucoma and diabetic retinopathy have also been suggested.[28,95] Goji berry extract ameliorated the high glucose-induced blood-retinal barrier disruption in human retinal pigment epithelial cells.[96] Studies reported that LBP showed significant neuroprotective effects on retinal ganglion cells in male C57BL/6N mice and

Sprague-Dawley rats with ocular hypertension.[97-99] In db/db mice with thin retina, goji berry extract restored the thickness of the retina, the ganglion cell number, and the integrity of retinal pigment epithelium after daily intake for eight weeks.[100]
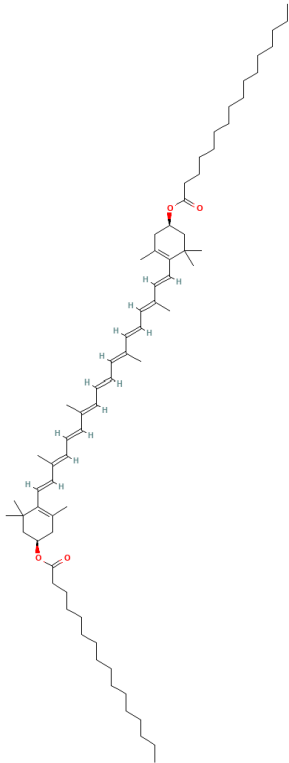
Figure 2. Chemical structure of zeaxanthin dipalmitate – the main zeaxanthin form exists in goji berries.[71]

Objective

Traditional uses of food in general, and fruits specifically, provide useful guides for modern research in both nutrition and pharmaceuticals. This literature review illustrates the evidence and certain proposed mechanisms of select fruits with a high phytonutrient profile on cardiovascular and eye health. The consumption of these fruits and bioactive compounds derived from them demonstrate great potential to protect against certain age-related diseases. Therefore, the

objectives of this dissertation are i) to investigate the effects of mango consumption on markers of cardiovascular diseases, ii) to examine the effects of goji berry consumption on macular pigment accumulation in human eyes, and iii) to review evidence for L, Z, and goji berries on eye health throughout the lifespan, with an emphasis on clinical studies.

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Chapter II

Goji Berry Intake Increases Macular Pigment Optical Density in Healthy Adults: A Randomized Pilot Trial

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Abstract

Age-related macular degeneration (AMD) is the third leading cause of blindness worldwide. Macular pigment optical density (MPOD), a biomarker for AMD, is a non-invasive measure to assess risk. The macula xanthophyll pigments lutein (L) and zeaxanthin (Z) protect against blue light and provide oxidant defense, which can be indexed by MPOD. This study examined the effects of Z-rich goji berry intake on MPOD and skin carotenoids in healthy individuals. A randomized, unmasked, parallel-arm study was conducted with 27 participants, aged 45–65, who consumed either 28 g of goji berries or a supplement containing 6 mg L and 4 mg Z (LZ), five times weekly for 90 days. After 90 days, MPOD was significantly increased in the goji berry group at 0.25 and 1.75 retinal eccentricities ($p = 0.029$ and $p = 0.044$, respectively), while no changes were noted in the LZ group. Skin carotenoids were significantly increased in the goji berry group at day 45 ($p = 0.025$) and day 90 ($p = 0.006$), but not in the LZ group. Regular intake of goji berries in a healthy middle-aged population increases MPOD may help prevent or delay the development of AMD.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness among seniors in developed countries, and third worldwide after uncorrected refractive errors and cataracts [1,2]. In early stages, the disease is characterized by small to intermediate drusen with pigmentary changes that may progress rapidly to more advanced forms such as choroidal neovascularization or central geographic atrophy with loss of central vision [3]. Lutein (L), zeaxanthin (Z), and the isomer meso-zeaxanthin (meso-Z) are macular pigments that filter damaging blue light and provide oxidative defense in the macula. These pigments are found in plants as xanthophylls, with increased dietary intake proposed to reduce the development and progression of AMD [4]. The relative concentration of xanthophyll carotenoids in the retina can be measured non-invasively by psychophysical and objective methods, expressed as macular pigment optical density (MPOD) [5]. Numerous epidemiological studies report that individuals with a low MPOD level are at an increased risk of AMD [6].

Dietary L and Z are found in certain fruits and vegetables with red, yellow, or orange color, egg yolk, and in some green leafy vegetables [7,8]. The dietary intake of Z is lower than L in all age groups and ethnicities in the U.S. [9]. Dietary intakes of L and Z are strongly associated with their serum levels, as well as with MPOD [10]. Previous studies have shown that high intakes of these carotenoids from dietary sources or supplements can increase plasma L and Z, and MPOD [11]. Once early AMD has progressed to the intermediate stage, dietary supplements are indicated, but no clinical evidence yet exists for interventions that can address the prevention of small-intermediate drusen with pigmentary changes, the initial clinical signs of macular disruption [12].

Goji berry (*Lycium barbarum* L. and *L. chinense*), also termed wolfberry or Go Chi Zi, has been used in traditional Chinese medicine for more than 2000 years [13]. The bright red berry contains the highest amount of Z among all known dietary sources and is mainly present in a dipalmitate form [14,15]. The intake of zeaxanthin dipalmitate (ZD) extracts from goji berry increases plasma Z to a greater extent than non-esterified Z supplementation [16]. The berries also contain unique carbohydrates that are present as conjugates with peptides or proteins, which are often referred to *L. barbarum* polysaccharides *(LBP)*. These have shown anti-inflammatory and neuroprotective effects in animal and cell culture studies [17].

The typical adult human eye has approximately 2.4 times more Z than L in the central fovea of the macula [18], making goji berry intake a prime candidate for increasing MPOD. Nevertheless, there is a paucity of clinical evidence on goji berry and MPOD particularly for the prevention or delay of progression from early to intermediate AMD. In individuals from China with signs of early AMD, 25 g of daily consumption of goji berries for 90 days significantly increased both serum Z and MPOD [19]. However, this study had a broad age range (51 to 92 years of age), some participants smoked, and others had certain pre-existing medical conditions. Additionally, the authors only reported central MPOD values up to 0.5 retinal eccentricity (RE), whereas macular pathology and visual dysfunction in AMD may extend beyond that central region. Therefore, to provide a more complete understanding of the influence of goji berry intake on the progression AMD, data is needed on for different population groups that measures MPOD at eccentricities over the entirety of the macula.

In the current study, we prospectively evaluated if the daily intake of 28 g of goji berries or a commercially available supplement providing 6 mg of L and 4 mg for 90 days can improve

MPOD and skin carotenoid levels, an index of total carotenoid intake, among healthy middle-aged adults, 45 to 65 years old, with no signs of drusen or early AMD.

Materials and Methods

2.1. Participants

Eighty-eight volunteers, ages from 45 to 65 years old, were recruited from an online website and public advertisements in the area of greater Sacramento, California. Participants provided informed consent and were screened with a questionnaire. Inclusion criteria were being generally healthy (not currently under medical supervision, free from self-reported diabetes, cancer, heart, kidney or liver diseases and gastrointestinal disorders), having a normal macular condition as verified by an optometrist, and if relevant, being prescribed the same medication regimen for at least 6 months that was not related to carotenoid metabolism and was approved by the study physician. Exclusion criteria were a dislike of, or allergy to goji berries, diseases of the eye, malabsorption problems, substance or alcohol abuse, smoking, drugs for management of lipids, glucose, or blood pressure, use of dietary supplements other than multivitamins and minerals that provided greater than 100% of the U.S. Dietary Reference Intake, or any supplement containing L or Z. The intervention was registered on ClinicalTrials.gov (NCT03983525) (accessed on July 21, 2020), with the first posted date of 6 December 2019, complied with the tenets of the Declaration of Helsinki, was approved by the Institutional Review Board of the University of California (UC), Davis (IRB #1220178) and was conducted at the UC Davis Ragle Human Nutrition Research Center.

2.2. Study Design

Qualified participants were randomized into a prospective, parallel-arm, unmasked study to consume either 28 g of goji berries or a commercially available supplement of L and Z five days per week for 90 days. Study measurements were collected at baseline (prior to supplement or goji berry intake; day 0), at 45 ± 2 days and 90 ± 2 days after intake.

Twenty-eight grams of goji berries is considered a single serving size [20]. The berries in this study were USDA-certified organic goji berries grown in the Ningxia region of northern China and provided by Navitas Organics, Novato CA, USA. The goji berries were portioned into clean, single-serving plastic bags and provided in 45-day allotments. The commercially available supplements (Source Naturals, Scotts Valley, CA, USA, lot #FG-91753) were purchased online, contained 6 mg of L and 4 mg of Z per serving and were repackaged into 45-day supplies in clean plastic bottles. Compliance was monitored by a self-administered log. Habitual dietary information was collected with the Automated Self-Administered 24 h dietary assessment web-based tool (ASA24; https://epi.grants.cancer.gov/asa24, accessed on August 10, 2020) once between day 0 and 45, and once again between day 45 and 90.

The MPOD was assessed by the psychophysical method of customized heterochromatic flicker photometry using a macular densitometer (Macular Metrics, Providence, RI, USA). After participants viewed a 5-minute video detailing the measurement procedures, they were dark-adapted for 7 minutes and then began the test. The light intensity of each relevant wavelength was calibrated with a photodiode. The flicker frequency was selected based on a preliminary test of the participant's sensitivity. The task was to eliminate or minimize the flicker in the visual field three times by turning a dial that changed the intensity of a 460 nm light. Each participant performed the test while looking directly at the flickering light at 0.25, 0.5, 1, and 1.75 RE degrees, representing the MPOD level from the center to the periphery of the macula.

Skin carotenoid content was measured by reflection spectroscopy ("Veggie Meter", Longevity Link Corporation, Salt Lake City, UT, USA). After cleaning, the tip of the right index finger was inserted into the spectrophotometer and three measurements were collected. A skin carotenoid score was calculated by the system software. Carotenoids that exist in human plasma, including β-carotene, lycopene, L, Z, and their isomers have been successfully detected in toto and quantified by this device [21,22], which has been validated to reflect fruit and vegetable consumption [23].

2.3. Statistical Analysis

Sample size was based on a study that assessed the impact of a Z supplement on MPOD in 24 healthy people [24]. Statistical analyses were performed with JMP version 16 (SAS Institute Inc., Cary, NC, USA). Two-tailed *t*-tests evaluated potential between-group differences at baseline. The MPOD and skin carotenoid data were analyzed with mixed-effects models using time and treatment as the main factors, with age and sex as the covariates, and participant ID as the random effect. For main effects, student *t*-tests determined significance within group pairs. *p*-Values of 0.05 or less were considered statistically significant. Correlation coefficients between the outcome measures were determined via Spearman's method. The mean values of the dietary intake data were compared by two-tailed *t*-tests, which were log-transformed when necessary, and presented as the mean ± S.E.M. or the back-transformed mean with 95% confidence intervals (CI).

Results

Thirty-one healthy, middle-aged adult males and females (mean age of 56 years) met the inclusion criteria between May 2019 and Jan 2020. The participants consumed either goji berries

(n = 16) or the LZ supplement (n = 15) 5 days per week for 90 days. Twenty-eight individuals

completed the intervention, after which two in the goji berry and one from the LZ group were

excluded from the data analysis due to measurement errors. Furthermore, data from one was

subsequently removed after learning of a major change in dietary patterns that included a low

intake of macronutrients between days 45 and 90 (Figure 1).
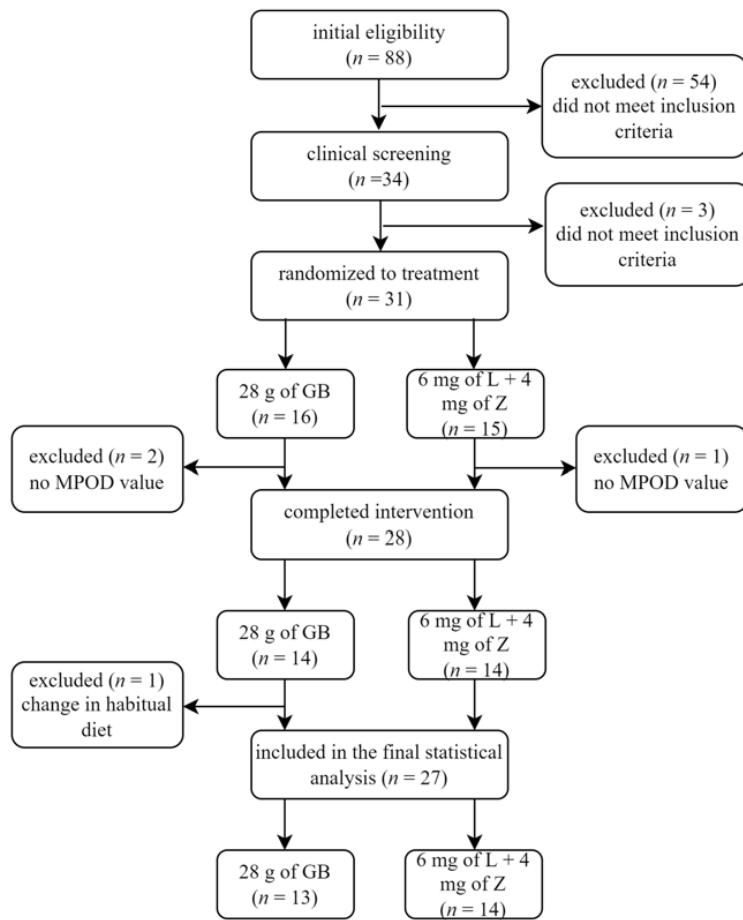
Figure 1. Participant flow diagram. Thirty-one participants were randomly assigned to consume either 28 g of goji berries (GB) or a supplement containing 6 mg of lutein (L) and 4 mg of zeaxanthin (Z), five times per week for 90 days. Twenty-eight individuals completed the study. An $n = 13$ in the GB group and an $n = 14$ in the LZ group were used in the statistical analysis.

Reported protocol compliance was greater than 96% for both groups, and no adverse symptoms

were noted other than minor intestinal gas from one participant in the goji berry group. Table 1

presents the reported average intake of select nutrients in the habitual diet that may have affected eye health over the study period. No significant differences between groups were noted. The composition of the goji berries is presented in Table 2. A daily goji berry serving provided 28.8 mg of Z, which was substantially higher than the 4 mg of Z present in the supplement. Although sufficient extraction of L from our goji berry samples could not be obtained, previous work by others estimated a L content of 0.15 mg in 28 g of goji berries from six different goji berry samples collected in the Ningxia province of China, the same region from which the goji berries used in this study were obtained [25].

Table 1. Mean intake of select dietary nutrients, apart from intake of goji berries (GB) or lutein and zeaxanthin (LZ) supplementation, collected once between day 0 and day 45, and again between day 45 and day 90.

