The Influence of Mango Fruit Intake on Skin and Vascular Health in Humans

By

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ABSTRACT

The role of nutrition in dermatology has been investigated for a long time, with a history of research focused on the effects of nutritional disorders on the skin. Some classic examples include pellagra, scurvy, and acrodermatitis enteropathica. Although these conditions have been mainly eradicated from developed countries due to fortification of food with the essential nutrients of concern or supplementation upon early detection, they can still be found among individuals or communities who are not receiving adequate nutrition due to factors such as economic instability, alcoholism, or eating disorders. As skin diseases caused by nutrition deficiencies become under control, current research focuses on managing dermal disorders such as dermatitis and acne, or promoting esthetics by decreasing wrinkles and hyperpigmentation as well as increasing hydration, collagen, and elasticity.

Epidemiological studies suggest that a diet pattern rich in plant-based foods such as fruits and vegetables is associated with improved skin esthetics, such as fewer wrinkles, and lowers the risk of dermatological disorders. Plant-based foods are typically rich in bioactive compounds such as carotenoids, vitamins, and polyphenols that provide oxidant defense, protect against DNA damage and promote structural integrity to the skin. Deficiencies of select micronutrients such as vitamin A, C, E, and K have also been associated with certain skin disorders such as thickening of the skin, poor wound healing, and dermatitis. Unlike health conditions such as cardiovascular disease and diabetes, official dietary recommendations for specific skin disorders or esthetic concerns do not currently exist. The 2020 – 2025 Dietary Guidelines for Americans recommend consuming whole fruits and a variety of vegetables, with specific reference amounts for those with dark green, red, and orange colors, which are typically rich in carotenoids. This

general recommendation aligns with the epidemiological studies that suggest beneficial effects to the skin by adopting a dietary pattern that consists of more plant-based foods. However, each fruit and vegetable has a unique nutrition profile and may elicit different skin benefits.

This dissertation aimed to explore and understand the benefits of different plant-based food consumption on the skin. A particular emphasis was placed on mango, a tropical fruit that is widely consumed, and its relation to skin and inflammation. Chapter I provides some historical background on nutrition and skin, contextual information on the structure and functions of the skin, as well as a literature review of the reported effects of dietary carotenoids, vitamins, and polyphenols that can be found in mango on skin health and their potential mechanisms. The promising effects of mango intake led to the development of a study as detailed in Chapter II, which is a published manuscript that reported on a clinical trial that investigated the effects of fresh-frozen mango fruit intake on facial wrinkles in the lateral canthi and erythema in the cheeks of postmenopausal women with fair to beige skin tones. Chapter III is a manuscript that will be submitted for publication that reviewed the current evidence for consumption of plant-based foods and extracts, including fruits, vegetables, nuts, and legumes, on skin protection assessing parameters related to collagen, elasticity, erythema, hydration, roughness, and wrinkle. The narrative review focused on clinical dietary interventions with an aim to enable dietitians to provide better dietary recommendations with regards to select dermatological concerns. Chapter IV discussed the role of inflammatory markers on cardiovascular health and describes a study that investigated the effects of mango intake on endothelial function and pro-inflammatory markers. Appendix A is an accepted manuscript that described the development, utilization, and efficiency of a nutrition education game that aimed to elicit and reinforce healthier snack choices in children aged 9 to 13 years old. Appendix B details a newly funded proposed study to

investigate the effects of mango intake on wrinkles and other skin measurements and changes in the gut microbiome by comparing it to a control group. In part, to confirm the findings from the study reported in Chapter II as the lack of a control group was a major limitation. Finally, a summary of this work and a discussion on future research directions will be presented in the Perspectives and Conclusions section.

CHAPTER I

LITERATURE REVIEW

1. Introduction

The relationship between nutrition and skin health has come a long way since the 1700s. Initially, research was focused mainly on the effects of nutritional disorders on the skin. One classic example is pellagra, a deadly photosensitive skin disease caused by niacin (vitamin B3) deficiency that was first described in 1735 by Gaspar Casal(1). Fortification of flour with niacin has mostly eliminated pellagra from developed countries. However, it is still a disease that can be found among the impoverished who are not receiving adequate nutrition(2), or malnutrition caused by other conditions such as alcoholism(3) or eating disorders (4). Scurvy, caused by a deficiency in vitamin C (ascorbic acid), is another well-known morbid skin disease with descriptive reports of the signs dating back to 1550 BC but mostly connected to the history of sea voyages in the 15th to 18th centuries(5). This disease, characterized by swollen bleeding gums and reopening of healed wounds, has been largely eradicated by simply consuming foods or supplements with vitamin C(5). Acrodermatitis enteropathica is a rare autosomal recessive disorder caused by a mutation in a gene that reduces the synthesis of zinc transporters and causes an inability to effectively absorb dietary zinc leading to a deficiency(6). Once diagnosed, this condition can be effectively treated with life-long supplementation of zinc sulfate(7).

As science advances and skin diseases caused by nutrition deficiencies and malabsorption become manageable, the focus has turned to identifying the role of diet with respect to skin health on new disorders and enhancing esthetics. Present skin disorders of concern are often complicated

by various factors including genetics. Take for example, the risk of eczema increases by three-fold in those with a filaggrin gene deficiency, which disrupts the epidermis structure and causes increases in transepidermal water loss (TEWL) and dry skin(8). A reduction in collagen, hydration, and elasticity, along with an increase in wrinkles, has been linked to a decline in estrogen as women approach peri- and postmenopausal status(9). Women have been found to have deeper wrinkles in the perioral region than men in a similar age range(10). Skin elasticity and distensibility decrease gradually over time in men but sharply diminish in women in the 40 – 74 year age range(11). Skin type also impacts pathologies, with Fitzpatrick skin phototype (FSPT) I, II, and III, which have less melanin, tend to be more sensitive to UVR and have a higher risk of developing skin issues such as wrinkles and sunburn(12).

In addition, skin diseases have been associated with other health conditions, as evidenced by a significant correlation between psoriasis and hypertension, diabetes mellitus, and metabolic syndrome(13). Psoriasis has also been found to significantly increase the risk of stroke and myocardial infarction(14). Inflammation is typically the link between skin disorders and other health conditions. Patients with moderate-to-severe atopic dermatitis were found to have significantly increased serum high sensitivity c-reactive protein (hsCRP) levels compared to those without the skin disorder(15). Polycystic ovary syndrome, an endocrine disorder in adult women of reproductive age, increases the prevalence of skin disorders that includes acne and acanthosis nigricans (dark, velvety patches)(16). A study also found that participants with acne had an increased likelihood of having a family history of the skin disorder, severe or morbid obesity, and consumed greater amounts of milk, dairy, chocolate, sweets, and ice cream(17).

Habitual lifestyle is another factor that can affect the skin. Smoking significantly disrupts the skin, as demonstrated by non-smokers having a lower wrinkle depth(18), fragmented elastic

fibers(19), and interstitial collagenase matrix metalloproteinase (MMP)-1(20) compared to heavy smokers. Total alcohol intake has also been significantly associated with a higher risk of squamous(21) and basal cell carcinoma(22) in both men and women. A higher intake of milk, sugary beverages, milk chocolate, snacks, and fast foods, and fatty and sugary products, as well as significantly less meat, fish, vegetables, fruits, and dark chocolate was found in people with current acne compared to those who never had the skin disorder(23). Furthermore, youths with a recent diagnosis of atopic dermatitis were found to consume more convenience and fast foods, as well as carbonated and energy drinks(24). Better adherence to the Dutch Healthy Diet Index guidelines and higher fruit intake was associated with fewer wrinkles in women(25). Higher fruit and vegetable consumption has also been associated with lower plasma concentrations of hsCRP, homocysteine, and gene expression of pro-inflammatory markers(26). In addition, higher total dietary antioxidant capacity showed lower plasma hsCRP and expression of pro-inflammatory markers(26). Women aged 40 to 75 with a wrinkled appearance were found to have significantly lower intakes of protein, total dietary cholesterol, phosphorus, potassium, vitamin A, and C compared to those without(27). A significantly lower intake of linoleic acid and vitamin C was also observed in only those with dry skin. In Japanese women aged 20 to 74, the Daniell wrinkling score was significantly inversely associated with green and yellow vegetable intake and positively associated with saturated fat consumption(28). Modifications in dietary intake have great potential to prevent or alleviate specific skin disorders symptoms. In order to truly comprehend the effects of nutrition on the skin, it is essential first to understand its functions.