	GB	LZ	*p*-Value
Energy (kcal)	2146.4 ± 187.7	1984.3 ± 151.5	0.51
Protein (g)	89.3 ± 10.1	72.7 ± 7.2	0.18
Total Fat (g)	84.2 ± 9.3	84.4 ± 8.8	0.98
Carbohydrate (g)	256 ± 26	241 ± 17	0.6
Vitamin A (mcg)	807.8 ± 120.6	578.3 ± 58.4	0.07
Vitamin C (mg)	120.0 ± 18	103.9 ± 13.8	0.48
Vitamin E (mg) [1]	14.4 (8.6, 24.1)	11.0 (9.0, 13.3)	0.21
Zinc (mg)	11.8 ± 0.8	9.5 ± 0.9	0.08
Retinol (mcg)	307.1 ± 51.5	265.4 ± 40.5	0.52
β-carotene (mcg)	5127.6 ± 874.0	3408.1 ± 680.4	0.13
α-carotene (mcg) [1]	300.3 (81.3, 1109.0)	205.9 (87.7, 483.4)	0.58
β-cryptoxanthin (mcg) [1]	156.9 (33.6, 732.2)	91.0 (53.1, 156.0)	0.4
Lycopene (mg) [1]	7.2 (3.1, 15.0)	3.6 (1.7, 7.3)	0.2
Lutein + zeaxanthin (mg) [1]	3.1 (1.7, 5.5)	1.9 (1.1, 3.2)	0.2
DHA (g) [1]	44.9 (12.9, 156.4)	37.0 (15.8, 86.5)	0.77
DPA (g) [1]	16.2 (7.1, 36.6)	9.3 (5.1, 16.9)	0.23
EPA (g) [1]	11.8 (2.5, 56.5)	13.5 (6.0, 30.2)	0.86

Statistical analysis performed by two-tailed *t* test; values are the mean ± S.E.M. or back transformed mean[1] (95% CI) of the log data obtained from ASA24. DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; EPA: eicosapentaenoic acid.

Baseline MPOD measures were similar between the goji berry and supplement groups (Table 3). No significant interaction effects for treatment and time were observed in any REs. A significant main effect of time was found for MPOD at 0.25 RE (*p* = 0.023). In a sub-analysis, intake of goji

berries, but not LZ, significantly increased MPOD at 0.25 RE at day 90 compared to baseline (p = 0.029; Figure 2a). There was also a significant main effect of time for MPOD at 1.75 RE (p = 0.039), with a significant increase at day 45 compared to the baseline (p = 0.021), and again between day 90 (p = 0.044; Figure 2b). No significant MPOD changes were noted at any REs in the LZ group.

Table 2. Composition of select nutrients and carotenoids in 28 g of goji berries. * Lutein estimated from goji berries cultivated in Ningxia province, China (Zhao et al., 2013).

Nutrient	Amount
Calorie (Kcal)	95.1
Total Carbohydrate (g)	21.4
Fat (g)	0.4
Protein (g)	2.8
Fiber (g)	2.7
Total sugars (g)	15.1
Carotenoids	
Zeaxanthin (mg)	28.8
β-carotene (μg)	225
Trans β-carotene (μg)	110
α-carotene (μg)	13.8
Lycopene (μg)	<5.6
Lutein estimate* (mg)	0.15

Baseline skin carotenoid scores were not significantly different between the goji berry and LZ groups (Table 3). No significant interaction effects for treatment and time were observed. However, the main effect of time was significant (p = 0.011). This corresponded to a significant increase from baseline after 45 and 90 days in the goji berry group (p = 0.025 and 0.006, respectively), while no significant changes were noted in the LZ group (Figure 3). The absolute values of MPOD and skin carotenoid scores are shown in the supplementary Table S1.

Overall, skin carotenoid scores were significantly correlated with MPOD at 0.25 (ρ = 0.33, p = 0.004), 0.5 (ρ = 0.41, p = 0.0002), and 1 RE (ρ = 0.38, p = 0.0007; supplementary Figure S1.1). The skin carotenoid score was not correlated with MPOD at any of the REs for the goji berry

group (supplementary Figure S1.2). In contrast, for the LZ group, the skin carotenoid score was significantly correlated with MPOD at 0.25, 0.5, and 1 RE (0.25 RE: $\rho = 0.55$, $p = 0.0003$; 0.5 RE: $\rho = 0.57$, $p = 0.0002$; 1 RE: $\rho = 0.54$, $p = 0.0004$), with a trend at 1.75 RE ($\rho = 0.31$, $p = 0.06$; supplementary Figure S1.3).

Table 3. Baseline measurements of participants in the goji berry (GB) and the lutein and zeaxanthin supplement (LZ) group.

	GB Group (n = 13)	LZ Group (n = 14)	*p*-Value
Age (years)	55.9 ± 1.7	55.8 ± 1.4	0.94
Sex (F), n (%)	9 (69.2)	10 (71.4)	-
MPOD			
0.25 RE	0.67 ± 0.06	0.68 ± 0.06	0.88
0.5 RE	0.54 ± 0.07	0.58 ± 0.05	0.51
1 RE	0.36 ± 0.03	0.39 ± 0.03	0.32
1.75 RE	0.16 ± 0.02	0.16 ± 0.02	0.77
SC Score	369.5 ± 44.9	397.8 ± 39.6	0.64

Variables were not significantly different between the two groups. Statistical analysis was performed by two-tailed *t*-tests; data are presented as mean ± S.E.M. MPOD: macular pigment optical density; RE: retinal eccentricity degrees; SC: skin carotenoid.
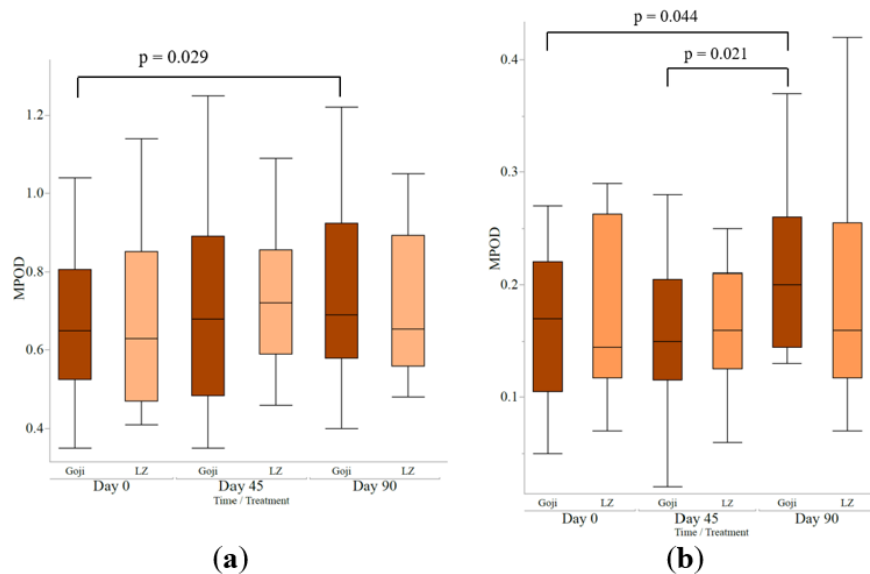
Figure 2. (**a**) Three months of goji berry intake increased macular pigment optical density (MPOD) at 0.25 retinal eccentricity (RE) degrees, at Day 90 compared to baseline (Day 0) and at day 45. (**b**) Three months of goji berry intake increased macular pigment optical density (MPOD) at 1.75 retinal eccentricity (RE) degrees, at Day 90 compared to baseline and at day 45. Statistical analysis performed by mixed models using time and treatment as the main factors, and age and sex as the covariates with Student's *t*-test for pairwise comparisons; boxplots are the median and interquartile range.
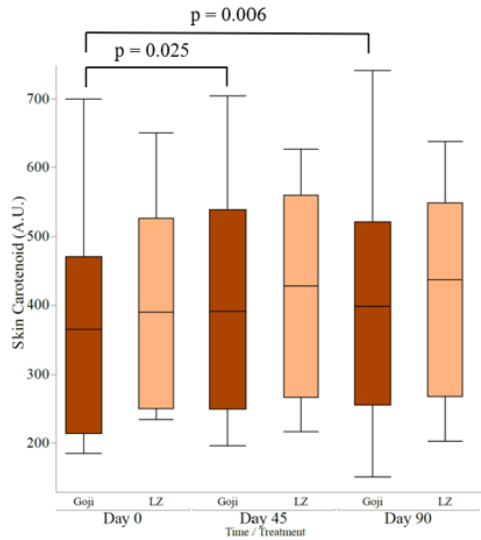
Figure 3. Three months of goji berry intake increased skin carotenoid score at Day 45 and Day 90 compared to Day 0. No changes in the lutein and zeaxanthin supplement (LZ) group were noted. Statistical analysis performed by mixed models using time and treatment as the main factors, and age and sex as the covariates with student *t*-test for pairwise comparisons; boxplots are the median and interquartile range.

Discussion

Ninety days of 28 g of goji berry intake significantly increased the optical biomarker MPOD in healthy adults at 0.25 and 1.75 REs. These results suggest that even in a healthy population with no evidence of small drusen or early AMD, goji berry intake can improve eye health. Our results are consistent with data of improved MPOD after a similar amount and intake period of goji berry in a Chinese population at risk for intermediate AMD [19]. Moreover, our trial is consistent with reports of protection against macular hypopigmentation and drusen development in a population of generally healthy and older (65 to 75 years of age) individuals who were provided Z at approximately a third of the amount of Z provided in the current trial (i.e., 10 mg/d of Z derived from goji berries) [26]. Our findings suggest that a higher intake of Z relative to L may be useful in reducing the risk of AMD. This is consistent with increased MPOD levels after 4 months of supplementation with 20 mg Z or 26 mg Z with 8 mg L plus 190 mg of mixed omega-3 fatty acids by young healthy adults [27]. Interestingly, we observed a significant

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increase in MPOD at 1.75 RE, but not at 0.5 or 1 RE, in the goji berry group. A possible explanation for this trend is the relatively low macular pigment at 1.75 RE compared to the other REs, which may increase the potential for improved MPOD in this peripheral area of the macula. Our results are also consistent with data from 11 randomized controlled trials where supplementation with at least 10 mg of the macular carotenoids was effective at increasing MPOD [28].

Significant correlations were observed between the overall skin carotenoid score and MPOD, which is consistent with clinical results of carotenoid supplementation [29]. Further analysis demonstrated that L and Z, but not goji berry intake, was significantly influencing this trend. Previous work has shown an association between serum L and Z in skin and blood with macular pigment carotenoid accumulation [29]. Data from the current trial are consistent with this observation as goji berry intake was significantly associated with the skin carotenoid score. However, in contrast to data with L and Z supplements, MPOD score was not correlated with changes in skin carotenoids with goji berry intake. The skin photometer detects overall carotenoid content, and as goji berries are also rich in β-carotene, neoxanthin, and cryptoxanthin [30], these carotenoids likely influenced the skin measurements, and would not reflect the selective carotenoid accumulation of L and Z in the macula. Other goji berry components such as taurine, vitamin C, zinc, and LBP may influence the results by lowering oxidant stress and improving eye health [31–33]. For example, studies in animals and cell lines suggest that LBP can protect against AMD by reducing oxidative stress and cell apoptosis in retinal pigment epithelium [34]. Taken together, under the conditions tested, it is reasonable that MPOD may not fully correlate with skin carotenoids in the goji berry group.

To our knowledge, the impact of goji berry intake on MPOD in healthy middle-aged people has not been previously reported. While others have noted improved MPOD after LZ supplementation among people with low MPOD baseline levels [35], our findings suggest that even in populations with normal MPOD values, a significant increase can be detected after goji berry consumption at the most central part of the macula (0.25 RE). A meta-analysis regarding the effects of L, Z, and meso-Z supplementation noted that the MPOD at baseline was inversely associated with macular responses, suggesting individuals with a relatively lower macular pigment status may receive more benefit with higher amounts of L or Z [36].

The Age-Related Eye Disease Study 2 (AREDS2) trial assessed the impact of dietary supplements containing 10 mg of L, 2 mg of Z, 500 mg of vitamin C, 400 IU of vitamin E, 80 or 25 mg of zinc, 2 mg of copper, and/or 350 mg of docosahexaenoic acid plus 650 mg of eicosapentaenoic acid [37]. The results showed a significantly reduced rate of progression from intermediate- to late-stage AMD after 5 years [38,39]. Secondary analyses of the study indicated protective roles of L and Z [38]. We did not use the AREDS2 supplement for the comparison group because this formula has only been shown to be effective for those with intermediate AMD [39], and no clinical evidence exists for its efficacy in our study population of healthy people. In addition, we note that 80 mg of zinc in the AREDS2 supplement is twice the upper limit of recommended daily intakes for zinc [40].

In epidemiological studies, L and Z intakes have been inversely associated with the development of AMD [37,41]. In the current study, the reported dietary intake of L plus Z, not including the berries or supplement, was 3.1 and 1.9 mg/d in the goji berry and supplement groups, respectively, which is higher than the typical estimated intakes in the US of 1.6–1.86 mg/d [42].

Three to five mg/d of L and Z have been recommended to help support normal macular function, although no recommended dietary allowance values yet exist [8].

A few studies have explored the effects of L and Z from a whole food on MPOD. Daily consumption of one Hass avocado containing 0.5 mg of L over 6 months was associated with a significant increase in MPOD in healthy adults [43]. In contrast, no increase in MPOD was observed after consuming one Hass avocado daily for 3 months [44]. Daily consumption of egg yolks providing 1.38 mg L and 0.21 mg Z resulted in a significant increase in MPOD and other measures of visual acuity in older adults with signs of early-stage AMD after 12 months [45]. Another study giving older adults two egg yolks/day for 5 weeks, followed by four egg yolks/day for 5 weeks, reported increases in MPOD, but only among those with low baseline MPOD values [46]. The addition of either spinach (10 mg L, 0.3 mg Z) or corn (0.4 mg L, 0.3 mg Z), or the combination, for 14 months significantly increased the MPOD among the majority of healthy individuals [47].

Our study has some limitations. Choice of a control is always a challenge in whole food studies, since masking is an issue. A commercially available LZ supplement was used, rather than an inert capsule, since our research design was intended to compare options available to consumers and explore the role of goji berries over and above the intake of purified L and Z. The actual amount of L and Z in the supplement was not confirmed. A previous report noted that the carotenoid content of some powder-based supplements tested in 2017 did not meet label claims, while oil-based supplements did [48]. Since L and Z are preferentially deposited at different eccentricities in the retina, the different amounts of Z in the goji berries and supplement may not be ideal. Volunteers were not screened for low MPOD as an inclusion criterion. Although the relatively modest number of participants in each group may raise some concerns, these numbers

are similar to those reported by Obana et al. and are consistent with an initial probe study [49]. Finally, although MPOD was the primary outcome measure, other ocular measurements such as contrast sensitivity and best corrected visual acuity were not assessed. Future studies on goji berry intake and eye health ideally should combine functional and anatomic measurements.

Conclusions

In conclusion, this study shows that 90 days of goji berry consumption was associated with an increase in MPOD in healthy, middle-aged adults. In addition to L and Z, other bioactive compounds in goji berries may be involved in the increase in MPOD. Further research on goji berries is warranted as both a dietary strategy to reduce the risk of AMD and to serve as part of an integrative approach to mitigate the consequences of this disorder.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Table S1: Macular pigment optical density (MPOD) and skin carotenoid (SC) scores in the goji berry (GB) and lutein + zeaxanthin supplement (LZ) groups at Day 0 (SV1), Day 45 (SV2), and Day 90 (SV3). Values are the mean ± S.E.M. RE: retinal eccentricity. Figure S1.1: Positive correlations between skin carotenoid and MPOD at 0.25, 0.5, and 1 RE degrees in data from the goji berry GB and LZ groups combined. Scatter plots and 95% CI (blue shades) of the linear relationship between skin carotenoid and MPOD at 0.25 RE (a), 0.5 RE (b), 1 RE (c), and 1.75 RE (d). Figure S1.2: The skin carotenoid score and MPOD were not correlated in the GB group at any of the four RE degrees. Scatter plots and 95% CI (blue shades) of the linear relationship between skin carotenoid and MPOD at 0.25 RE (a), 0.5 RE (b), 1 RE (c), and 1.75 RE (d). Figure S1.3: Positive correlation between skin carotenoid and MPOD at 0.25, 0.5, and 1 RE degrees in the LZ group. Scatter plots and 95% CI (blue shades) of the linear relationship between skin carotenoid and MPOD at 0.25 RE(a), 0.5 RE (b), 1 RE (c), and 1.75 RE (d).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of University of California, Davis (IRB #1220178, approved from 4 May 2018).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement:

Conflicts of Interest: The authors declare no conflict of interest.

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Supplementary Materials

Supplementary Table S1. Macular pigment optical density (MPOD) and skin carotenoid (SC) scores in the goji berry (GB) and lutein + zeaxanthin supplement (LZ) groups at Day 0 (SV1), Day 45 (SV2), and Day 90 (SV3). Values are the mean ± S.E.M. RE: retinal eccentricity.

		Day 0	Day 45	Day 90	p within treatment Day 0 vs. Day 45	Day 45 vs. Day 90	Day 0 vs. Day 90	p overall
MPOD								
0.25 RE								
	GB	0.67 ± 0.06	0.74 ± 0.06	0.76 ± 0.06	0.16	0.42	**0.029**	0.93
	LZ	0.68 ± 0.06	0.74 ± 0.06	0.74 ± 0.06	0.11	0.88	0.14	
0.5 RE								
	GB	0.54 ± 0.07	0.55 ± 0.05	0.58 ± 0.05	0.65	0.3	0.14	0.65
	LZ	0.59 ± 0.05	0.59 ± 0.05	0.60 ± 0.05	0.83	0.71	0.54	
1 RE								
	GB	0.36 ± 0.03	0.39 ± 0.03	0.40 ± 0.03	0.2	0.51	0.06	0.74
	LZ	0.40 ± 0.03	0.41 ± 0.03	0.39 ± 0.03	0.36	0.37	0.97	
1.75 RE								
	GB	0.16 ± 0.02	0.15 ± 0.03	0.21 ± 0.03	0.75	**0.044**	**0.021**	0.99
	LZ	0.17 ± 0.02	0.17 ± 0.02	0.19 ± 0.03	0.86	0.39	0.29	
SC								
	GB	369.5 ± 44.9	421.4 ± 44.7	431.4 ± 44.7	**0.025**	0.56	**0.006**	0.73
	LZ	397.8 ± 39.6	442.2 ± 43.9	435.6 ± 43.8	0.17	0.7	0.3	
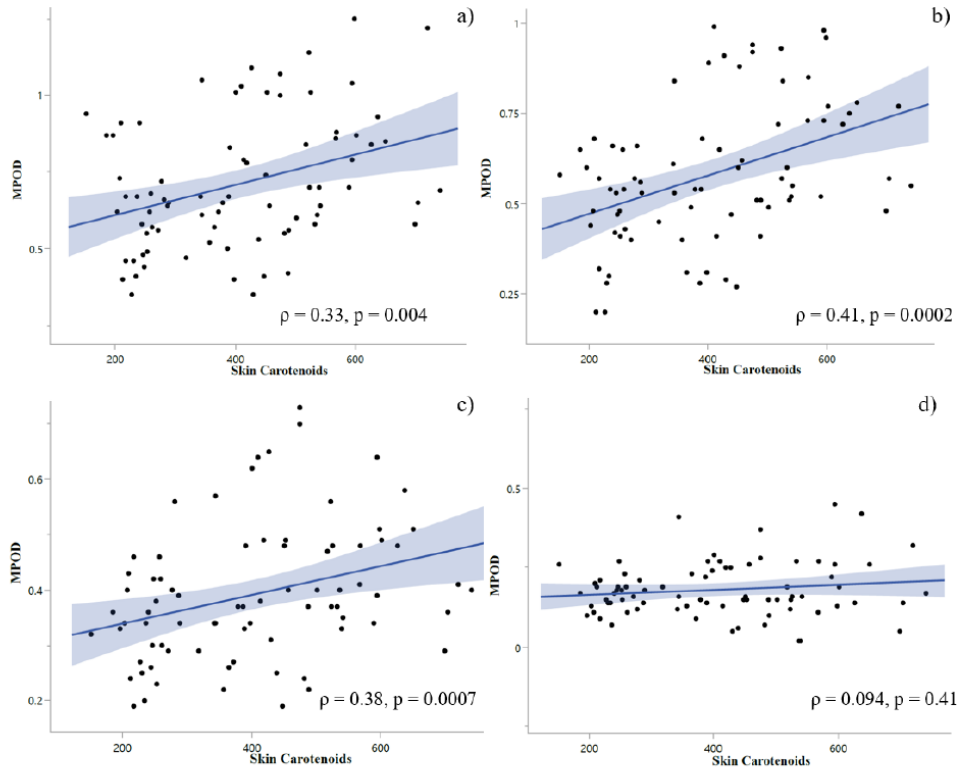
Supplementary Figure S1.1. Positive correlations between skin carotenoid and macular pigment optical density (MPOD) at 0.25, 0.5, and 1 retinal eccentricity (RE) degrees in data from the goji berry (GB) and lutein and zeaxanthin supplement (LZ) groups combined. Scatter plots and 95% CI (blue shades) of the linear relationship between skin carotenoid and MPOD at 0.25 RE (a), 0.5 RE (b), 1 RE (c), and 1.75 RE (d).
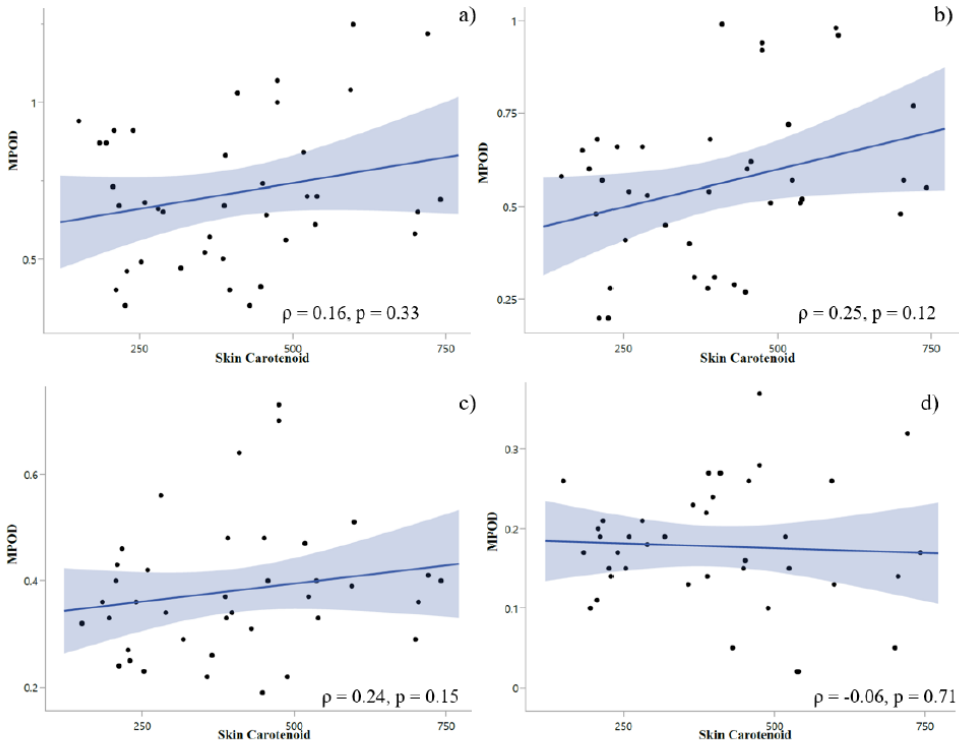
Supplementary Figure S1.2. The skin carotenoid score and macular pigment optical density (MPOD) were not correlated in the goji berry (GB) group at any of the four retinal eccentricity (RE) degrees. Scatter plots and 95% CI (blue shades) of the linear relationship between skin carotenoid and MPOD at 0.25 RE (a), 0.5 RE (b), 1 RE (c), and 1.75 RE (d).
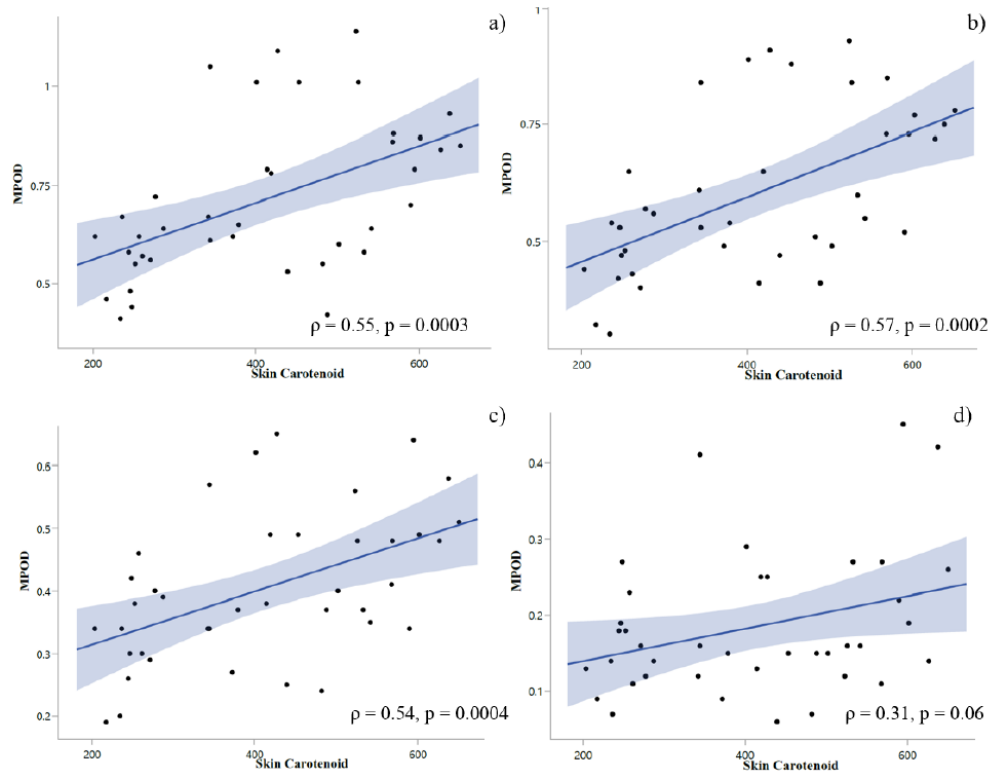
Supplementary Figure S1.3. Positive correlation between skin carotenoid and macular pigment optical density (MPOD) at 0.25, 0.5, and 1 retinal eccentricity (RE) degrees in the lutein and zeaxanthin supplement (LZ) group. Scatter plots and 95% CI (blue shades) of the linear relationship between skin carotenoid and MPOD at 0.25 RE (a), 0.5 RE (b), 1 RE (c), and 1.75 RE (d).

Effects of two weeks of mango intake on vascular function and blood pressure in

postmenopausal women

Introduction

Cardiovascular diseases (CVD) have been the leading cause of death globally, with mortality estimated to be 17.9 million people in 2019, accounting for 32% of all deaths.[1] Common modifiable risk factors for CVD include hypertension, hyperlipidemia, diabetes, harmful alcohol use, sedentary lifestyle, smoking, and male sex.[2,3] In women, menopause is a risk factor for CVD due to the loss of the cardioprotective effects of estrogen and an increased likelihood of developing endothelial dysfunction, dyslipidemia, glucose resistance, and vascular inflammation.[4] Endothelial dysfunction may also lead to an increased risk of cardiovascular events due to impaired control of vascular tone.[5]

Abundant consumption of fruits and vegetables is known to reduce the risk of CVD.[6,7] Fruits and vegetables contain a variety of nutrients and bioactive compounds, many of which have vasculo-protective effects. Insights from traditional diets offer clues regarding which plant foods might confer vascular health benefits. For example, Kuna Indians living on their native islands off of the coast of Panama consuming a traditional diet rich in cocoa, fish, and fruits such as mangos (*Mangifera indica*) showed virtually no CVD or hypertension. However, when they migrated to an urban environment and ate a diet rich in processed foods and fats, and low in their traditional fruits and vegetables, the incidence of vascular diseases increased significantly.[8] While these

trends helped to identify cocoa as a potentially vasculoprotective food, other foods with traditional use history for heart health, such as mango have been less studied.

Mangos are abundant in β-carotene, and vitamins C and E,[9] the intake of which are associated with reduced risk for CVD.[10,11] Mango also contains numerous phenolic acids and polyphenols.[12,13] Mangiferin, a unique xanthone polyphenol found in mango pulp, bark, and leaves has been shown to have bioactive properties such as anti-inflammatory and anti-diabetic effects.[14] In hyperuricemic rats, mangiferin intake significantly reduced systolic blood pressure (SBP), serum uric acid and inflammatory markers, and increased the expression of endothelial nitric oxide synthase (eNOS).[15] In overweight individuals, mangiferin intake was associated with a reduction in serum triglycerides, free fatty acids, and the insulin resistance index.[16] Moreover, insulin sensitivity was improved after mango fruit powder supplementation compared to a high-fat control group in male C57BL/6 mice with early signs of metabolic syndrome.[17] In overweight and obese individuals, the postprandial glucose and insulin levels after 100 kcal of fresh mango intake were significantly lower than the consumption of isocaloric low-fat cookies.[18] These outcomes suggest that mango intake may have vasodilation and glucose control effects in humans.

The vasodilation function of the vascular epithelium in response to reactive hyperemia is a useful measure of vascular function, and is measured non-invasively using flow mediated dilation (FMD) or peripheral arterial tonometry (PAT).[19] Reactive hyperemia is the shear stress induced from an increased blood flow caused by the release of occlusion (ischemic) of an artery.[20] Using PAT, the changes in digital arterial pulse volume are assessed at baseline and after ischemia through digital probes placed on the fingertips, with the contralateral (non-ischemic) arm serving as the control. Although both methods may predict CVD events, a reduced reactive hyperemia

value measured by FMD was associated with higher body mass index (BMI), age, and SBP, while PAT was associated with higher BMI, hypercholesterolemia, and diabetes.[21,22]

The aim of the current study was to investigate the effects of two weeks of daily mango consumption on changes in microvascular function, platelet aggregation, blood pressure, blood lipids, and blood glucose in postmenopausal women. Based on the results for the two-week study, a follow-up probe was conducted to assess the acute (2 hour) effects of mango intake on blood pressure, blood glucose and insulin.

Materials and Methods

<u>Participants</u>

Postmenopausal women aged 50 to 70 years with BMI of $25 - 40$ kg/m^2 were enrolled. Postmenopausal status was defined as a lack of menses for at least two years or at least six months with a follicle-stimulating hormone (FSH) level of $23 - 116.3$ mIU/mL. Other inclusion criteria were an overall body weight equal to or greater than 100 pounds, and agreement to comply with all study procedures. Exclusion criteria included BMI greater than 40 kg/m^2, blood pressure greater than or equal to 140/90 mm Hg, abnormal values from a lipid panel, complete blood count (CBC), or comprehensive metabolic panel (CMP), use of prescription medications other than thyroid, daily use of anticoagulation agents such as aspirin and nonsteroidal anti-inflammatory drugs, or use of dietary supplements other than a general formula of multivitamins/minerals that provided up to 100% of the recommended dietary allowances. Additional exclusion criteria were vegetable consumption greater than or equal to 3 cups/day, fruit consumption greater than or equal to 2 cups/day, fatty fish intake greater than or equal to 3 times/week, dark chocolate intake greater than or equal to 3 oz/day, coffee and/or tea intake

greater than or equal to 3 cups/day, alcohol intake greater than 3 drinks/week. Women were also excluded if they followed a non-traditional diet (e.g., vegan, vegetarian), engaged in routine high-intensity exercise, self-reported diabetes, renal or liver disease, malabsorption or gastrointestinal diseases, cancer within the last five years, or heart disease, including cardiovascular events or stroke.