2. Structure and functions of the skin

The skin is the largest organ in the human body and forms a protective physical barrier against environmental, mechanical, and chemical insults that can generate free radicals(29). Homeostasis within the skin is also maintained by combating reactive oxygen species (ROS) that can be produced from metabolic processes. The skin consists of three layers, the epidermis, dermis, and hypodermis (Figure 1). These three components undergo degenerative changes with aging, with alterations in the dermis being the most obvious(29,30). Measurements of six skin parameters (TEWL, surface pH, stratum corneum (SC) hydration, skin surface temperature, skin color) at 16 anatomical sites demonstrated inconsistencies among people of different ages(31).

The epidermis is the first layer of the skin with direct contact to the external environment that is composed of dense keratinocytes(30). The epidermis comprises five layers, with the outermost layer known as the *stratum corneum* or the horny layer which is filled with large flat corneocytes embedded in a lipid matrix. Below it lies a transitional layer called the *stratum lucidum*, followed by the *stratum granulosum* or granular cell layer which is composed of flattened granular keratinocytes. The fourth layer is the *stratum spinosum* or spinous layer, which consists of polygonal keratinocytes connected by desmosomes. Lastly, *stratum basale,* which is a single layer of keratinocyte stem cells, sits at the bottom and produces progenitors and keratinocytes that migrate to the more superficial layers in about 28 days. Melanocytes that produce melanin pigments can also be found in this section of the epidermis. The epidermal permeability barrier varies with age, sex, body sites, and pigment types(32). A depletion in the major lipids of the stratum corneum has been observed in skin disorders such as psoriasis, atopic dermatitis, ichthyosis, and xerosis(33).

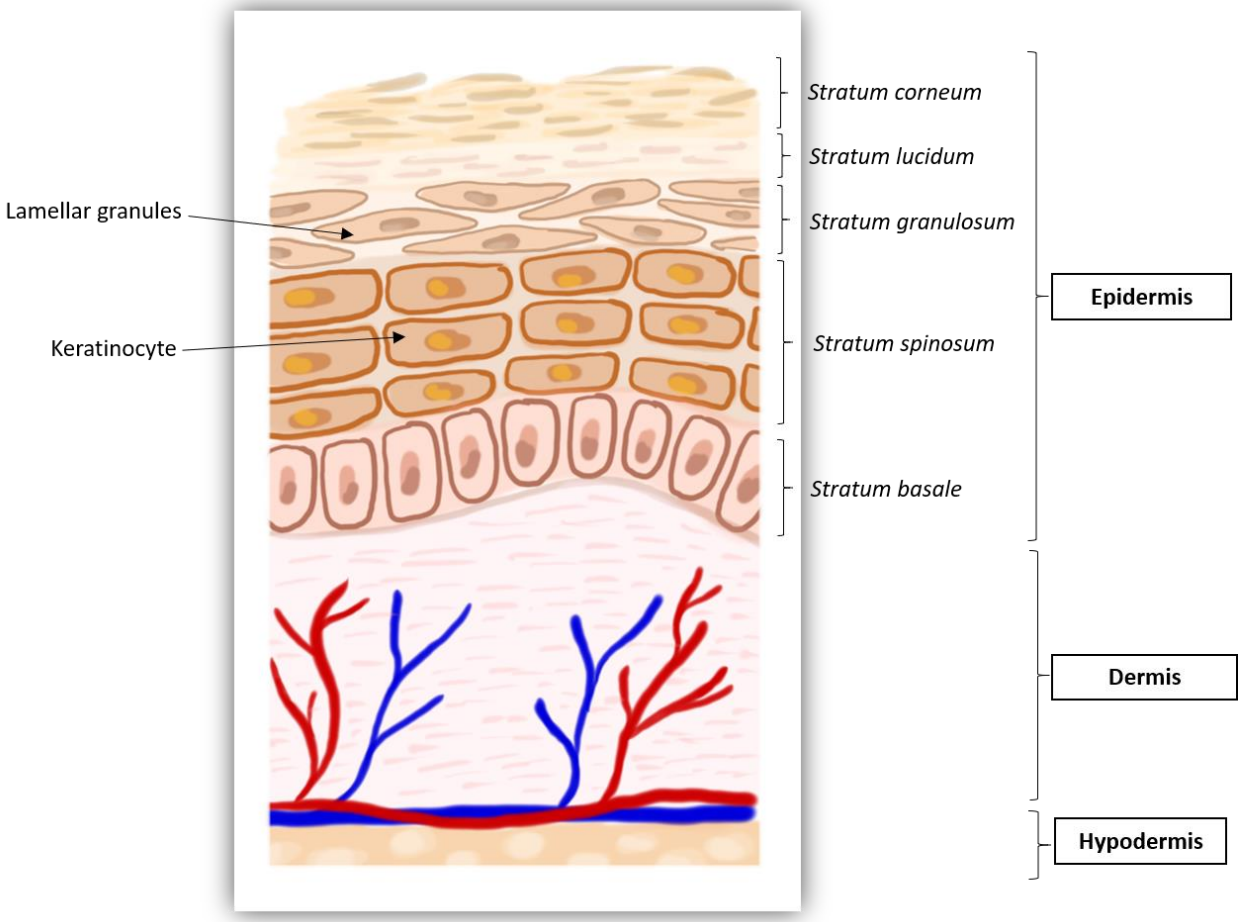


Figure 1. Human skin structure

The dermal-epidermal junction connects the keratinocytes in the epidermis to the dermis via anchoring filaments(34). The network of interconnecting proteins and finger-like projections of rete ridges in the dermal-epidermal junction helps to provide structural integrity and mechanical stability to the skin. Flattened dermal-epidermal junction and shortened rete ridge were found in aged compared to young skin. Major components such as collagen IV, VII, and XVII, as well as integrin β4 and laminin-332 provide stability to the dermal-epidermal junction and have been observed to significantly decrease in the skin of older people as compared to the young.

The dermis is comprised mainly of collagen fibers, a major component of the extracellular matrix (ECM) accounting for 75% of the dry weight of skin and provide tensile strength and elasticity(30). In human skin, type I collagen contributes to 80 to 90% of the total collagen, while type III accounts for 8 to 12%, and type V is less than 5%. Typically the collagen bundles increase in size deeper in the dermis. Elastic fibers contribute only 0.2% of the dry weight of the dermis but can absorb water up to 1000 times their volume and function to return the skin to its normal structure after being stretched or deformed. The other ECM components are proteoglycans and glycosaminoglycans, which surround and embed the fibrous and cellular matrix elements in the dermis. Fibroblasts are dermal-resident cells responsible for the synthesis and degradation of ECM proteins. Other cellular components of the dermis include immune cells like histiocytes, mast cells, dermal dendrocytes, endothelial cells, and skin appendages.

The hypodermis, located below the dermis and the thickest layer of the skin, connects the upper layers to the bones and muscles at the bottom(29). The hypodermis consists of connective tissue of collagen and elastin, adipocyte, and blood vessels, allowing it to protect against mechanical shock, provides thermal insulation, and contributes to energy metabolism and fatty acid storage.

2.1 Intrinsic and extrinsic factors of aging

Aging affects the structure and functions of the skin. Extrinsic aging is characterized by deep wrinkles, skin laxity, and hyperpigmentation and is mainly caused by chronic sun exposure(30). Intrinsic aging occurs with advancing age and is characterized by fine wrinkles and

a thinning epidermis. Regardless of aging type, wrinkles and reduced elasticity are typical skin aging outcomes due to progressive deterioration of the dermis.