After determining initial eligibility through telephone screening, participants were further screened at the laboratory in the morning after an overnight fast. After informed consent was obtained, anthropometric measurements were taken, including body weight, height, and waist circumference. Blood pressure and resting heart rate were measured three times, five minutes apart, after 15 minutes of sitting quietly. Volunteers also completed a diet and health habits questionnaire (HHQ). A fasting blood sample was collected for a CMP, a CBC, and a lipid panel (LP). If participants reported menses occurring within two years prior to the telephone screening, FSH was measured. Volunteers were excluded if their low-density lipoprotein (LDL) value was greater than or equal to 190 mg/dL, or for those with zero to one major cardiovascular risk factors apart from high LDL cholesterol if their LDL was greater than or equal to 160 mg/dL, for those with two major cardiovascular risk factors apart from elevated LDL cholesterol greater than or equal to 130 mg/dL, or for those with two major cardiovascular risk factors apart from high LDL cholesterol and a Framingham 10-year risk score of 10 to 20% (calculated using the National Cholesterol Education Program calculator at http://cvdrisk.nhlbi.nih.gov/calculator.asp).

Study designs

Study I was a single-arm, four-week trial (Figure 1). Baseline values were collected at study visit 1 (SV1), which then began a run-in period of two weeks during which no mangos were

consumed. At SV1, baseline (0h) anthropometry, blood pressure, PAT, and blood was collected, and taken again two hours (2h) later. At the end of two weeks, study visit 2 (SV2) began with baseline measures taken, followed by ingestion of 330 gm (2 cups) of pre-packaged, fresh, frozen Ataulfo mangos, and data were collected two hours later. Participants then returned home with a 14-day supply of pre-packaged mangos and instructed to consume 330 gm of mangos daily, with 165 g eaten before noon, and the other half consumed in the evening. Two weeks later, study visit 3 (SV3) ensued, which followed the same protocol as SV2 (i.e., measurements at 0h and 2h). Water was allowed *ad libitum* during all study visits.

Prior to each study visit, participants were instructed to refrain from strenuous exercise for 24 hours before arriving at the laboratory to reduce the potential impact on PAT measurements. Two 3-day food records (two weekdays and one weekend) were collected, once between SV1 and SV2, and again between SV2 and SV3. The records were analyzed using the Food Processor software (Version 11.3.x; ESHA; Salem, OR). Compliance and potential adverse symptoms were monitored by daily self-reported logs.

Study II was based on the findings from study I. This single-armed trial design is shown in Figure 2. After an overnight fast, at SV1, anthropometry, blood pressure, heart rate, and blood samples were collected at baseline (0h) and at one-hour (1h) and two-hour (2h) time points. At least 48 hours later, at SV2, baseline measures were taken, followed by ingestion of 330 gm of pre-packaged, fresh, frozen Ataulfo mangos, and data were collected 1h and 2h after intake. After at least two days, at SV3, baseline measures were taken, followed by ingestion of 113 g of white bread, which contained calories and carbohydrates similar to those found in 330 gm of mangos, and data were collected 1h and 2h after ingestion.

The inclusion and exclusion criteria were the same for both study I and II. Participants were instructed to refrain from consuming additional mangos before SV1 and throughout their enrollment. Procedures were performed at the same time of the day to minimize circadian effects. The screening and interventions were conducted at the UC Davis Ragle Human Nutrition Research Center. The UC Davis Institutional Review Board approved the protocol, and the study was registered at ClinicalTrials.gov (NCT03203187).

<u>Assessment of anthropometry, blood pressure, vascular function, and platelet aggression</u>

Microvascular function was assessed by PAT (Endo-PAT 2000; Itamar Ltd., Caesara, Israel). After resting in a supine position for 30 minutes, a non-invasive, sterile finger probe was fitted to each middle finger. A manual blood pressure cuff was placed on the distal forearm of the non-dominant arm. A baseline reading of peripheral arterial tone was recorded, and then the blood pressure cuff was inflated to a supra-systolic level approximately 60 mmHg above systolic blood pressure to induce occlusion of blood flow for five minutes. Then, the pressure was released, resulting in reactive hyperemia. Two consecutive blood pressure measures were taken immediately before and after the PAT assessment. The PAT software then automatically calculated the reactive hyperemia index (RHI), Framingham Reactive Hyperemia Index (fRHI), augmentation index (AI), and AI adjusted to 75 beats per minute (AI75).

Whole blood was collected and rested at room temperature for 15 minutes before centrifugation at 200 x g for 10 minutes. Half of the serum was then aliquoted for use as platelet rich plasma (PRP), and the remaining serum was further centrifuged at 1500 x g for 15 minutes to provide platelet poor plasma (PPP). An average platelet count of PRP was measured with a hemocytometer. Depending on the platelet number in the sample, a specific ratio of PRP and PPP was combined to create a test sample with a final cell count of 250,000 platelets per µL.

Then, the combined plasma was held at room temperature for 20 minutes, after which platelet aggregation was assessed (CHRONO-LOG 700 Whole Blood/Optical Lumi-Aggregometer; Chrono-log Corporation, Havertown, PA). After calibration using sterile water, 500 µL of the previously prepared combined plasma was placed into glass cuvettes and incubated at 37 °C for three minutes. Collagen was then added to the PRP to induce aggregation while the PPP was left untouched and served as a control. The collagen was added in separate cuvettes at either 1 or 3 µg collagen per 1 ml of PRP. The changes in aggregation were measured for amplitude (percent), slope (percent per minute), lag time (minutes and seconds), and area under the curve (AUC; percent x time).

Laboratory measurements

Study I collected blood samples at 0h and 2h at each of three study visits to test CBC, CMP (including blood glucose), and LP. Study II collected blood samples at 0h, 1h, and 2h at each of three study visits to test CMP and insulin. Blood and plasma samples were analyzed at the UC Davis Department of Pathology and Laboratory Medicine.

Mango variety and processing

The high-polyphenol Ataulfo variety (also termed honey mango) was used in both studies. Fresh mangos were from single shipments for study I and again for study II. The mangos ripened under ambient conditions until they were a light-yellow color with a medium-soft texture, after which they were washed, manually peeled, deseeded, cubed, weighed into daily portions (330 g), and frozen at –20°C until time of use.

Statistical Analysis

Microvascular function, calculated by the RHI, was the primary outcome for study I. Sample size calculation was determined based on a previous study from our laboratory assessing the effects of walnuts on vascular function.[23] Microvascular function values were assumed to have a standard deviation of 0.5. Therefore, a sample size of 20 was needed to detect significant differences in RHI with 80% power at a 5% level of significance. Data were checked for normality and homogeneity of variance using the Shapiro-Wilk or Brown-Forsythe tests. The two-week differences in microvascular function, anthropometric and biochemical measures, and nutrient intake were analyzed using paired-t tests. The 2h change values for microvascular function, BP, platelet aggregation, and blood glucose were analyzed by one-way repeated measure (RM) Analysis of Variance using treatment (no mango vs. mango) as the main factor and participant ID as the random effect.

For study II, the acute changes (1h and 2h) from baseline (0h) in BP, blood glucose, and insulin were analyzed by two-way RM ANOVA using time and treatment as the main factors and participant ID as the random effect. For main effects, Tukey's tests were used for post-hoc analysis, with student t-tests used to determine significance within group pairs. A $p < 0.05$ was considered statistically significant. Statistical analyses were performed with JMP version 16 (SAS Institute Inc., Cary, NC, USA).

Results

Study I

Participant characteristics

Twenty-eight overweight and obese postmenopausal women were enrolled in the study between September 2016 and August 2017 (Figure 3). Data from three participants with a PAT recording

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of less than 70% (n=3) were excluded due to low validity of the measurement, as was data from one who was lost to follow-up. Data from one participant was also not included in the analysis due to an abnormal platelet count.

Baseline (SV1) characteristics of participants are shown in Table 1. The mean age was 60.3 ± 1 years. Participants had borderline-high total cholesterol (202.7 ± 6.3 mmol/L) and borderline-low HDL-cholesterol levels (55.2 ± 3.8 mmol/L).

<u>Changes in blood pressure</u>

No significant changes were noted for blood pressure measures or heart rate before or after two weeks of daily mango intake. The 2h change value of SBP was significantly decreased after mango intake at SV2 compared to SV1 (-3.76 vs. 1.98 mmHg, p = 0.0015; Figure 4.1). The change value of SBP was also significantly decreased 2h after mango intake at SV3 compared to SV1 (-1.93 vs. 1.98 mmHg, p = 0.03). The acute (2h) change value of pulse pressure (PP; SBP – DBP) was significantly decreased after mango intake at SV2 compared to SV1 (-3.13 vs. 1.0 mmHg, p = 0.0009; Figure 4.2). The 2h changes in diastolic blood pressure (DBP), mean arterial pressure (MAP; [2 {DBP)} + SBP]/3), and heart rate (HR) were not significantly different among SVs.

<u>Changes in microvascular function and platelet aggregation</u>

No significant changes were noted for RHI, fRHI, AI, or AI75 before or after two weeks of daily mango intake. The 2h change values of RHI, fRHI, AI, and AI75 were not significantly different from SV1, SV2, or SV3. No significant changes were noted for platelet aggregation markers before and after two weeks of daily mango intake. The 2h change values of platelet aggregation markers were not significantly different from SV1, SV2, or SV3.

Changes in plasma cholesterol and blood glucose

No significant changes were noted for plasma cholesterol markers or blood glucose before and after two weeks of daily mango intake. At SV1, blood glucose was decreased 2h after baseline as expected, due to further fasting. However, the acute change values for glucose 2h after mango intake at SV2 and SV3 were not significantly different from the values at SV1 (Figure 5).

Dietary intake

Reported protocol compliance was 100%, with no adverse symptoms noted. Estimated dietary intakes of energy, macronutrients, and micronutrients during the no-mango phase (between SV1 and SV2) and during two weeks of daily mango consumption (between SV2 and SV3) are shown in Tables 2 and 3. The total caloric intake was not significantly different between periods of no mango and daily mango intake. The intakes of soluble fiber, total sugar, monosaccharides, and disaccharides were significantly higher, while other carbohydrates were significantly lower during daily mango consumption. Intakes of β-carotene, vitamin C, vitamin E, and folate were significantly higher in the period of daily mango intake, compared to the no mango period.

Study II

Participant characteristics

A total of six participants completed the study and were included in the statistical analysis. Baseline participant characteristics are shown in Table 4. The mean age was 63 ± 2.1 years old. Participants had borderline high fasting blood glucose (103.8 ± 4.6 mg/dL).

Pulse pressure and blood pressure

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The 2h change in PP at SV1 was significantly higher than after mango intake at SV2 (2.06 vs. -2.72 mmHg, p = 0.036), as well as SV3 (2.06 vs. -2.78 mmHg, p = 0.034; Figure 6.1). The 1h and 2h change in SBP, DBP, and MAP were not significantly different between treatments and time points. Interestingly, the 1h and 2h changes in HR after white bread intake at SV3 were significantly higher than after water intake at SV1 (1h: 4.89 vs. -4.06 bpm, p = 0.021; 2h: 5.67 vs. -4.22 bmp, p = 0.012; Figure 6.2).

<u>Blood glucose and insulin</u>

The change in blood glucose 1h after white bread intake at SV3 was significantly greater than that at 2h (32 vs. 8 mg/dL, p = 0.044; Figure 7.1). The 1h change in blood glucose at SV3 was significantly higher than water intake at SV1 (32 vs. 0.67 mg/dL, p = 0.01). The 1h and 2h changes in blood glucose after mango intake at SV2 were not different compared to SV1 or SV3.

The change in insulin 1h after white bread intake at SV3 was significantly increased compared to 2h (57.3 vs. 20.53 µU/ml, p = 0.0023; Figure 7.2). The 1h change in insulin at SV3 was significantly increased compared to that at SV1 (57.3 vs. -1.7 µU/ml, p < 0.001) as well as at SV2 after mango intake (57.3 vs. 20.5 µU/ml, p = 0.0024). The 2h change in insulin at SV3 was significantly greater than at SV1 (20.47 vs. -2.78 µU/ml, p = 0.042), but not different from the value at SV2. The change in insulin at SV2 was not significantly different from SV1 at any time point.

Discussion

During the two-week mango intake period, the estimated increases in soluble fiber, total sugar, monosaccharides, disaccharides, β-carotene, vitamin C, vitamin E, folate, were expected, compared to the reported intakes during the run-in, no-mango period. Despite these increases in

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carbohydrates during the mango feeding period, fasting glucose and plasma lipid levels, body weight, and waist circumference, did not change. Some animal and human studies suggest that mango intake may benefit blood glucose control. The blood glucose levels after an oral glucose tolerance test were significantly decreased in obese Wistar rats fed a high-fat diet and supplemented with 35 ml of mango juice with or without peel extract for seven days, compared to controls.[24] Another study reported a significant decrease in fasting blood glucose in diabetic but not normal male Wistar rats 30 days after consuming a diet mixed with dried Tommy Atkins mango powder at 5% of diet weight.[25]

The RHI, fRHI, AI, AI75, and platelet aggregation did not differ two weeks after daily mango intake, which may have been due to the relatively short intervention period.[26] In a randomized double-masked, placebo-controlled, four-week trial among healthy individuals aged 40-70 years with a BMI of 19-30 kg/m^2, the RHI as measured by PAT was significantly increased after daily intake of 100 mg intake of unripe mango fruit powder made from the Kili-Mooku cultivar, compared to their baseline levels. When fed the same powder at 300 mg per day, the RHI was significantly increased, but only among individuals with compromised endothelial function. Another study reported that the daily intake of 400 g of fresh frozen Ataufo mango pulp for six weeks significantly decreased SBP in lean individuals aged 18-65 with BMI 18-26.2 kg/m^2, and significantly decreased hemoglobin A1C, plasminogen activator inhibitor-1, interleukin-8, and monocyte chemoattractant protein-1 in participants with BMI > 28.9 kg/m^2.[27] While intriguing, the results need interpreted cautiously since the BMI numbers were not the standard values used for healthy, overweight, and obese criteria.[28]

The SBP was significantly reduced in the first two hours after the first mango intake in Study I, compared to baseline or run-in values. In contrast, the SBP was unchanged in Study II at one and

two hours after mango intake. The discrepancy between values from Studies I and II may be due to a low number of participants in study II. However, the change in PP was significantly reduced 2h after mango intake in both study I and II. Importantly, the PP also changed after white bread intake, suggesting that the response might be due to a postprandial effect. In study II, although the postprandial changes of SBP and DBP were not significantly different between the mango and white bread groups, the HR changes 1h and 2h after white bread intake were significantly increased compared to no mango intake. This finding is consistent with a report that both supine and standing HRs were significantly increased 1h and 3h after a 790 kcal meal in the morning after an overnight fast.[29] However, the calorie content in mango and white bread in this current study was only 298 kcal. Studies regarding the consumption of fruits and postprandial BP and HR are scarce. Future research is encouraged to investigate whether fruit intake will induce similar hemodynamics as meals.

In study I, the 2h change in blood glucose was not different between mango or no mango intake, despite the difference in sugar intake from the fruit. This observation was reinforced further in study II, where the blood glucose was significantly increased 1h after white bread intake but not after eating an isocalorically-matched amount of mango. The insulin level was also significantly increased 1h after white bread intake compared to 1h after no mango or mango intake. In addition, although the 2h change in blood glucose after eating white bread returned to a level similar to baseline values, the 2h change of insulin was still significantly elevated compared to the 2h value seen in the no mango group. These data are consistent with other reports regarding mango consumption and glucose regulation. For example, in obesity-prone mice fed a high-fat diet, the fasting blood glucose, insulin, and homeostatic model assessment for insulin resistance (HOMA-IR) score were significantly decreased after 10 weeks of mango fruit powder intake at

each of three levels (18, 54, or 108 mg/kg body weight).[17] In obese male C57BL/6J mice

consuming a high-fat diet, daily supplementation freeze-dried mango (Tommy Atkins) at either

1% or 10% of the weight of the diet significantly reduced body fat compared to those consuming

a non-supplemented control diet. Curiously, only the 1% mango group showed significantly

decreased fasting blood glucose and postprandial blood glucose responses after tolerance tests,

but no difference was noted for insulin or HOMA-IR, compared to those consuming the 10%

supplementation or control diets.[30] In overweight and obese humans, plasma insulin was

significantly increased 45 min after consuming 100 kcal of mango (Tommy Atkins, Kent, or

Haden cultivars), compared to their baseline levels, but did not increase as much as when the

participants consumed an to isocaloric low-fat cookie.[31] The same study also noted that capillary

blood glucose levels were significantly elevated 30 min after mango intake compared to their

baseline values, while returning to the baseline range at 60, 90, or 120 min after intake, whereas

intake of the low-fat cookie showed significantly increased blood glucose at both 30 and 60 min,

which is consistent with trends from our study. However, the above study measured insulin at

baseline and 45 min after food intake, so the postprandial insulin levels cannot be compared

directly with our study. Future research may consider assessing the association between

postprandial BP, glucose, and insulin resistance at multiple timepoints.

This study has several limitations. The Ataulfo mangos were not analyzed for nutrients or

phenolic contents. Different mango cultivars vary in macronutrients, micronutrients, as well as

phytonutrient content. Among commonly consumed mango cultivars, Ataufo mango pulp

contains the highest concentration of β-carotene, ascorbic acid, total phenolics, gallotannins, and

mangiferin, in comparison to Haden, Keitt, Kent, and Tommy Atkins.[32] The high concentrations

were used in the selection of Ataulfo. The amount of white bread as an isocaloric control was

calculated based on the USDA food database, which does not identify the cultivar or cultivars

that were tested. Finally, the postprandial blood glucose and insulin responses in study II were

not measured at 30 min, which may have missed the possible peak levels. Future studies may

take the measurements at more frequent time points, as well as insulin resistance indicators, such

as HOMA-IR, to better understand the role of mango in blood glucose management.

In conclusion, two weeks of daily mango intake was associated with a decrease in SBP and PP.

The glucose and insulin responses after mango intake were also moderated, compared to

ingesting of an isocaloric amount of white bread. While the effects of mango intake on

microvascular function were not as significant as the response from other whole foods, other

measures of cardiovascular health, as well as glucoregulatory benefits, warrant further study.

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Figures and Tables

Figures 1. Study design – study I
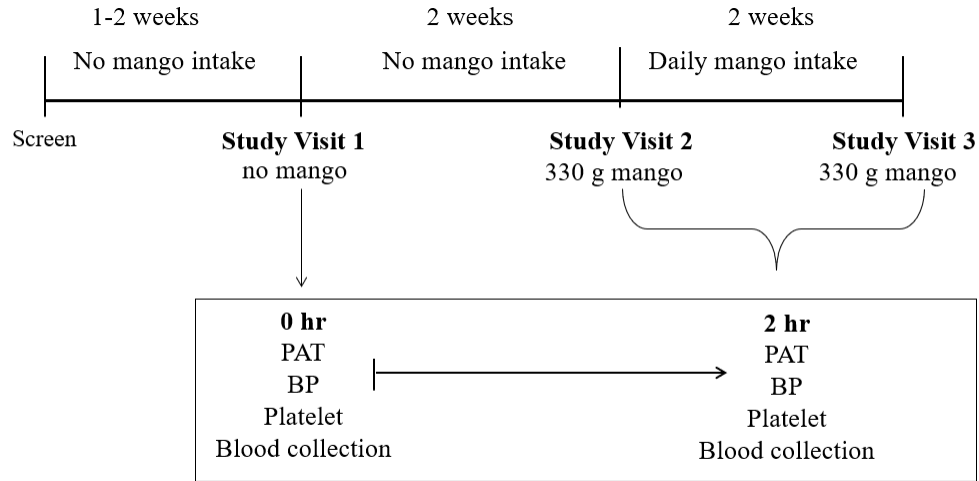
Figure 2. Study design – study II

Figure 3. Study I flow


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┌──────────────────────┐
│ Telephone Screening  │
│      n = 213         │
└──────────┬───────────┘
           │          ┌─────────────────────────────┐
           ├─────────→│ Excluded (n = 162)          │
           │          │ Not meeting inclusion criteria│
           │          └─────────────────────────────┘
           ↓
┌──────────────────────┐
│  Clinical Screening  │
│      n = 51          │
└──────────┬───────────┘
           │          ┌─────────────────────────────┐
           ├─────────→│ Excluded (n = 23)           │
           │          │ Not meeting inclusion criteria│
           │          └─────────────────────────────┘
           ↓
┌──────────────────────┐
│     Enrolled         │
│      n = 28          │
└──────────┬───────────┘
           │          ┌─────────────────────────────┐
           ├─────────→│ Lost to follow up (n = 1)   │
           │          └─────────────────────────────┘
           │          ┌─────────────────────────────┐
           ├─────────→│ PAT signal validity < 70% (n = 3)│
           │          └─────────────────────────────┘
           ↓
┌──────────────────────┐
│Completed Intervention│
│      n = 24          │
└──────────┬───────────┘
           │          ┌─────────────────────────────┐
           ├─────────→│ Abnormal platelet count (n = 1)│
           │          └─────────────────────────────┘
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│   Data Analysis      │
│      n = 23          │
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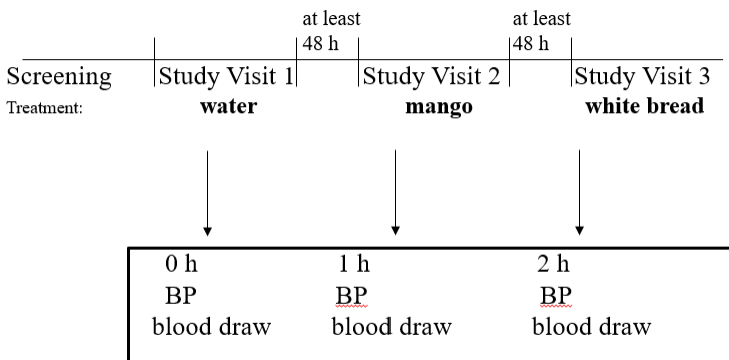
Figure 4.1. Study I – two-hour change in systolic blood pressure before mango intake (SV1), after the first intake (SV2) and after two weeks of daily mango intake (SV3)
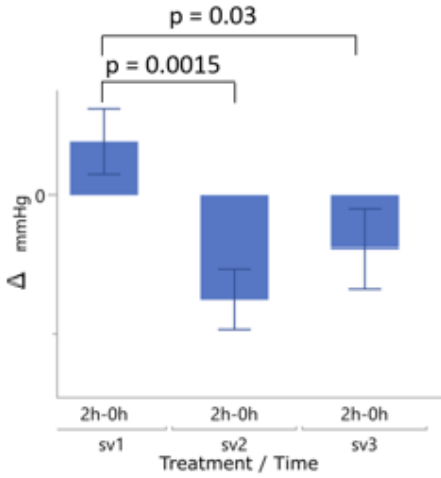
Figure 4.2. Study I – two-hour change in pulse pressure before mango intake (SV1), after the first intake (SV2) and after two weeks of daily mango intake (SV3)
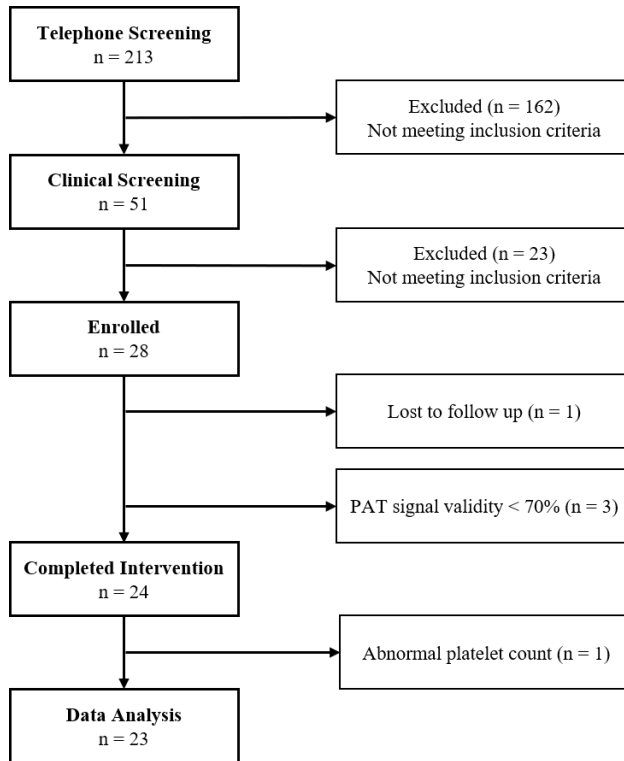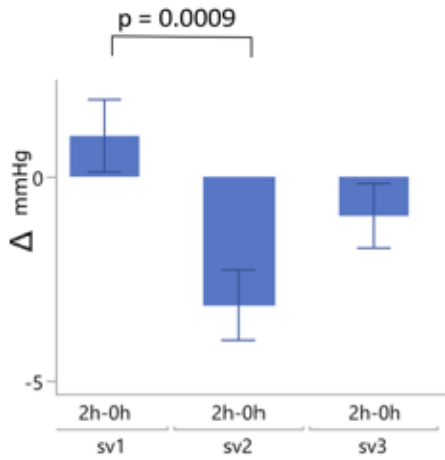
Figure 5. Study I – two-hour change in blood glucose before mango intake (SV1), after the first intake (SV2) and after two weeks of daily mango intake (SV3)

Figure 6.1. Study II – one-hour and two-hour changes in pulse pressure after no mango intake (SV1), after mango intake (SV2), and after white bread intake (SV3)

Figure 6.2. Study II – one-hour and two-hour changes in heart rate after no mango intake (SV1), after mango intake (SV2), and after white bread intake (SV3)
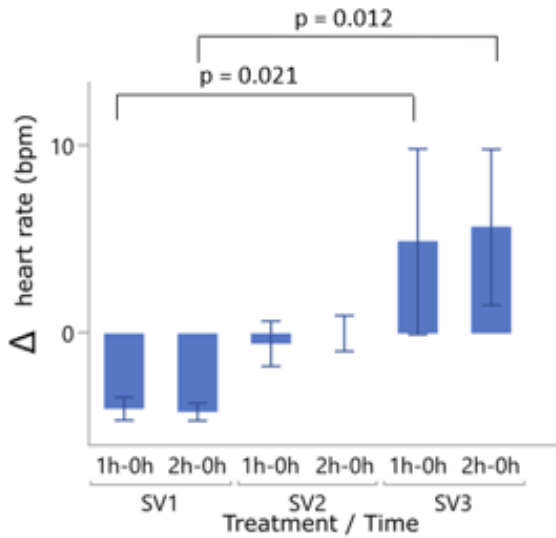
Figure 7.1. Study II – one-hour and two-hour changes in blood glucose after no mango intake (SV1), after mango intake (SV2), and after white bread intake (SV3)
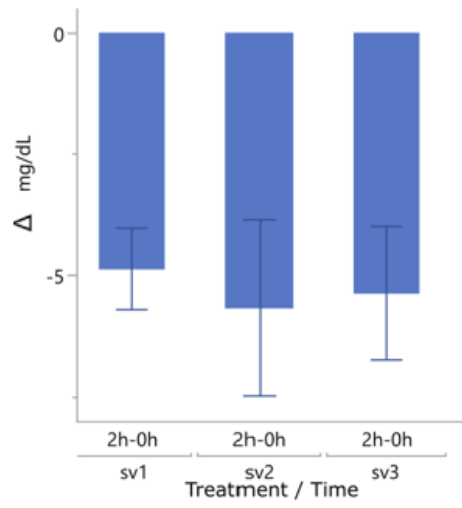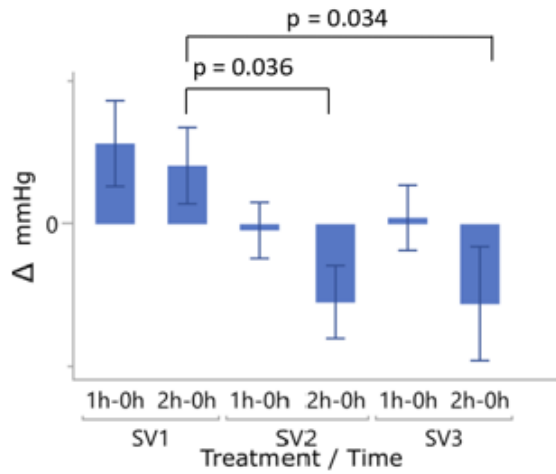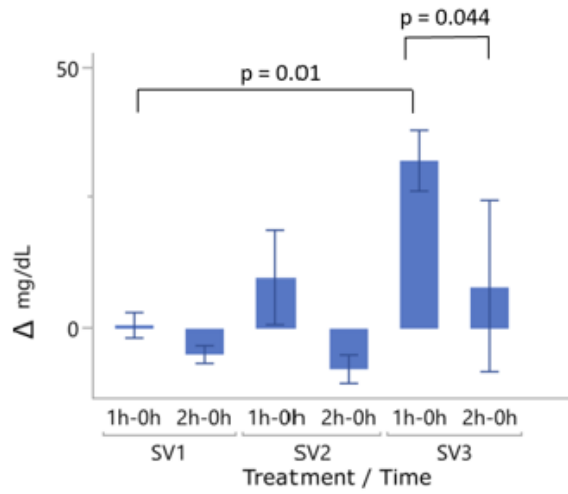

Figure 7.2. Study II – one-hour and two-hour changes in insulin after no mango intake (SV1), after mango intake (SV2), and after white bread intake (SV3)
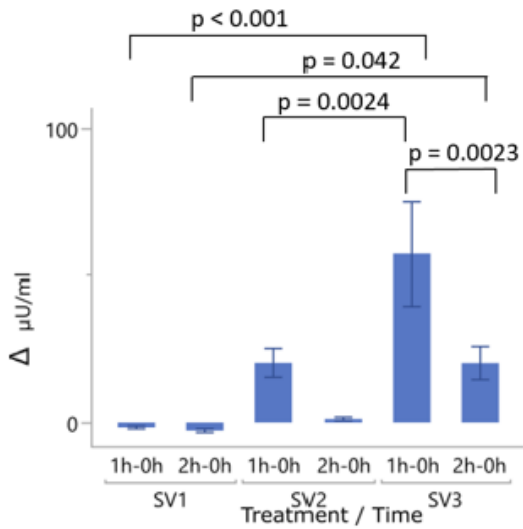
Table 1. Study I – Baseline characteristics of participants (n=23)