Extrinsic factors

Environmental factors such as ultraviolet radiation (UVR) and pollutants are common contributing factors to skin aging. Both ultraviolet(UV)-A and -B irradiation can inflict damage on the skin via different mechanisms(35). Ultraviolet A rays have the longest wavelengths and mainly produce free radicals or ROS through interaction with endogenous photosensitizers (Figure 2). The ROS will then indirectly damage DNA, protein, and membranes, contributing to the photodamage of dermal connective tissue cells and proteins. Ultraviolet B wavelength can be directly absorbed by DNA, which disrupts it. Free radicals and ROS have a major role in lipid peroxidation, which disrupts the cell membrane. Ultraviolet radiation can also stimulate the production of neutrophil extracellular traps (NETs) that are released by neutrophils, resulting in NETosis, a form of programmed cell death(36). NETosis caused by UVR-induced ROS production that acts as signal mediators, was much faster than activation by chemical or biological factors. Endogenous and dietary antioxidants can successfully inhibit UV-induced NETosis. Air pollutants exist outdoors and indoors and can enter the skin via nanoparticles, inhalation, or ingestion, generating chemicals that produce ROS(37). Concentrated air particles are a type of atmospheric pollutant that have been shown to increase inflammation, alter phospholipids, and increase ROS and oxidative stress in the skin(38). Air pollutants have also been associated with signs of premature aging such as wrinkles, hyperpigmentation, lentigos, melasma, atopic dermatitis, skin barrier dysfunction, psoriasis, acne, and skin cancer.

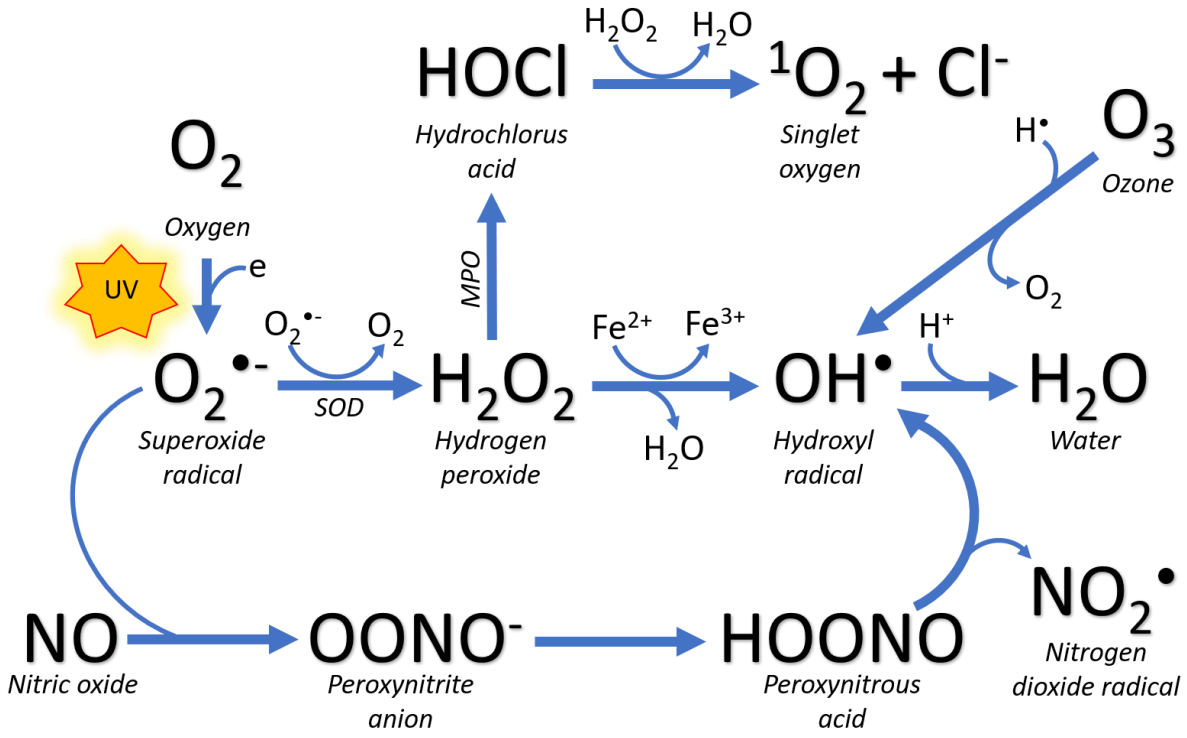


Figure 2. Reactive oxygen species (ROS). SOD = superoxide dismutase, MPO = myeloperoxidase

Intrinsic factors

The genetic constitution of each individual determines intrinsic aging. Caucasian, African American, East Asian, and Hispanic skin each have distinguishing features of aging, but all populations share dyspigmentation, fine lines, and loose skin(39). Increased melanin content predisposes darker skin to a greater degree of hyperpigmentation, but skin thickness may protect against the formation of fine lines. Telomere length was observed to decrease in the epidermis and dermis with age but did not differ between sun-exposed and sun-protected sites of the body, suggesting an association of telomere shortening with only chronological aging but not UV exposure(40). However, telomerase activity, which maintains the length of telomeres that shorten

with every cell division, can be reduced by extrinsic factors such as air pollution(41) and chronic oxidative stress(42). In addition, the formation of interstitial type I collagen was found to significantly decline with age, while degradation of the protein remained relatively consistent throughout(43). Type I collagen is the main component in the ECM, and a decreased turnover rate indicates a reduction in skin support and regulation.