	Mean ± SEM
Age (yrs)	60.3 ± 1.0
Height (cm)	162.4 ± 1.4
Weight (kg)	76.8 ± 1.9
BMI (kg/m^2)	29.1 ± 0.6
WC (cm)	98.2 ± 1.5
SBP (mmHg)	114.7 ± 2.3
DBP (mmHg)	74.8 ± 1.4
HR (bt/min)	60.3 ± 1.4
PP (mmHg)	40.1 ± 1.3
MAP (mmHg)	87.9 ± 1.7
RHI	2.1 ± 0.1
fRHI	0.53 ± 0.06
AI	22.6 ± 3.6
AI75	15.3 ± 3.2
Blood Glucose (mg/dL)	98.1 ± 1.8
Total Chol. (mg/dL)	202.7 ± 6.3
HDL (mg/dL)	55.2 ± 3.8
LDL (calc)	128.9 ± 4.5
Chol: HDL	4.0 ± 0.2
TG (mg/dL)	93.8 ± 11.2
non-HDL (mg/dL)	143.9 ± 5.5
MPV (fL)	8.9 ± 0.3
Platelets (k/mm)	237.8 ± 10.6

BMI: body mass index; DBP: diastolic blood pressure; fRHI: Framingham reactive hyperemia index; HDL: high-density lipoprotein; HR: heart rate; LDL: low-density lipoprotein; MAP: mean arterial pressure; MPV: mean platelet volume; PP: pulse pressure; RHI: reactive hyperemia index; SBP: systolic blood pressure; SEM: standard error of the mean; TG: triglycerides; WC: waist circumference

Table 2. Study I – Differences in reported energy and macronutrients intake before and after mango intake

	No mango intake		Daily mango intake	
	Mean	CI	Mean	CI
Calories (kcal)	1980	(1730, 2220)	1890	(1630, 2160)
Calories from fat (kcal)	735	(622, 849)	636	(519, 754)
Calories from saturated fat (kcal)†	220	(183, 265)	187	(159, 220)
Protein (g)	88.1	(73.5, 103)	87.3	(72.1, 102)
Carbohydrates (g)	221	(183, 259)	231	(199, 263)
Dietary fiber (g)	20.4	(16.4, 24.4)	23.1	(19.3, 26.9)
Soluble fiber (g)†	0.65	(0.41, 1.03)	3.87	(3.54, 4.24) **
Total sugars (g)	80.2	(63.5, 96.8)	108	(98.0, 119) **
Monosaccharides (g)	10.4	(6.67, 14.2)	30.2	(27.7, 32.7) **
Disaccharides (g)	7.77	(4.97, 10.6)	30.1	(26.1, 34.2) **
Other carbohydrates (g)	107	(85.9, 128)	87.0	(70.0, 104) *
Fat (g)	81.8	(69.1, 94.5)	70.7	(57.7, 83.8)
Saturated fat (g)†	24.5	(20.4, 29.4)	20.8	(17.7, 24.5)
Monounsaturated fat (g)	14.6	(12.2, 17.1)	14.5	(10.1, 18.9)
Polyunsaturated fat (g)	7.75	(6.31, 9.19)	7.43	(4.55, 10.3)
Trans fatty acid (g)†	0.63	(0.47, 0.85)	0.56	(0.30, 1.04)
Cholesterol (mg)	309	(240, 379)	288	(218, 358)

†: log-transformed data. Data shown are the means and 95% CI, or back-transformed means and 95% CI of two independent analyses. Values between no mango (SV1–SV2) and daily mango intake (SV2–SV3) were compared by two-tailed paired t-tests (*: $p < 0.05$, **: $p < 0.001$).

Table 3. Study I – Differences in reported micronutrients intake before and after mango intake

	No mango intake		Daily mango intake	
	Mean	CI	Mean	CI
Vitamin A, RAE (µg)†	347	(214, 562)	466	(363, 597)
Carotene, RE (µg)	555	(307, 804)	737	(491, 983)
Beta carotene (µg)†	731	(348, 1540)	3020	(2420, 3770) *
Thiamine (mg)	0.74	(0.55, 0.92)	0.80	(0.62, 0.92)
Riboflavin (mg)	1.09	(0.92, 1.26)	1.17	(0.96, 1.37)
Niacin (mg)	11.4	(8.86, 13.9)	12.8	(10.4, 15.2)
Vitamin B6 (mg)	0.99	(0.72, 1.26)	1.22	(0.95, 1.49)
Vitamin B12 (µg)	2.68	(1.76, 3.60)	2.33	(1.43, 3.22)
Biotin (µg)†	11.0	(6.67, 18.1)	6.84	(4.16, 11.2)
Vitamin C (mg)	100	(68.4, 132)	178	(158, 198) **
Vitamin D (IU)	91.4	(40.6, 142)	78.2	(46.0, 110)
Vitamin E (mg)†	4.42	(2.63, 7.43)	6.78	(5.06, 9.08) *
Folate (µg)	219	(166, 273)	334	(282, 386) *
Calcium (mg)	768	(639, 898)	716	(573, 858)
Iron (mg)	10.25	(8.47, 12.0)	9.31	(7.93, 10.7)
Phosphorus (mg)	625	(515, 736)	622	(495, 749)
Potassium (mg)	1470	(1200, 1740)	1850	(1530, 2160)

†: log-transformed data; RAE: retinol activity equivalent; RE: retinol equivalent. Data shown are the means and 95% CI, or back-transformed means and 95% CI of two independent analyses. Values between no mango (SV1–SV2) and daily mango intake (SV2–SV3) were compared by two-tailed paired t-tests (*: $p < 0.05$, **: $p < 0.001$).

Table 4. Study II – Baseline characteristics of participants (n=6)

	Mean ± SEM
Age (yrs)	60.3 ± 1.0
Height (cm)	162.4 ± 1.4
Weight (kg)	76.8 ± 1.9
BMI (kg/m^2)	29.1 ± 0.6
SBP (mmHg)	114.7 ± 2.3
DBP (mmHg)	74.8 ± 1.4
HR (bt/min)	60.3 ± 1.4
PP (mmHg)	40.1 ± 1.3
MAP (mmHg)	87.9 ± 1.7
Blood Glucose (mg/dL)	98.1 ± 1.8
Insulin (uU/ml)	10.6 ± 1.6

BMI: body mass index; DBP: diastolic blood pressure; HR: heart rate; MAP: mean arterial pressure; PP: pulse pressure; SBP: systolic blood pressure; SEM: standard error of the mean

Chapter IV

Potential Roles of Dietary Zeaxanthin and Lutein in Macular Health and Function: Focus on Goji Berries

Abstract

Lutein, zeaxanthin, and meso-zeaxanthin are three xanthophyll carotenoid pigments that selectively concentrate in the center of the retina. The human body cannot synthesize carotenoids so these compounds must be obtained by food. Macular pigments protect the retina by filtering blue light and oxidant defense. The accumulation of these three xanthophylls in the central macula can be assessed through non-invasive methods and quantified through the index of macular pigment optical density (MPOD), which serves as a useful tool to assess risk for, and progression of age-related macular degeneration (AMD). Dietary surveys consistently report that the intakes of lutein and zeaxanthin are below the amount shown to improve MPOD. In addition to low dietary intake, pregnancy and lactation may compromise the lutein and zeaxanthin status of both the mother and infant. Lutein is found in modest amounts in some orange and yellow-colored vegetables, and in egg yolks, but rich sources of zeaxanthin are not commonly consumed. Goji berries contain the highest known levels of zeaxanthin of any food, and regular intake of these bright red berries may help protect against the development of AMD through an increase in MPOD.

Introduction

Epidemiological studies suggest that diets rich in carotenoids can be beneficial for vision, heart, bone health, cognitive performance, and cancer prevention.[1] The current review focuses on the potential role of the xanthophyll carotenoids lutein (L) and zeaxanthin (Z) in eye health, specifically their potential role in reducing risk of age-related macular degeneration (AMD). We review the absorption, distribution, and metabolism of L and Z, and the current dietary recommendations for these carotenoids, then speculate about their putative role in maternal and infant health. Lastly, we discuss the potential value of goji berry within the diet as a food with the highest known amount of Z.

Carotenoids contribute to the bright red, orange, and yellow color in plants.[2] These fat-soluble phytochemicals are classified into two categories: carotenes, which include only hydrocarbons, and xanthophylls (e.g., L and Z) that also contain oxygen.[3,4] While some dietary carotenoids serve as vitamin A precursors (e.g., β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin)[5,6] most of the approximately 100 carotenoids found in plants do not.[7] Among the carotenoids devoid of vitamin A activity are L and Z, along with meso-zeaxanthin (meso-Z), a stereoisomeric metabolite of L.[8]

The intestinal absorption of xanthophylls includes both facilitated transport and passive diffusion.[9] Absorption involves enterocyte uptake by CD36, scavenger receptor class B type I (SR-B1), and Niemann-Pick C1-like transporter 1 at the apical membrane. Xanthophylls are then secreted through the basolateral membrane of the enterocyte, mainly by ATP binding cassette A1 and carried by lipoproteins to target tissues.[10] SR-B1, SR-B2, and CD36 transport L and Z into the tissues.[11] Steroidogenic acute regulatory domain protein 3 has been identified as a binding protein for L in the retina, and glutathione S-transferase pi isoform for Z.[12]

Macular Pigments

Lutein, Z, and meso-Z impart a distinctive yellow color to the fovea of primates – the specialized central area of the macular region of the retina that is rich in cone photoreceptors and optimized for high-acuity central color vision. The compounds have a maximal absorbance at a wavelength near 460 nm and are most concentrated in the inner and outer plexiform layers, which consists primarily of axonal connections between the retinal layers. Their combined density is greatest in the center of the macula and decreases with increasing retinal eccentricity.[13,14] In the central fovea, the concentration of Z and meso-Z is higher than L at a ratio of 2.4:1. Lutein is most abundant in the peripheral macula, with a Z + meso-Z to L ratio of 1:2 when measured by high-performance liquid charomatography.[15] However, a newer technique, confocal resonance Raman microscopy suggests that the Z + meso-Z to L ratio is as high as 9:1 at the central fovea.[16]

Protection from blue light is critical for eye health. Compared to longer wavelengths of visible light, short blue wavelengths are higher in energy and generate reactive oxygen species (ROS).[17,18] Zeaxanthin can provide stronger oxidant defense than L during photooxidation,[19] while lutein has a greater capacity to absorb short wavelength light irradiation in lipid membranes.[20]

Compared to other carotenoids (e.g., lycopene or β-carotene), L and Z are more effective in scavenging ROS and can also reduce phospholipid peroxidation.[21,22] The photoreceptor-retinal pigment epithelium (RPE) complex in the outer retina is particularly susceptible to ROS damage due to its high polyunsaturated lipid content (Figure 1).[23] Quenching of singlet oxygen appeared best when L, Z, and meso-Z were mixed in equal ratios rather than separately when assessed in an eye tissue model, suggesting some synergy between the these macular pigments in their antioxidant properties.[24]

The most common method to quantify xanthophylls in the retina is to assess macular pigment optical density (MPOD). This parameter is measured through techniques such as heterochromatic flicker photometry (HFP), a non-invasive psychophysical technique,[25] fundus reflectometry, resonance Raman spectroscopy, or autofluorescence imaging.[26] The MPOD index is associated with plasma levels of L and Z,[27-29] and has been used to assess the risk for AMD.[30] However, some studies report no correlation between MPOD and risk of AMD,[31] which suggests that other ocular measures may be useful to obtain a more complete profile of AMD risk. In human donor eyes, the amount of L and Z was inversely associated with AMD.[32] Supplementation of L, Z, and meso-Z have been shown to significantly increase MPOD in both healthy individuals and patients diagnosed with AMD.[33,34] However, studies using foods rich in L and Z have produced inconsistent results,[35] which may be due to the relatively modest amounts of these carotenoids in foods compared to supplements. Importantly, the plasma concentration of L and Z has been more strongly associated with MPOD than the correlation between MPOD and dietary intake.[36]

Age-related Macular Degeneration

Age-related macular degeneration is the third leading cause of blindness worldwide after uncorrected refractive errors and cataracts.[37] An estimated 288 million people worldwide are projected to suffer from AMD by 2040.[38] In the United States, the prevalence of early-stage AMD was 9.1 million in 2010, and this number is projected to increase to 17.8 million by 2050.[39] AMD is characterized by a gradual loss of eyesight from the central visual field.[40] Although the exact etiology of AMD is not clear, common pathologic progress includes oxidative stress, lipofuscin toxicity, lipid accumulation, immune dysregulation, and choroidal hyperperfusion.[41] Age-related processes such as a decrease in retinal neuronal elements, alterations in the size and shape of RPE cells, and thickening of Bruch's membrane also

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participate in the pathology of AMD.[42] Damage to mitochondria in RPE cells has also been suggested to play a role.[43] Dry AMD, also termed non-exudative AMD, involves the formation of drusen, which are mainly lipid and protein deposits that accumulate between the RPE and Bruch's membrane in the macula.[44] In contrast, wet AMD, also termed exudative or neovascular AMD, is a consequence of abnormal blood vessel formation arising from the choroid, known as choroidal neovascularization (CNV).[45] Clinically, AMD is classified as early or intermediate stage based on the size and number of drusen, as well as presence of pigmentary changes.[46] The AMD is considered late or advanced stage in the presence of CNV, where fluid accumulation may result in damage to the neurosensory retina and fibrous scarring, or geographic atrophy (GA), where loss of the RPE result in damage to overlying photoreceptors and underlying choriocapillaris causing irreversible vision loss.[47]

The main risk factors for AMD are aging and smoking,[48] although some studies have shown no difference in MPOD between healthy older individuals and healthy young.[49,50] Other risk factors may include race, obesity, previous cataract surgery, presence of cardiovascular disease, and hypertension.[51,52] According to the U.S. National Institutes of Health, the prevalence of AMD is highest among Caucasians as compared to other races, and higher in females than in males.[53] Genetic factors are also associated with AMD,[54] with several high-risk single-nucleotide polymorphisms identified from genome wide association studies.[55-57] The strongest risk variants include the Y402H variant of complement factor H gene as well as those in the age-related maculopathy susceptibility 2 locus.[58-60] Whether the color of the iris or sunlight exposure are related to the risk of AMD is still being explored.[51,61-65]

Dietary interventions using L- and Z-rich foods have generated inconsistent results regarding the risk of AMD.[66,67] In a cohort study that assessed dietary carotenoid consumption among

individuals without AMD at baseline over more than 20 years, increased predicted plasma carotenoid score of L, Z, β-carotene, α-carotene and β-cryptoxanthin were associated with a lower risk of advanced, but not early or intermediate AMD.[68] Similarly, a meta-analysis of six longitudinal cohort studies found that the dietary intake of L and Z significantly reduced the risk of GA by 26% and CNV by 32%, with no apparent impact on early stages.[69] Another meta-analysis concluded that supplementation with L, Z, and meso-Z significantly increased MPOD levels in both AMD patients and healthy individuals in a dose-response manner.[70] However, whether the improvement in MPOD could be sustained after L and Z supplementation is discontinued remains unclear.

The Age-Related Eye Disease Study (AREDS) was a multi-center study that assessed the efficacy of a dietary anti-oxidant supplements on subjects who are 50 to 80 years old, with and without AMD or cataracts, for more than seven years.[71] The initial study used a formula containing 15 mg of β-carotene, 500 mg of vitamin C, 400 IU of vitamin E, with or without 80 mg of zinc and 2 mg of copper. Lutein and Z were not included because the scientific evidence to include these two carotenoids was not yet clear. Compared to the placebo group, participants consuming the antioxidants plus zinc and copper showed a 28% reduction in progression to advanced AMD after five years.[72] Subsequently, the AREDS2 was conducted with a newer formulation that included vitamins C and E, either 10 mg of L plus 2 mg of Z, and either 350 mg of docosahexaenoic acid (DHA) plus 650 mg of eicosapentaenoic acid (EPA), or both. Patients were also given either 25 or 80 mg of zinc, each with 2 mg of copper. Beta-carotene was eliminated from the supplement due to a potential increased risk of lung cancer among smokers, who were already at high risk for AMD.[73] Primary analyses of the AREDS2 formula found no additional benefit in reducing progression to advanced AMD, in comparison to the original

AREDS formula. However, in a secondary analysis of combined data from AREDS and AREDS2, the progression risk in those receiving L and Z was significantly lower than in other groups.[74] Neither formulation reduced the progression from early to intermediate AMD. These clinical trials did not monitor the MPOD status over time, thus limiting our understanding of the link between L and Z intake, retinal accumulation, and AMD development or progression. To date, the AREDS2 formula remains the standard of care for management of patients with intermediate AMD.

Maternal Lutein and Zeaxanthin in Infant Development

The accumulation of L and Z in the macula starts *in utero* in primates and plays a critical role in visual development and maturation later in life.[75] Lutein and Z were detected as early as 20 weeks of gestation in macular tissue from human fetuses inspected at autopsy.[15] Unlike fully matured human eyes, L is the dominant macular pigment in infants under the age of two regardless of eccentricities. The retina is less mature at birth compared to other eye structures, with complete differentiation requiring four to five years.[76] The maturation of the macula is associated with a change in the L:Z ratio over the first four years of life, which correlates with the development of cone photoreceptors.[77]

Studies in premature infants illustrate the importance of these L and Z in visual development.[78] In preterm human neonates, extremely low levels of serum L and Z are associated with an undetectable MPOD.[79] When a carotenoid-fortified formula containing 211 µg/L of combined L and Z was given to preterm infants, plasma carotenoid levels became comparable to breastfed preterm infants, and were significantly higher than those fed formulas without L or Z fortification.[80] In a small study that monitored the concentration of L and Z in various infant formulas and breastmilk from different mothers, Z was not detected in any formula but was

present in all breastmilk samples, while L was consistently higher in breastmilk.[81] Serum L was also noted to be six-fold higher in breastfed infants compared to those fed with a formula devoid of L.[82] Further studies are warranted to assess the prospective effects of L- and Z-fortified formula on MPOD and visual development in infants as they enter adulthood.

Lutein and Z may also protect against oxidative damage in premature infants, especially those with retinopathy of prematurity (ROP).[83] Premature infants with ROP usually have poor visual acuity, even after laser treatment or intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) agents.[84] In a model of oxygen-induced retinopathy, mouse pups given L showed less vessel leakage and lower avascular area compared to those given a L-free control.[85] The authors suggested that the anti-oxidant properties of L may have contributed to these results, although ROS levels were not measured. Studies that investigate L and Z supplementation in ROP babies have produced inconsistent results. In a multi-center, double-blind, randomized controlled trial of very-low-birth-weight infants, no difference was found in the incidence of ROP between those supplemented with daily oral L and Z (0.14 mg L plus 0.6 µg Z) or placebo.[86] However, the progression rate of threshold ROP showed a lower trend in the supplemented group. No adverse events were noted with L and Z supplementation, suggesting that they were well-tolerated. Another study examining the effects of daily oral L (0.5 mg/kg) and Z (0.02 mg/kg) supplementation in preterm infants from the seventh day after birth until 40 weeks of age or until hospital discharge found no change in the rate or severity of ROP compared to placebo.[87] Further, a meta-analysis of three randomized controlled trials also found no protective association between L and Z supplementation and the risk of ROP.[88] Additional studies are needed to assess the role of prenatal L and Z supplementation in pregnant women at-risk for premature delivery.

During development, L and Z are not interchangeable. Serum Z in newborns and in their mothers is strongly correlated with the MPOD of the babies, but no relationship was noted for either maternal or infant levels of L.[89] During delivery, a high maternal plasma Z, but not plasma L, was significantly associated with a lower risk of visual acuity problems in children at three years of age.[90] Further investigations that can accurately distinguish and quantify dietary and plasma Z from L are needed to better understand the role of these two carotenoids in visual performance during development.

The L and Z in human milk is particularly important for infant eye and brain development, and may provide long term benefits to vision and cognition.[91] Since humans cannot synthesize carotenoids, the fetus and breastfed infants must obtain these compounds from the mother through the placenta and the breast milk. During gestation, maternal lipoprotein synthesis increases, which accelerates the transport of carotenoids to the fetus.[92] This transfer may deplete maternal stores if the dietary intake of carotenoids in general, and L and Z specifically, is inadequate to maintain body stores. Low maternal skin and serum carotenoid levels have been reported in mothers of newborn infants.[89]

The prevalence of AMD is higher in women than in men, even though on a global basis more men smoke.[93] At the same time MPOD levels are lower in females.[94,95] The potential reasons for an increased lifetime risk for AMD in women are complex and multifactorial in nature and may include maternal depletion of L and Z during pregnancy and lactation (recognizing that not all females are parous or lactate). Importantly, the average dietary consumption of L and Z among females in the US (1.76 mg/d) is far below the amount of 10 mg/d known to increase MPOD.[34] Therefore, either the intake of supplements containing L and Z, or increased intake of foods rich in these two carotenoids for the duration of pregnancy and lactation may be of value.

The concentration of β-carotene, lycopene, L and Z, the main carotenoids in breastmilk are associated with maternal dietary intake over the first six months of lactation.[96] Daily maternal supplementation of either 6 mg of L with 96 µg of Z, or 12 mg of L with 192 µg of Z, over six weeks resulted in a dose-dependent increase in L and Z levels in the breastmilk and of the mothers and their infants when assessed three to four months postpartum.[97] Another study reported that more carotenes were present than xanthophylls in maternal plasma, whereas more xanthophylls such as L and Z were presented in breastmilk, in comparison to carotenes.[98] These findings support the notion that maternal-infant transfer of carotenoids may occur, possibly at the expense of the mother. Future studies are needed to clarify if breastfeeding or L and Z intake may impact their AMD risk.

The L-ZIP supplementation trial is currently exploring whether prenatal supplementation of 10 mg of L and 2 mg of Z will maintain maternal body stores, prevent potential macular pigment depletion during pregnancy, or enhance systemic and ocular carotenoid stores for both mothers and infants.[99] Clinical trials on the long-term effects of perinatal L and Z intake on MPOD changes among mothers and infants are also warranted.[100] Unfortunately, longitudinal studies on AMD in females often do not include breast-feeding history. A useful study design would be to investigate MPOD levels and relative risks of AMD between multiparous and nulliparous women, and in mothers practicing breastfeeding compared to formula feeding. Dietary intake of L and Z would be important to assess. Recognizing that such a study would take decades, shorter term studies could be conducted in non-human primates. Another challenge in retrospective studies is that breastfeeding history may not be accurate. Therefore, studies on the maternal transfer of L and Z during pregnancy and lactation with MPOD changes in infants and throughout the lifespan, could be important but difficult to conduct. Future research should also

focus on the measurement of L and Z status and MPOD in mother-infant pairs of twins or short birth intervals.

Last, when accessing AMD risk in women, reproductive hormone status may be a confounding factor. The pathogenesis of AMD involves oxidative stress and immune dysregulation.[42] Estrogen has been shown to reduce oxidative stress and inflammation in RPE cells as well as systemically.[101,102] Lifetime estrogen exposure such as the number of pregnancies, menopause, reproductive period, oral contraceptive use, and hormone replacement therapy may all influence the risk of developing AMD.[103] Current evidence regarding estrogen exposure and risk of AMD is inconsistent. One study reported that postmenopausal hormone use decreased the risk of neovascular AMD but increased the risk of early AMD, while parous women showed a reduced risk of early AMD but not neovascular AMD.[104] Two nationwide studies from South Korea among postmenopausal women noted that exogenous estrogen exposure was not a protective factor for AMD. A cohort study found that hormone replacement therapy (HRT) and a longer reproductive period was associated with an increased risk of neovascular AMD.[105] A cross-sectional study showed that oral contraceptive use was associated with an increased risk of late AMD.[106] In addition, a review summarizing the effect of estrogen exposure and the risk of all age-related eye diseases concluded that HRT, or the use of oral contraceptives, could be either positively or negatively associated with the risk of AMD.[103] In contrast, some studies have reported that a longer duration of breastfeeding may be protective from late but not early AMD,[106,107] even when the estrogen level was low during lactation. Future studies on the interaction of different reproductive and estrogen exposure histories and AMD risk are needed.

Dietary L and Z Intake and Challenges

Humans cannot synthesize carotenoids, and the best dietary sources are fruits, vegetables, egg yolks, and dairy products. Consuming a diet rich in green leafy vegetables and fish is recommended by the National Eye Institute for the high carotenoid and DHA and EPA contents.[33] Nevertheless, in the carotenoid group, L and Z are not yet considered essential, or even conditionally essential, so no dietary reference intakes (DRIs) for these two compounds exists. The US intake of L and Z has been decreasing. According to the U.S. National Health and Nutrition Examination Survey (NHANES), the average intakes of L plus Z were 2.15 mg/d in males and 2.21 mg/d in females in 1987, and 2.15 mg/d in males and 1.86 mg/d in females in 1992.[108] In NHANES 2013-2014, the average intakes of L and Z in males and females was 1.58 mg/d and 1.76 mg/d, respectively.[109] Moreover, based on data from NHANES 2003-2004, the reported intake of L was significantly higher than Z in all age groups and ethnicities. Importantly, the Z to L ratio was also lower in females than males older than 31 years of age, which may result in a higher risk of AMD in women than in men.[110] However, due to difficulties in analyzing dietary L and Z separately, most studies analyze both carotenoids together.[111] Since the amount of L in most foods is significantly greater than Z, precise quantification of Z has been a challenge.

The amount of L and Z in foods and dietary supplements appears to be safe.[112] No adverse events were found in clinical trials giving L at 30 mg/d for 120 days or 40 mg/d for 63 days.[113,114] The only reported adverse effect after a daily supplementation of 15 mg L in a 20-week trial was a single case of self-reported carotenodermia, a reversible condition of orange skin color.[115] Although a higher amount has been used in human studies, after assessing the potential risks, the observed upper safety level for L has been proposed as 20 mg/d.[116] The European Food Safety

Authority concluded safe upper limits for L and Z for use in dietary supplements were 1 mg/kg body weight/d and 0.75 mg/kg body weight/d, respectively.[117,118]

In primate models, rhesus monkeys fed a xanthophyll-free diet for 3 to 6.5 years developed extremely deficient or absent macular yellow pigment and drusen-like bodies.[119] When 3.9 µmol/kg per day of L or Z for 24 to 101 weeks were supplemented, the rhesus monkeys showed significant increases in their corresponding serum, retinal, and adipose tissue concentrations.[120] In their retina samples, L and meso-Z, but not Z, appeared in the L-supplemented group, while only Z was found in the group supplemented with Z. In humans, a study investigating the serum and macular responses of L, Z, and meso-Z from dietary supplements found that 13.13 mg/d for 12 weeks provided maximum MPOD improvement, whereas 7.44 mg/d was the amount that increased serum levels at the highest efficacy.[121] These and other studies support the need for dietary recommendations for L and Z, particularly as conditionally essential nutrients due to their protective effects on eye health.[122]

Lutein and Z fulfill many criteria as essential nutrients, including high concentrations in select tissues, biological plausibility for eye health, depletion outcomes such as vision impairment in primates, and inverse associations with certain diseases.[123] In addition to their role in eye health, L and Z are involved in cognitive function at all stages of life.[124] A randomized controlled trial reported that L and Z supplementation improved neural efficiency and learning performance by increasing the interaction of numerous brain regions in older adults.[125] Other reports have demonstrated an association between L intake or circulating levels and preserving age-related cognitive decline, reducing the risks of certain cancers, coronary heart disease, stroke, metabolic syndrome, and achieving higher levels of physical activity.[126][127][128] A systematic review of *in vivo*, *ex vivo*, and *in vitro* studies concluded that L may benefit vascular health by improving

endothelial function, reducing inflammation, regulating favorable lipid profiles, and maintaining glucose homeostasis.[129] Systematic reviews summarizing the amount of L needed for cognitive functions and enhancement of gray matter volume estimated that at least 10 mg/d for 12 months could be beneficial.[130,131]

Age must be considered when creating DRI values, since MPOD values are lower in older compared to younger individuals.[132] Whether the proposed intake of L and Z should be based on the amount that can reduce AMD risk, benefit visual maturation in newborns, protect cognitive health, or reduce the risk of other diseases requires further consideration. Sex differences in AMD prevalence must also be considered, especially in relation to pregnancy and lactation, as discussed above. Nevertheless, L and Z are not included for DRI consideration due to inadequate details from food databases, limited large-scale dietary intake studies, and insufficient knowledge regarding their metabolism and biological functions.[133] Many continue to advocate for a DRI for L, since it satisfies all nine criteria for bioactive compounds.[134,135]

Goji Berries and Eye Health

A rich dietary source of L and Z is goji berry, (*Lycium barbarium* and its closely related species *Lycium chinese*), also called wolfberry or Gou Qi Zi. The bright orange-red colored oval fruit, has been used for millennia in traditional Chinese medicine (TCM) for its role in visual health, to provide immunoregulatory, neuro-protective, and anti-inflammatory benefits, and to help regulate liver and kidney meridians (Figure 2).[136137138] Commercially-available goji berries and their products come primarily from the Ningxia and Xinjiang autonomous regions in western China.[139] Goji berry is known for its high amount of carotenoids, with the Z content higher than any other known food.[140,141] In addition to carotenoids, other bioactive compounds found in goji berries include Lycium barbarum polysaccharides (LBP), flavonoids, vitamins, minerals, betaine,

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cerebrosides, phenolic acids, and certain amino acids which may also support the overall health of the eye, particularly when working synergistically.[137,139,142] Although the TCM use of goji berry also includes the leaves and bark of the plant, this review will discuss the potential benefits of the fruit (*Fructus lycii*) on eye health.