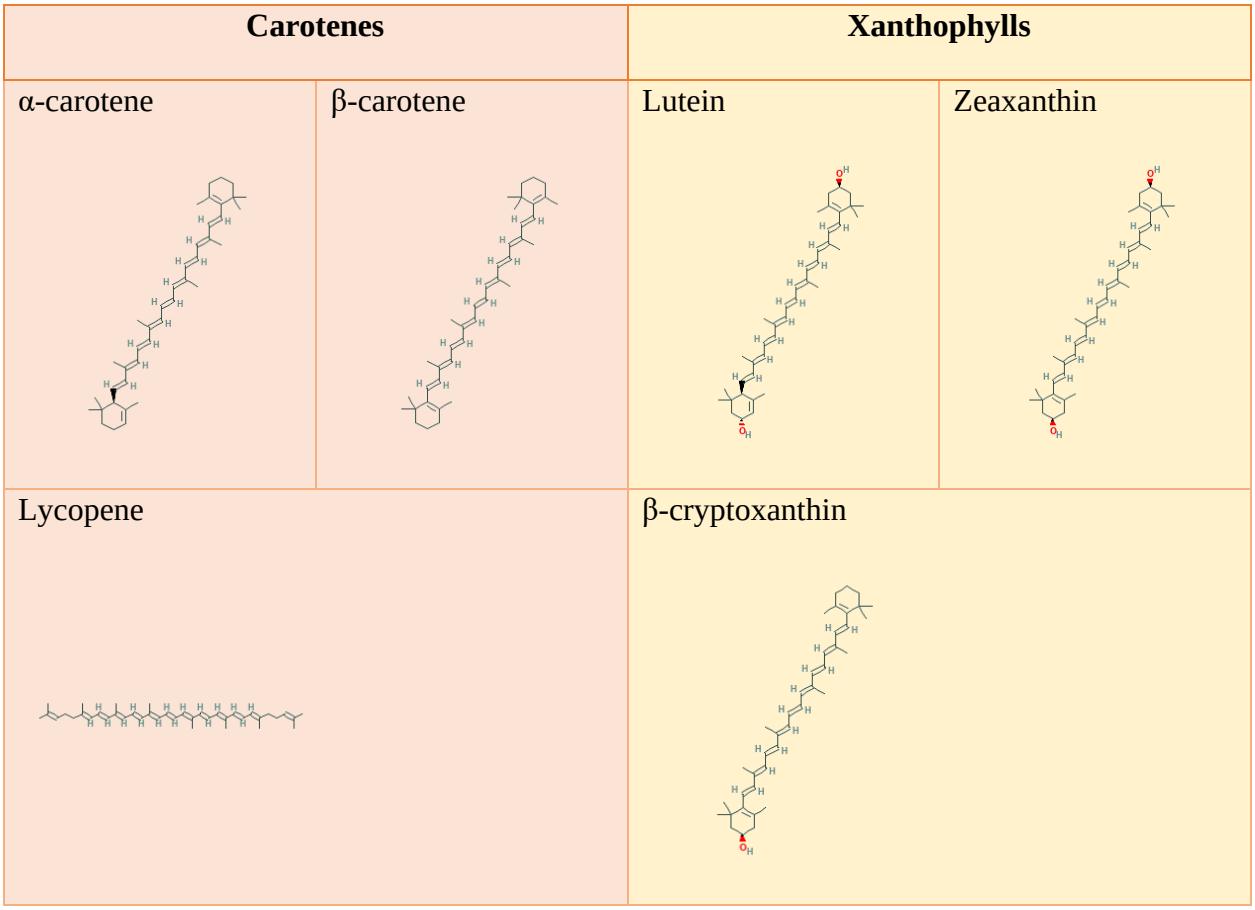
3. Mango and its bioactive compounds

The 2020 – 2025 Dietary Guidelines for Americans recommends a variety of fruit intake, especially whole fruit, with a daily goal of two cups(44). Mango is mentioned as one example of fruit to consume, but there are no specific recommendations on the amount. Research on the effects of mango consumption on human health is currently limited, but the nutritional value of the fruit and its potential benefits have garnered interest among researchers in recent years. Consumption of freeze-dried mango pulp powder for 12 weeks, at 10 g/day which is equivalent to 100 g of fresh fruit, significantly reduced blood glucose in obese adults(45). In addition, daily intake of 400 g of mango pulp for six weeks significantly decreased systolic blood pressure in lean individuals and reduced inflammatory cytokines interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 in the obese group(46). A non-significant reduction in hemoglobin (Hb)A1c and plasminogen activator inhibitor (PAI)-1 was also observed in obese participants. Circulating PAI-1 levels can predict the development of insulin resistance, type 2 diabetes mellitus, and atherosclerosis. In order to achieve a comprehensive understanding of mango and its benefits, it is necessary to recognize the fruit's nutritional composition and its bioactive compounds. This literature review aims to describe the carotenoids, vitamins, and polyphenols in mangos and explore the dietary benefits to the skin.

3.1 Carotenoids

Carotenoids are naturally occurring pigments that can be found in vegetables and fruits that have red, orange, or yellow colors and green leafy vegetables. Carotenoids are generally classified into either carotenes or xanthophylls based on their molecular structure(47). Carotenes do not contain oxygen atoms and include α-, β-, and γ-carotene, as well as lycopene, phytoene, and phytofluene(47). Xanthophylls contain oxygen atoms and include lutein, zeaxanthin, β-cryptoxanthin, astaxanthin, and fucoxanthin(47). The molecular structures of carotenoids in mangos are presented in Figure 3.

Figure 3. Molecular structure of carotenoids in mangos



Molecular structures derived from PubChem(48).

The levels and types of carotenoids present in vegetables and fruits depend on multiple factors such as cultivar or variety of the plant, maturation or ripeness, climate exposed during growth, farming practice, post-harvest storage, and food processing(49). According to the USDA Nutrient Database (United States Department of Agriculture, Standard Reference report 28), 100 g of raw mango (#09176), based on Tommy Atkins, Keitt, Kent, and/or Haden cultivars, contains 640 mcg β-carotene, 9 mcg α-carotene, 10 mcg β-cryptoxanthin, 3 mcg lycopene, 23 mcg lutein and zeaxanthin(50). However, the nutrient value varies among cultivars. A study found that Tommy Atkins contained the lowest (4.9 ± 1.5 mg/kg of fruit weight [FW]) average β-carotene, while Ataulfo had the highest amount (26.1 ± 4.4 mg/kg of FW) compared to Keitt, Kent, and Haden varieties(51). That would equate to about 2610 mcg β-carotene per 100 g of Ataulfo mango, which is four times higher than the standard amount reported by USDA.

Correlation among dietary pattern and carotenoid levels in skin and plasma

Lycopene and β-carotene are two of the most common plasma and skin carotenoids(52). Plasma lycopene and β-carotene were significantly correlated with skin levels, but this was not observed with xanthophyllic carotenoids (zeaxanthin, lutein, and β-cryptoxanthin). A study that observed 52 women aged 40 to 60 with BMI of 18 to 35 kg/m^2 for 12 months found that skin carotenoid measured by reflection spectroscopy (RS) was highly correlated with resonance Raman spectroscopy (RRS) and plasma levels, while weak correlations were observed with reported dietary fruit and vegetables or carotenoid intake(53). Resonance Raman spectroscopy uses a laser for blue light excitation of tissue carotenoids, while pressure-mediated RS uses broadband white light. Seasonal differences did not affect RS, but RRS scores were lower in the summer than in

spring or fall. Similarly, total plasma carotenoid was found to be the lowest in the summer and highest in spring.

In children aged three to five, skin carotenoid was significantly and positively associated with reported fruit and vegetable intake, while an inverse association was observed with BMI percentile(54). In adults, reported carotenoid and vitamin C intakes were positively correlated with plasma concentrations(55). A lower plasma carotenoid concentration was also observed with increasing BMI categories(55,56). Furthermore, a dietary pattern high in plant-based foods such as vegetables, fruits, legumes, and nuts was significantly and positively correlated with all plasma carotenoids measured in postmenopausal Norwegian women aged 50 to 69 (57). Current evidence demonstrates that carotenoids can be measured non-invasively through the skin and has been positively correlated with plasma levels and a high plant-based dietary intake.

Skin carotenoids have been found to be altered in certain dermal disorders, as evidenced by significantly lower levels in adults with psoriasis compared to those without(56,58). Significantly lower levels of plasma lutein, zeaxanthin, retinoic acid, and retinol were observed in participants with atopic dermatitis compared to healthy individuals(59). Plasma lutein and zeaxanthin were also negatively correlated with markers of atopic dermatitis, suggesting that intake of foods rich in these carotenoids may potentially be beneficial for this skin disorder.

Effects of dietary carotenoid interventions on skin and plasma levels

Several clinical studies have demonstrated the effects of carotenoid intake on skin and plasma levels. Carotenoid supplementation in the form of a juice, equivalent to 1/3 to 1 1/3 cups of cooked carrots daily for eight weeks, significantly increased skin levels in children compared

to controls(60). Similarly, the consumption of 500 mL of orange juice daily for 18 days significantly increased skin carotenoid concentration in 38 adults aged 19 to 66(61). However, skin carotenoid levels returned to baseline three days after the cessation of juice consumption. In addition, a significant increase in skin carotenoids was observed after three weeks of a variety of juice intake compared to controls in 47 adults aged 22 to 54(62). The study randomized participants to consume 300 mL of tomato, tomato-apple, strawberry-apple, or grape juice, or a control thrice a week for six weeks. Carotenoid content in tomato was the highest, followed by strawberry, grape, and apple. Antioxidant activity was found to be the highest in tomato and grape juice.

In addition to carotenoid supplementation through juice, intake of an extract has also demonstrated beneficial effects. A study found that among 120 participants categorized into young and middle-age groups with ages under 25 and over 50, respectively, the latter had a significantly different serum lycopene isomer profile compared to the former(63). The study also provided 15 middle-aged participants with 7 mg/day of lycopene supplementation for four weeks and found a significant increase in serum levels, and the isomer profile became close to the pattern observed in the young group. The two groups were different in age as well as weight range, with young participants being mostly normal weight while the middle-age category was overweight. Therefore, lycopene supplementation may have benefited either the participants who were older or overweight or both. Lycopene concentration also significantly increased in the desquamated corneocytes located at the outermost dermal layer throughout the entire intervention, but levels in skin sebum peaked at two weeks and plateaued.