In addition to a robust amount of Z, goji berries contain modest amounts of β-cryptoxanthin, β-carotene, neoxanthin and L.[136,140,143] The Z and L content among different varieties of dried goji berries cultivated in Ningxia province ranged from 25 to 152 mg/100g, and 0.3 to 1.9 mg/100g, respectively.[143] According to the United States Department of Agriculture food database, one serving of goji berries is 28 g, which would provide up to 42.6 mg of L + Z, depending on the cultivar.[144] Moreover, the predominant form of Z in goji berries is a dipalmitate, found with a diester linkage.[145] The ratio of Z dipalmitate to total carotenoids was up to 55% and 88%, in fresh and dried goji berry fruit, respectively.[145,146] This esterified form of Z showed a significantly higher intestinal absorption than monoester and free Z due to the high efficacy of hydrolysis, mainly by carboxyl ester lipase.[147] Plasma Z was significantly increased in individuals consuming 15g goji berries daily for 28 days in comparison to those on a habitual diet.[148] Participants consuming 5 mg of Z dipalmitate extracted from goji berries showed a higher plasma Z concentration than when they consumed the same amount as unesterified Z over a nine to 24 hour period.[149]

The high Z content of goji berries has been proposed as a dietary source to reduce the risk of AMD, although studies are limited.[150] In one study, circulating Z levels were significantly higher in healthy older individuals who consumed 10 mg of Z extract from goji berries daily for 90 days.[151] No change in macular pigmentation or soft drusen was observed, but MPOD was not measured. In an uncontrolled trial, individuals with early stage AMD who consume a beverage

containing 12 mg of L and 2 mg of Z derived from marigold flower and goji berry, respectively, daily for five months, showed higher circulating levels of L and Z, lower intraocular pressures, and better best-corrected visual acuity (BCVA) scores.[152] Unfortunately, the study lacked a control group, did not test the effect of Z separately, and did not clarify whether the form of Z extracted from goji berry was the dipalmitate. Another study investigating the effects of an herbal formula among healthy adults with dry eyes noted that those chewing tablets containing L (6, 10, or 14 mg), Z (1.2, 2.2, or 2.8 mg), extracts from blackcurrant, chrysanthemum, and goji berry showed dose-dependent reductions in eye fatigue symptoms, improved tear secretion as well as MPOD, compared to placebo.[153] The basis of this formula was derived from TCM, so the multicomponent formulation could not directly inform the role of any single ingredient. A study in patients with early AMD reported that the MPOD was significantly higher in those consuming 25 g/day of goji berries (containing approximately 15 mg of Z and 2.5 mg of L) for 90 days, compared to their baseline levels and to a habitual diet control group. The BCVA was also significantly improved in the goji berry group compared to their baseline values.[154] We recently reported that MPOD and skin carotenoid scores were increased in healthy middle-aged individuals consuming 28 g/day of goji berries (containing approximately 28.8 mg of Z and an estimated 0.15 mg of L) five times a day for 90 days compared to a group taking a supplement with 6 mg of L and 4 mg of Z.[155] These results illustrate that MPOD levels can increase in healthy individuals even without early signs of AMD. While these results are encouraging, longer intervention periods with a larger number of participants are necessary.

In addition to AMD, goji berries have been studied as a therapy for retinitis pigmentosa, an inherited retinal disease. Patients who consume 0.35 g/d of LBP for 12 months showed a significant improvement in visual acuity and macular thickness, compared to control subjects

who did not consume L or Z.[156] Examples of human studies that evaluated the effects of supplements containing goji berries on retinal health are shown in Table 1.

Based on preclinical evidence, potential benefits of goji berry intake on glaucoma and diabetic retinopathy may also exist. Goji berry extract ameliorated the high glucose-induced blood-retinal barrier disruption in human retinal pigment epithelial cells.[157] Studies reported that LBP showed significant neuroprotective effects over retinal ganglion cells in male C57BL/6N mice and Sprague-Dawley rats with ocular hypertension.[158-160] In db/db mice, goji berry extract restored the thickness of the retina, the ganglion cell number, and the integrity of RPE after daily intake over eight weeks.[161]

Although research on the upper limit of goji berry intake is scarce, goji berry allergy risk has been associated with the existence of cross-reactivity to nonspecific lipid transfer proteins from peaches, tomatoes, tobacco, tree nuts, and select pollens.[162,163] In addition, bleeding symptoms after consuming goji berry juice, tea, or wine have been described in case reports among patients taking warfarin, an anticoagulant medicine.[164,165] Although the potential value of foods high in L and Z during pregnancy and lactation has been discussed above, the utilization of goji berry products during these unique periods in a woman's life needs special caution.

Clinical studies of goji berries on eye health have been conducted primarily in Asia, with emerging research reported from Italy, Lithuania, and Switzerland.[166-168] Potential gene-nutrient interactions must be considered when comparing results from Asian with Caucasian populations.

Conclusion

Macular xanthophylls cannot be synthesized *de novo* in primates. The oxidative defense and blue light filtering characters of L, Z, and meso-Z are important for visual function and potentially

reducing the risk of AMD. Because L and Z storage in the maternal body during pregnancy and lactation may become depleted due to active transfer to the offspring, more attention to safe and robust intake of foods and dietary supplements containing these xanthophylls is warranted. Since many infant formula products are not fortified with L or Z, addition of these two xanthophylls should also be considered.

The impact of foods and dietary supplements rich in L and Z on MPOD and visual function among AMD patients and in healthy individuals deserves further attention. Since Z may play a different role than L in terms of macular pigment development or protection, better analytical techniques are needed to reassess food composition databases to distinguish these compounds, with greater attention paid to Z as a stand-alone food component, rather than grouping it with other xanthophylls.

Goji berries have the highest known content of Z of any commonly consumed food, which also comes in a unique dipalmitate form. Given the increasing rates of AMD worldwide, goji berries, along with L and Z supplements, may help reduce this escalation. A reexamination of the US DRI status is warranted.

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Figures and Tables

Table 1. Human studies using goji berries directly or in supplements on eye health. AMD: age-related macular degeneration; BCVA: best corrected visual acuity; L: lutein; MPOD: macular pigment optical density; Z: zeaxanthin.

Authors and year	Sample	Intervention	Frequency and duration	Outcome measures	Results
Bucheli et al. (2010)[151]	150 healthy seniors (ages 65-70 years)	13.7 g/d of a milk-based formulation with 10 mg Z and 68.5 mg vitamin C derived from goji berry OR placebo	Daily for 90 days	Funduscopic exams	The placebo group showed increased hypopigmentation and soft drusen accumulation in the macula, while the treatment group remained stable
Chan et al. (2019)[156]	42 patients with retinitis pigmentosa (ages 26-69 years)	Goji berry OR placebo granules	Daily for 12 months	Visual acuity, Ganzfeld full-field electroretinogram, Humphrey Visual Field Analysis, and Spectral-domain Optical Coherent Tomography	The treatment group maintained contrast visual acuity and macular thickness while the placebo group showed a decline in all measures
Kan et al. (2020)[153]	303 healthy individuals with dry eye symptoms (ages 18-65 years)	Three treatment arms giving chewable tablets with the highest L of 14 mg, Z of 2.8 mg, goji berry extract of 175 mg, crysanthemum extract of 175 mg, and blackcurrent extract of 233 mg, OR placebo	Daily for 90 days	Eye fatigue symptom score, MPOD, Schirmer test, optical coherence tomography, and keratography	The treatment groups showed significantly reduced eye soreness, blurred vision, dry eye, foreign body sensation, and tearing, improved tear secretion and increased first tear break-up time, average tear break-up time, tear meniscus height, and MPOD, compared to the placebo group
Li et al. (2018)[154]	114 patients with early AMD (ages 51-92 years)	25 g/d of dried goji berry OR habitual diet	Daily for 90 days	MPOD, serum L+Z, BCVA	Increased serum Z and MPOD in the goji berry group compared to their baseline levels or to the control group

Li et al. (2021)[155]	27 healthy individuals (ages 45-65 years)	28 g/d of dried goji berry OR supplement with 6 mg L and 4 mg Z	Five times a week for 90 days	MPOD, skin carotenoids	Increased MPOD and skin carotenoid score in the goji berry group compared to no significant changes in the supplement group
Peng et al. (2016)[152]	56 patients with early AMD (ages 35-50 years)	60 ml/d of beverage containing 12 mg of L from marigold flower and 2 mg of Z from goji berry	Daily for 5 months	Serum L and Z, plasma oxidative indices, antioxidant enzymes in erythrocytes, anti-inflammatory markers, BCVA, intraocular pressure, photostress recovery, ocular comfort index, MPOD	The treatment group showed significantly increased serum L and Z, antioxidant capacity, antioxidant enzymes, ocular comfort index, and MPOD, and a decreased oxidative stress index, inflammatory markers, BCVA, and interocular pressure compared to baseline values

Figure 1. A. Spectral domain-optical coherence tomography of a macular region, showing anatomical layers. B. Fundus image of a healthy adult retina. The dark area is the macula; the light area is the optic nerve.
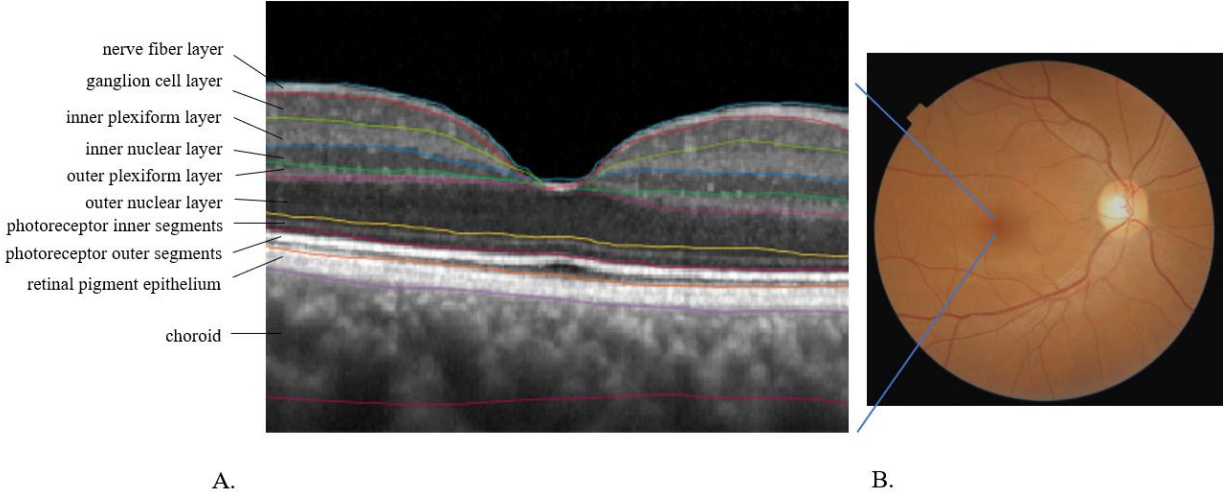

A.

B.

Figure 2. Dried goji berries

Chapter V

Perspectives and Conclusions

Many regions of the world have a history of using fruits as part of traditional medicine. The epidemiological, animal, and clinical studies on the health benefits of select fruits have been demonstrated, but more human research is needed.[1,2] Besides carbohydrates, vitamins, minerals, and fiber, fruits are rich in phytonutrients. Some fruits such as mango and mangosteen are particularly high in xanthonoids, while others, such as goji berries, are rich in certain carotenoids.[3] The consumption of certain fruits has been associated with a decreased risk of cardiovascular diseases and age-related eye diseases.[4,5] However, according to the Dietary Guidelines for Americans 2020 – 2025, 80% of the adult population does not achieve the goal of consuming two cups of fruits per day.[6]

As described in Chapter I, (–)-epicatechin in cocoa, and anthocyanins in blueberries can act as bioactive compounds for their anti-hypertensive and vasodilatory effects. In cell culture and animal studies, mangiferin and carotenoids in mango may be main factors that contributes to reported vascular protection. However, clinical intervention trials investigating the efficient amount, bioavailability, and impacts of mango or mango extracts with purified mangiferin and other bioactive compounds on vascular-related outcome measures, such as flow mediated dilation, peripheral arterial tonometry, blood lipids, and platelet aggregation, are warranted. Reports have shown that the composition of phenolic compounds in mango and blueberries varied significantly among different cultivars.[7,8] Therefore, choosing fruit cultivars with high bioactive profiles for further research is important.

Chapter I also discussed evidence on goji berries and eye health, with an emphasis on clinical trials. Although clinical studies focus on the high concentration of carotenoids, especially zeaxanthin (Z) and lutein (L) in goji berries, other bioactive compounds such as lycium barbarum polysaccharides (LBP), vitamins, minerals, taurine, and betaine may have synergistic effects on eye-related diseases through different mechanisms. More clinical studies are encouraged to investigate the effects of goji berries to help prevent age-related macular degeneration as well as on the development of glaucoma and diabetic retinopathy.

The suggestion for more clinical research outlined in Chapter I is based, in part, on the study presented in Chapter II, which was conducted to investigate the effects of goji berry intake on eye health. The target population was comprised of healthy men and women aged 45 to 65 years old. The consumption of 28 g goji berries five times a day for 90 days increased macular pigment optical density (MPOD) and skin carotenoids, while a control group taking a commercially available supplement with 6 mg of L and 4 mg of Z showed no changes.[9] My study suggests that the concentration of macular pigments can increase, even in healthy individuals without early signs of age-related macular degeneration (AMD). Future research with a larger number of participants, a longer intervention period, and more visual health measurements is encouraged. Further, testing the effect of regular goji berry intake in a group of at-risk adults showing mild signs of AMD (e.g., small drusen) would be an exciting project.

Chapter III details two clinical studies investigating the effects of Ataulfo mango intake on markers related to cardiovascular health after a single intake over a two-hour assessment period, and then continuing with daily intake of the fruit for two weeks. The at-risk population in this study was postmenopausal overweight and obese women 50 to 70 years old. The study found that the consumption of 330 g fresh frozen mango after a single intake, and after two weeks of

daily intake, did not change microvascular function, platelet aggregation, or cholesterol markers, but did result in a significant decrease in systolic blood pressure. A second follow-up study noted that mango intake did not increase blood glucose or insulin two hours after intake, in contrast to consumption of an isocalorically similar ingestion of white bread. However, both white bread and mango intake decreased pulse pressure two hours after ingestion, suggesting this finding might be due to a postprandial effect. A stronger insulin fluctuation was noted after the intake of white bread compared to mango, which might be due to the blood glucose-tempering effects reported for mangiferin, a key constituent in mangos. Further clinical studies with a larger number of participants are needed to investigate the impact of mango more fully on blood glucose regulation. Additionally, although the amount of mangiferin in Ataulfo mango is higher than other cultivars, the overall content of this unique polyphenol is lower in the pulp compared to skin or to the mango tree bark.[10] The ripeness of mango also affects the phytonutrient profile.[11] Given the traditional use history of mango, including the fruit skin and the tree bark, further studies examining the efficacy of mango by-products or mango at different stages of ripeness are indicated.

Chapter IV reviewed the roles of L and Z regarding their ability to filter blue light and provide oxidant defense in the macula and retina. Such protection is crucial for eye health and for the treatment and possible prevention of AMD. With modern technology, the accumulation of macular xanthophylls such as L and Z can be measured noninvasively and quantified as MPOD. A meta-analysis concluded that the minimum amount of L and Z to increase MPOD was estimated to be 10 mg/day. However, the average reported dietary intake of these two carotenoids in the United States has been below 2 mg/day, with a decreasing trend over the last 30 years. A number of studies have reported that the supplementation of L and Z improves

MPOD in people with intermediate to advanced AMD, but no randomized controlled trials have proposed an effective strategy to prevent the occurrence of AMD or how to delay the progression from early to intermediate phases of the disease. Epidemiological date notes that the prevalence of AMD is higher in females than males. Although the exact mechanisms are not clear, Chapter IV presents a possible explanation due to the depletion of L and Z through maternal-infant transfer during both pregnancy and lactation. Together with the low dietary L and Z intake in this population, the optimal accumulation and long-term storage of these macular xanthophylls may become compromised. More research is needed to confirm this hypothesis. Building on my work detailed in Chapter II, Chapter IV also focused on goji berries, a dietary source with the highest known amount of Z of any commonly consumed food. Given the increasing rates of AMD worldwide, consumption of goji berries, along with L and Z supplements, may help reduce this acceleration.

In conclusion, in many cases the traditional use of select fruits as medicine has guided modern research in nutrition and medicine, and the identification of bioactive compounds for health promotion and disease prevention. Traditional medicine is largely based on the life experiences of healers, and from trial-and-error that has evolved over centuries. The scientific method has been used as a tool to examine and verify many traditional practices, though much more remains to be explored. Future research identifying the effective use of fruits, including the amount and frequency of intake and which populations might benefit most effectively, is warranted. Future dietary recommendations identifying specific fruits, and their bioactive compounds will predictably enhance health promotion and disease prevention.

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