An increase in plant-based foods has also been shown to enhance dietary carotenoids, as well as skin and plasma levels. A study randomized 29 adults aged 18 to 65 years with BMI 19 to 30 kg/m^2 to a 28 weeks intervention consisting of four phases(64). Phase 1 and 3 consisted of six

weeks of a low-carotenoid diet each. Phase 2 and 4 were eight weeks long and entailed a high vegetables and fruits diet and the participant's usual diet, respectively. Skin carotenoids actively responded to the amount of vegetables and fruits consumed, and the authors suggested that the biomarker could be used to assess adherence as soon as two weeks after the start of a dietary intervention. In addition, plasma and skin carotenoid concentrations were correlated at baseline and throughout the study. Overall, dietary carotenoid supplementation in the form of a juice, extract, or food has been found to effectively increase human skin levels.

Effects of dietary carotenoid intake on skin health

Dietary carotenoid supplementation has been shown to be photoprotective in experimental animals. A significantly lower number of UVB-induced tumors was found in mice fed for 35 weeks a carotenoid-rich tomato diet compared to controls(65). Additionally, a significant increase in plasma lycopene was observed, although the concentration of lycopene in tomato was not the highest compared to other carotenoids. The finding demonstrated that cells have a strong affinity for storing lycopene, the most common plasma carotenoid(54). In another animal model, guinea pigs were randomized to receive either 50 or 250 mg/kg body weight of citrus peel extract, containing β-cryptoxanthin or a control (water) and were irradiated with UVB three times per week for 14 days(66). Supplementation significantly reduced melanin content in both treatment groups compared to control, while skin luminosity only improved with a higher extract intake. The extract groups also showed reduced oxidative stress by decreasing UV-induced ROS production in a dose-dependently manner and inhibited tyrosinase activity, an enzyme involved in melanogenesis.

Beneficial skin effects from carotenoid intake have been demonstrated in clinical trials as well. Twenty adults aged 20 to 57 with FSPT I or II consumed either solely a carotenoid (25 mg) supplement or combined with α-tocopherol (335 mg) daily for 12 weeks and received UV irradiation on their dorsal skin before and after supplementation(67). Both groups had a significant increase in serum β-carotene and skin carotenoid levels as well as reduced UV-induced erythema. Those who received α-tocopherol also had a significant increase in serum vitamin E. In another study, supplementation with a mixture of carotenoids and probiotics was found to inhibit a decrease in Langerhans cells, production of CD45+ dermal inflammatory cells, and increased melanin density in women over 18 years of age with FSPT II, III, or IV(68). Participants were irradiated with UVR at the buttocks or upper back or exposed to natural sunlight. Minimal erythema dose (MED) and erythema index significantly increased post-supplementation, indicating photoprotection. Furthermore, supplementation prevented sunburn, sun intolerances, and the appearance of sunspots. Additionally, a significant decrease in UV-induced erythema with carotenoid supplementation was observed in adults aged 22 to 55 with FSPT II compared to baseline levels and the control group(69). Participants were randomized to a 12-week intervention to consume one of three capsules: 24 mg of carotenoid extract from alga *Dunaliella salina*, a mix of β-carotene, lycopene, and lutein, 8 mg each, or a control consisting of soybean oil. Serum β-carotene and lutein were significantly increased in both carotenoid supplementation groups compared to the control.

Supplementation with a 250 mL beverage containing 5.7 mg lycopene, 3.7 mg phytoene, 2.7 mg phytofluene, 1 mg β-carotene, and 1.8 mg α-tocopherol resulted in a significant increase in all plasma and lymphocyte carotenoids of young adults(70). Damage to DNA by oxidative stress was also significantly reduced and inversely correlated with plasma and lymphocyte carotenoids

(lycopene, phytoene, and phytofluene). Furthermore, a significant increase in skin carotenoids and inhibition of collagen I degradation was observed in a study that provided carotenoid-rich extracts containing a total of 1650 mcg of carotenoids to 29 women aged 40 to 56 with FSPT II (71).

Potential mechanism of carotenoids for skin protection

Carotenoids can inhibit active radicals by transferring electrons through the donation of hydrogen atoms or attaching to them(47). The superoxide inhibitory effects of carotenoids are also closely related to the number of conjugated double bonds in its molecular structure(47), enabling efficient absorption of UV in the visible light region(72). However, exposure to free radicals weakened this capability due to scavenging activity, which breaks down the polyene structure of carotenoids(72,73). A rapid decrease in absorption capability was observed when an extract of a commercial tomato juice, which had the same UV absorption rate as lycopene, was exposed to free radicals(73). Furthermore, both lycopene and β-carotene inhibited lipid peroxidation in a dose-dependent manner. Mice exposed to ozone had a significant increase in heme oxygenase (HO)-1 protein, a marker of oxidative stress, pro-inflammatory tumor necrosis factor (TNF)-α and macrophage inflammatory protein (MIP)2 in the skin, and inducible nitric oxide synthase (iNOS) mRNA levels(74). However, supplementation with 5 g/kg of beta-carotene protected against the ozone effects described(74). Carotenoids can also activate the antioxidant response element transcription system and have antiproliferative properties and anti-immunosuppressive actions(72). They also upregulate gap junctional cell-cell communication by connexin gene expression, independent of provitamin A or antioxidant properties. Taken together, the above shows that carotenoids can protect the skin through prevention and interception of ROS as well as reparation of the effects of oxidative stress.

3.2 Vitamins

Vitamins are generally categorized into fat and water-soluble and have essential functions in growth, reproduction, immunity, metabolism, and redox reactions(75). Vitamin A, D, E, K are fat-soluble and are stored in the body's adipose tissues and liver. Vitamin C and the B vitamins: thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and B12 are water-soluble and require constant replenishment through the diet as the excess is excreted in urine and not stored to any appreciable degree. Mangos contain a variety of vitamins, which are shown in Figure 4.

Figure 4. Molecular structure of vitamins in mangos

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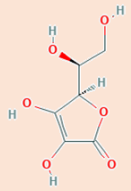
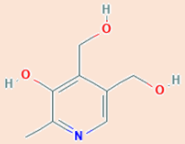
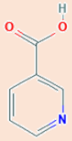
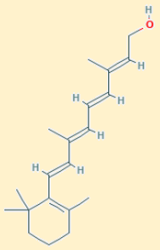
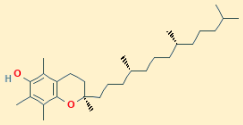
  </td><td>Pyridoxine
  </td><td>Niacin
  </td><td>Vitamin A
  </td><td>Vitamin E
  </td></tr>
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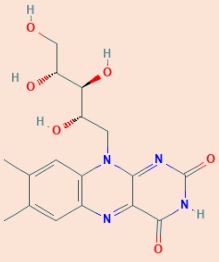
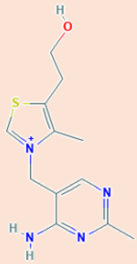
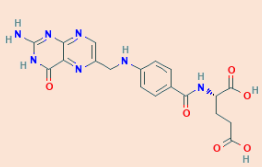
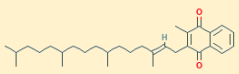
  </td><td>Thiamin
  </td><td>Folate
  </td><td colspan="2">Vitamin K
  </td></tr>
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Molecular structures derived from PubChem(48).

According to the USDA Nutrient Database, 100 g of raw mango (#09427) contains 36.4 mg Vitamin C, 0.028 mg Thiamin (B1), 0.038 mg Riboflavin (B2), 0.669 mg Niacin (B3), 0.119 mg Pyridoxine (B6), 43 mcg folate (B9), 54 mcg Vitamin A, 0.9 mg Vitamin E, and 4.2 mcg Vitamin K(50). Similar to β-carotene, the amount of vitamin C varies based on cultivar. Tommy Atkins was found to contain the lowest amount (19.3 ± 4.8 mg/100g of puree fruit) of vitamin C, while Ataulfo had the highest concentration (125.4 ± 6.4 mg/100 g of puree fruit)(51). The recommended dietary allowance (RDA) for adults and the percent of the RDA met from 100 g of mango intake for each vitamin are listed in Table 1. Depending on the cultivar, 100 g of mango consumption could meet 21% to 167% of the RDA for vitamin C in adults.

Table 1. Vitamins in mangos and %RDA met for men and women

Vitamins	100 g mango per USDA Nutrient Database	RDA for men	% RDA	RDA for women, not pregnant or lactating	% RDA
C (mg)	36.4	90	40.4	75	48.5
Thiamin (mg)	0.028	1.2	2.3	1.1	2.5
Riboflavin (mg)	0.038	1.3	2.9	1.1	3.5
Niacin (mg)	0.669	16	4.2	14	4.8
Pyridoxine (mg)	0.119	1.3 – 1.7	7 – 9	1.3 – 1.5	8 – 9
Folate (mcg)	43	400	10.8	400	10.8
A (mcg)	54	900	6	700	7.7
E (mg)	0.9	15	6	15	6
K (mcg)	4.2	120	3.5	90	4.7

Vitamin requirements vary across age groups and sexes, which is evident from the different RDAs for infants, children, men, and women. Requirements may also vary depending on external factors. Vitamin status can be affected by alcohol consumption as evidenced by a 4.6% decrease in serum α-tocopherol, and a 4.9% increase in isoprostane, a marker of oxidative stress, after consuming 30 g alcohol per day, equivalent to two alcoholic beverages, for eight weeks in postmenopausal women(76). Furthermore, consumption of 15 g or 30 g of alcohol, equivalent to one or two alcoholic beverages, was associated with a 5% decrease in serum vitamin B12(77). Skin hyperpigmentation has been reported in patients with B12 deficiency(78). A non-significant increase of 3% was also observed in homocysteine in those consuming 30 g alcohol compared to controls, and high levels are a risk factor of heart disease(77). A higher level of serum homocysteine was also observed in patients with psoriasis compared to controls(79). Environmental factors have been shown to affect vitamin levels in the skin, as evidenced by a significant decrease in α-tocopherol and vitamin C concentrations in the upper epidermis layer of mice skin when exposed to ozone compared to normal air(80). Malondialdehyde concentration, a marker of oxidative stress, was also ten-fold higher with ozone exposure compared to normal air in the upper epidermis.

Vitamin status has also been shown to affect skin conditions. Plasma vitamin A and E concentrations were significantly lower in patients with acne, especially in those with severe grades compared to those with moderate or mild conditions, or controls(81). Additionally, women with a wrinkled appearance were found to have significantly lower intakes of vitamin A and C compared to those without(27). A significantly lower intake of vitamin C was also observed only in those with dry skin. Although the literature on vitamins and skin is extensive, most studies were conducted with topical applications, and clinical investigations of vitamin intake on skin health

are remarkably limited. Nonetheless, the following sections explore the effects of dietary intake of vitamin C, A, E on the skin.

Vitamin C

The skin typically contains high levels of vitamin C (ascorbic acid), comparable to other body tissues such as the lungs, kidneys, as well as skeletal and heart muscles(82). Vitamin C is essential for the proper functioning of the skin through oxidant defense and promotion of collagen formation. As a potent antioxidant, vitamin C can neutralize and remove oxidants, effectively reducing oxidative stress and skin damage. Consumption of 100 mg or 180 mg of vitamin C daily for four weeks resulted in a significant increase in skin radical-scavenging activity, while no changes were seen in the controls(83). Vitamin C is also a co-factor for proline and lysine hydroxylases that stabilizes the collagen molecule structure and promotes gene expression of the protein(82). Supplementation with a mixture of vitamin C and pantothenic acid was shown to significantly increase the number of fibroblasts, cells that produce collagen, compared to controls(84). Vitamin C can also regenerate oxidized vitamin E by donating a hydrogen atom to the radical.

Surprising to many, photoprotection from vitamin C intake has not been clearly demonstrated in dietary clinical studies. Adults with FSPT II or III consumed vitamin C (500 mg ascorbic acid) daily for eight weeks and received UV irradiation to their buttocks along with skin biopsies collection before and after supplementation(85). Vitamin C intake significantly decreased total glutathione and malonaldehyde content in the skin before UVR exposure but did not inhibit an increase in these markers post-irradiation. Vitamin C supplementation also significantly

increased plasma and skin levels but did not affect UV-induced erythema. However, supplementation with vitamin C has been shown to reduce systemic inflammation effectively as evidenced by a significant and negative association between dietary ascorbic acid and α-tocopherol intake with inflammatory markers, specifically $PGF_{2\alpha}$, hsCRP, IL-6, and the formation of free radicals F_2-isoprostanes in a seven-year follow-up study(86). Furthermore, the consumption of 1 g of vitamin C per day for eight weeks significantly reduced hsCRP and IL-6 in adults with high baseline hsCRP (≥ 6 mg/L) levels(87).

Vitamin A

Vitamin A belongs to a group of fat-soluble substances and falls under the category of retinoids(88). Vitamin A and its derivatives are among the most effective substances slowing the aging process of the skin(88). Anti-wrinkle properties of retinoids promote keratinocyte proliferation, strengthen the protective function of the epidermis, restrain transepidermal water loss, protect collagen against degradation and inhibit metalloproteinase activity(88).

The intake of vitamin A has been associated with different forms of skin cancers. A research group followed adults for up to 14 years and found a non-significant inverse association between reported intake of retinol and folate and the risk of squamous cell carcinoma(89). A meta-analysis reported that an increased retinol intake was not associated with a reduced risk of malignant melanoma, a form of skin cancer that begins in melanocytes(90). In contrast, another study found that current but not former retinol supplementation was significantly associated with a decreased risk of melanoma(91). Timely intake of vitamin A may be required to effectively combat certain skin disorders.

Vitamin E

Vitamin E is mainly found in the form of α-tocopherol in the human skin, and lower levels of γ-tocopherol are also present(92). Exposure to UVR has been shown to deplete α-tocopherol in the human stratum corneum by 50%. Alpha-tocopherol is able to directly absorb UVB and form radicals, which in turn can be regenerated or converted into excited-state singlet oxygen or reactive oxygen intermediates.

The protective effects of vitamin E intake on the skin have also been demonstrated in clinical studies. Adults with Fitzpatrick skin type II or III consumed vitamin E supplementation for eight weeks, then received UVR radiation on their buttocks, and skin biopsy samples were collected(93). Both plasma and skin vitamin E concentrations were increased with supplementation. Vitamin E intake significantly decreased skin malondialdehyde concentration, before and after UVR exposure, and inhibited a UVR-induced decrease in glutathione concentration in the skin. Additionally, daily vitamin E (400 IU) supplementation in 96 participants aged 10 to 60 for eight months significantly improved or resulted in the complete remission of atopic dermatitis, defined by control of itching, while the control group had worsened conditions(94). A significant decrease in serum IgE was also observed in the vitamin E group, with a 62% reduction found in participants with great improvements and near remission of atopic dermatitis compared to baseline. In another study, 26 weeks of daily 400 mg vitamin E supplementation in participants exposed to arsenic effectively decreased skin lesion scores compared to controls(95).

In a 60-day clinical trial, 45 participants aged 13 to 45 with atopic dermatitis were randomized to four groups: 1) active vitamin D and placebo for vitamin E; 2) placebo for vitamin D and active vitamin E, 3) active vitamins D and E, and 4) placebo(96). Consumption of

supplements containing vitamins D and/or E increased serum 25 hydroxy vitamin D_3 [25(OH)D_3] and plasma α-tocopherol, respectively. Eczema severity was evaluated with the Severity Scoring of Atopic Dermatitis (SCORAD) index(97). Compared to baseline measures, groups 1, 2, and 3 showed a significant reduction of 34.8%, 35.7%, 64.3%, respectively, while the placebo showed a non-significantly reduction in SCORAD by 28.9%. A negative correlation between plasma α-tocopherol and SCORAD was also observed(96).

3.3 Polyphenols

The total phenolic content in Tommy Atkins, Kent, Keitt, and Haden mangos was found to be similar with a range of 19.5 to 49.4 mg of gallic acid equivalent (GAE)/100 g of puree(51). Ataulfo mango contained 109.3 ± 14.8 mg of GAE/100 g of puree, which is substantially higher than the other varieties. Mangiferin (Figure 3) was abundant in Ataulfo (227 – 996 mcg/g of puree) but present only in trace amounts in the Keitt cultivar. Gallotannins were also present in the highest quantity in Ataulfo mangos (342 – 708 mg/100 g of FW) and lowest in Tommy Atkins (54 – 160 mg/100 g of FW). Ellagic acid was also present in varying concentrations (26 – 187 mcg/g of puree) in Ataulfo mangos.

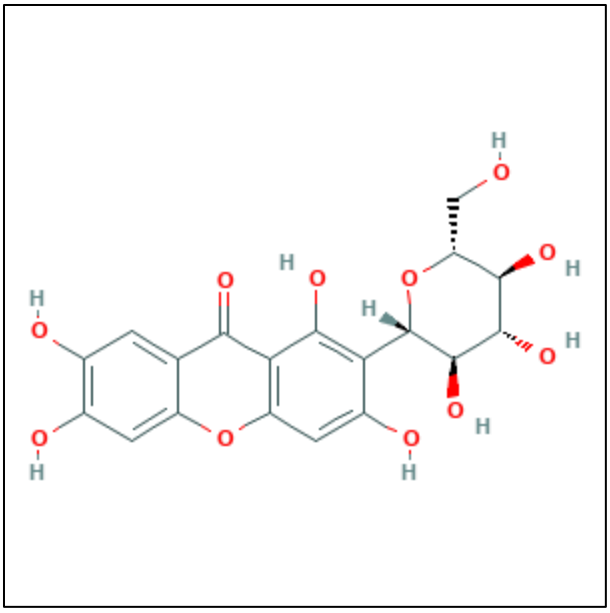

Figure 3. Molecular structure of mangiferin derived from PubChem(48)

Although mangiferin can be found in some herbs and vegetables, it is generally recognized as a polyphenol unique to the mango fruit. Several animal studies have demonstrated beneficial skin effects from mangiferin consumption. Mice exposed to UVB radiation resulted in an increased wrinkle length and depth, as well as epidermal thickness, but mangiferin intake at 100 mg/kg body weight five days a week for 12 weeks inhibited wrinkle formation and epidermal thickening(98). Supplementation resulted in less collagen fiber damage compared to UVB treated controls, and an increase in collagen bundles was noted. Mangiferin also inhibited the expression of UVB-induced MMP-9, an enzyme involved in the degradation of the ECM. In another study, mice with oxazolone-induced contact dermatitis were fed 50 mg/kg body weight of mangiferin or phosphate-buffered saline daily for two weeks(99). Mangiferin supplementation inhibited dermatitis-induced epithelial thickening and reversed the suppression of CD68, a protein produced by macrophages, indicating protection against the innate immune system. Supplementation with mangiferin also

decreased pro-inflammatory indices, including iNOS, IL-1β, and IL-6. Inhibition of NF-kB2 and IkB phosphorylation were also observed, indicating that mangiferin intake diminished the activation of the NF-kB pathway in dermatitis and impeded the production of pro-inflammatory cytokines.

Clinical studies on the effect of mangiferin intake on the skin have not been reported. However, a study demonstrated that a single oral dose of mangiferin effectively increased plasma concentrations in adults, peaking at 10 hours post-consumption and remained higher than baseline values for a day(100). Additionally, a 12 weeks study that provided overweight adults with 150 mg/day of mangiferin found a significant increase in serum levels of the xanthone and a decrease in triglycerides, free fatty acid, and high-density lipoprotein levels(101).

3.4 Objectives

This literature review illustrates the potential impact of diet on the skin. Although limited, the consumption of mango and its bioactive compounds has demonstrated great potential to protect against skin disorders and inflammation. Therefore, the objectives of this dissertation were to investigate the effects of mango consumption on markers of skin aging and inflammation in humans, and to briefly review evidence for plant-based foods and extracts on skin protection, with an emphasis on clinical studies.

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CHAPTER II

Prospective Evaluation of Mango Fruit Intake on Facial Wrinkles and Erythema in Postmenopausal Women: A Randomized Clinical Pilot Study

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Abbreviations: A=average, AL=average length, AS=average severity, AW=average width, BMI=body mass index, D=deep, DBP=diastolic blood pressure, DL=deep length, DS=deep severity, DW=deep width, E=emerging, EL=emerging length, ES=emerging severity, EW=emerging width, F=fine, FL=fine length, FS=fine severity, FW=fine severity, HDL=high density lipoprotein, L=length, LDL=low density lipoprotein, SBP=systolic blood pressure, SCs=skin carotenoids, UV=ultraviolet radiation, W=width.

ABSTRACT

Mangos are rich in β-carotene and other carotenoids, along with several phenolic acids that may provide oxidant defense and photoprotection to the skin. The objectives of this study are to investigate the effects of Ataulfo mango intake on the development of facial wrinkles and erythema. A randomized two-group parallel-arm study was conducted to assess 16 weeks of either 85 g or 250 g of mango intake in healthy postmenopausal women with Fitzpatrick skin type II or III. Facial photographs were captured at weeks 0, 8, and 16, and wrinkles at the lateral canthi and erythema at the cheeks were quantified. Skin carotenoid values were measured with reflection spectroscopy. Deep wrinkle severity decreased significantly in the 85 g group after 8 ($p = 0.007$) and 16 ($p = 0.03$) weeks compared to baseline measures. In contrast, those in the 250 g group showed an increase after 16 weeks in average wrinkle severity ($p = 0.049$), average wrinkle length ($p = 0.007$), fine wrinkle severity ($p = 0.02$), and emerging wrinkle severity ($p = 0.02$). Erythema in the cheeks increased with 85 g of mango intake ($p = 0.04$). The intake of 85 g of mangos reduced wrinkles in fair-skinned postmenopausal women, while an intake of 250 g showed the opposite effect. Further studies feeding 85 g of mangos are warranted.

1. INTRODUCTION

Skin aging is generally classified as intrinsic and extrinsic. Intrinsic aging includes genetic factors that influence pigmentation of the skin, skin composition and thickness, and hormonal composition [1]. Extrinsic aging leads to premature aging of the skin and includes lifestyle and environmental factors such as smoking, temperature and humidity, and ultraviolet (UV) radiation [1]. Photoaging involves changes in the skin induced by repeated exposure to UV radiation and is most often seen as the primary form of extrinsic aging [2]. Wrinkles are a common result of these factors [3].

Numerous dietary factors can modulate skin health. For example, consumption of carotenoid-rich kale extracts in humans reduced radical formation, prevented collagen I degradation, and improved the extracellular matrix [4,5], while supplementation with both carotenoids and vitamin C has been reported to decrease wrinkles at the lateral canthi and improve skin hydration [6,7]. Consumption of lycopene-rich tomato nutrient complex and lutein in humans has also been shown to provide protection against damage from UV radiation at the molecular level [8]. Beta-carotene and other carotenoids may provide photoprotection through direct chemical reactions with UV-induced reactive oxygen species (ROS) and interfere with UV-induced gene expression [9]. In addition, β-carotene can be converted into Vitamin A in the liver. The role of Vitamin A and ascorbic acid on skin health is widely recognized and has been recently reviewed elsewhere [10]. Ascorbic acid is present in high concentration in the skin and functions both as a powerful reducing agent to combat the effects of ROS and UV radiation and is essential in the synthesis of collagen [10]. Vitamin A helps to regulate cellular differentiation by increasing epidermal proliferation to counteract photoaging [10].

Mangos may be particularly suited to provide compounds that benefit the skin, particularly Ataulfo mangos, which have a robust carotenoid profile (mainly β-carotene), along with phenolic acids and ascorbic acid, which are generally higher compared to other mango cultivars typically found in the US [11,12]. Gallic acid, chlorogenic acid, protocatechuic acid, and vanillic acid are the major phenolics identified in Ataulfo mangos [13]. Mice fed gallic acid demonstrated photoprotection by a reduction in the degradation of collagen [14], and the consumption of chlorogenic acid from coffee was correlated with lower UV pigmented spots in middle-aged Japanese women [15]. Mangos also contain mangiferin; a xanthone reported to possess anti-inflammatory properties and provide protection against UV radiation [16]. In a study with human cadaver skin, elastase and collagenase activity were inactivated by mangiferin in a dose-dependent manner [17].

While a mango extract fed to mice inhibited UVB-induced wrinkle formation through inhibition of epidermal thickening and increasing collagen bundles, the effects of mango consumption on human skin remain unknown [18]. We therefore assessed the effects of Ataulfo mango intake at 85 g (0.5 cup) or 250 g (1.5 cups) for 16 weeks on the development of wrinkles and erythema, and changes in skin carotenoids, in postmenopausal women.

2. MATERIALS AND METHODS

2.1. Participants

Healthy postmenopausal women aged 50 to 70 were recruited from the greater Sacramento area. Inclusion criteria were Fitzpatrick skin type I, II, or III, and a body mass index (BMI) between 18.5 and 35 kg/m2. Exclusion criteria included allergy to mangos, self-reported

malabsorption, daily intake of more than two cups of fruits and three cups of vegetables, fruit juice consumption of more than one cup per day, use of statins or anti-inflammatory drugs, medical or cosmetic procedures to the face within the past six months, current or recent (less than one year) cigarette smoking, and the use of antioxidant supplements. This intervention was conducted between August 2018 and June 2019 and was registered at ClinicalTrials.gov (NCT03590756), with the protocol approved by the Institutional Review Board of the University of California, Davis (IRB #1185928). All participants gave their informed consent for inclusion before they participated in the study.

2.2. Study Design

Eligible participants were randomized by block design into an open-label, two-arm parallel clinical trial consuming either 85 g or 250 g of Ataulfo mango, four times per week for 16 weeks. A no-mango control group was not utilized as no human studies investigating the effects of fresh-frozen mango intake on skin exist, and thus relevant reference data was unknown. The two amounts of mango used in the present study were based on the 2015–2020 Dietary Guidelines for Americans [19]. The 250 g (1.5 cups) amount was close to the recommendation of two cups of fruit per day, while still allowing participants to consume other fruits during the 16-week intervention. The 85 g (0.5 cup) comparison amount was selected to provide sufficient differentiation from the 250 g portion size. The duration of the intervention was established to allow sufficient time for a new dermal and epidermal layer of skin to develop. Study assessments were conducted in the morning after a 12-h fast at weeks 0, 8, and 16 in the Ragle Human Nutrition Center at the University of California, Davis, CA, USA.

2.3. Ataulfo Mangos

Fully ripe, fresh Ataulfo mangos were washed, peeled, cut, portioned, and immediately frozen and stored at −20°C. Participants were given frozen mangos prepackaged into 85 g (0.5 cup) or 250 g (1.5 cups) daily servings and instructed to store them frozen and consume one serving, four times per week. Women could decide how to consume the mangos, as long as the fruit was not heated, since cooking could lead to losses in ascorbic acid and other vitamins [20].

2.4. Wrinkles and Erythema

High-resolution facial photographs were obtained in a dark room without skincare products or makeup (Mini Research 3D Clarity System, BrighTex Bio-Photonics, LLC, San Jose, CA, USA). Perspectives of the front, left, and right facial profiles were taken, rasterized, and classified based on the pixel image contrast. Lines with a minimum length of 2.6 mm with high contrast were termed deep (D), while those with medium contrast were grouped as fine (F) and with low contrast as emerging (E) (Figure 1). For each of these classifications, the wrinkle length (L) and width (W) were determined, along with a severity (S) score calculated as contrast multiplied by L. The average (A) values for L, W, and S were then calculated from the mean contrast of all pixels.

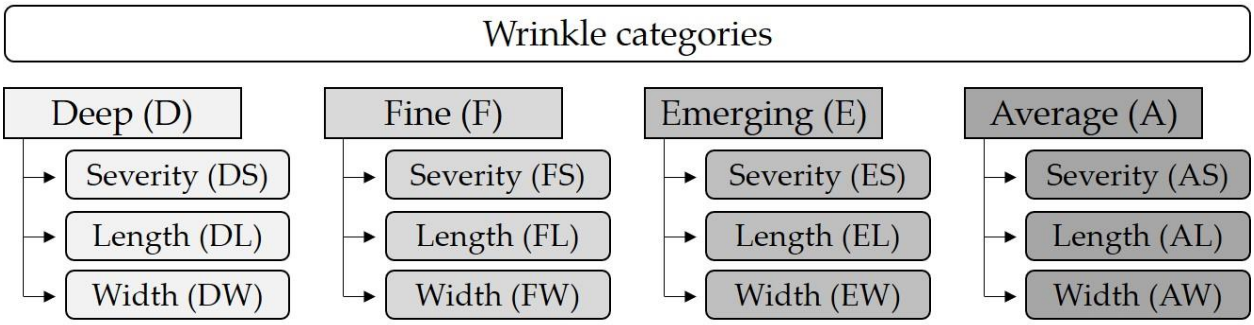


Figure 1. Classification of wrinkles. Deep wrinkles are high contrast, fine wrinkles are medium contrast, and emerging wrinkles are low contrast lines. Average wrinkles are the mean of all three categories. Severity is calculated as contrast multiplied by length.

2.5. Skin Carotenoids

Skin carotenoids (SCs), units in mm wavelength, were measured in the right index finger after cleaning with alcohol by reflection spectroscopy (Veggie Meter®, Longevity Link Corporation, Salt Lake City, UT, USA). The device has been validated to correspond with plasma carotenoid levels and was calibrated before each use [21].

2.6. Blood Pressure and Lipids

Three blood pressure readings were obtained, five minutes apart after a fifteen-minute seated rest using an automated oscillometric unit and averaged to obtain systolic (SBP) and diastolic (DBP) values (Vital Spot, VSM 300,Welch Allyn, Skaneateles Falls, NY, USA). Plasma lipids were analyzed for cholesterol, low-density lipoprotein (LDL), non-high-density lipoprotein (HDL), and triglycerides at the UC Davis Department of Pathology and Laboratory Medicine.

2.7. Dietary Intake

A 24-h recall was conducted at each study visit using the validated Automated Self-Administered 24-h (ASA24) dietary assessment tool (https://epi.grants.cancer.gov/asa24). A compliance log was maintained, showing the date, time, and format of mango consumption.

2.8. Statistical Analysis

An a priori power analysis showed that there was greater than 80% power to detect a 10% difference in wrinkle severity between the 250 g and 85 g mango groups at Week 16, with recruitment of at least 15 subjects in each group with alpha set to $p = 0.05$. Statistical analyses were performed with JMP version 15 (SAS Institute Inc., Cary, NC, USA). p values of 0.05 or less were considered statistically significant. Each parameter was assessed for normality, or transformed (Log, Log10, or Johnson) to achieve normality before analyses. Data are presented as mean ± SD. Baseline participant characteristics were analyzed with the t-test or Wilcoxon signed-rank test as appropriate. All other outcome measures were analyzed using the Fit Model to perform Two Way ANCOVA, with treatment and time as factors, and BMI as a covariate. Post-hoc analyses were performed with Contrast tests. The Pearson and Spearman correlation coefficients were used to analyze the correlation between outcome measures that were normally and non-normally distributed, respectively.

3. RESULTS

3.1. Baseline Characteristics

Thirty-six healthy postmenopausal women were enrolled. Thirty-two individuals completed the study (Figure 2). Analysis of skin carotenoids, blood pressure, and dietary records included all participants. For the wrinkle analysis, four sets of data were removed due to technical errors in image capture. Those that showed no deep wrinkles at baseline were removed from the analysis of both deep and the average wrinkle score (which used deep wrinkle values), resulting in data from eight and nine participants for the left lateral canthus, and ten and eight for the right lateral canthus for the 85 g and 250 g groups, respectively. Data for fine and emerging wrinkles were available from 13 to 15 individuals in the 85 g and 250 g groups, respectively. Analysis of plasma lipids and glucose included 21 participants (13 and eight in the 85 g and 250 g group, respectively).

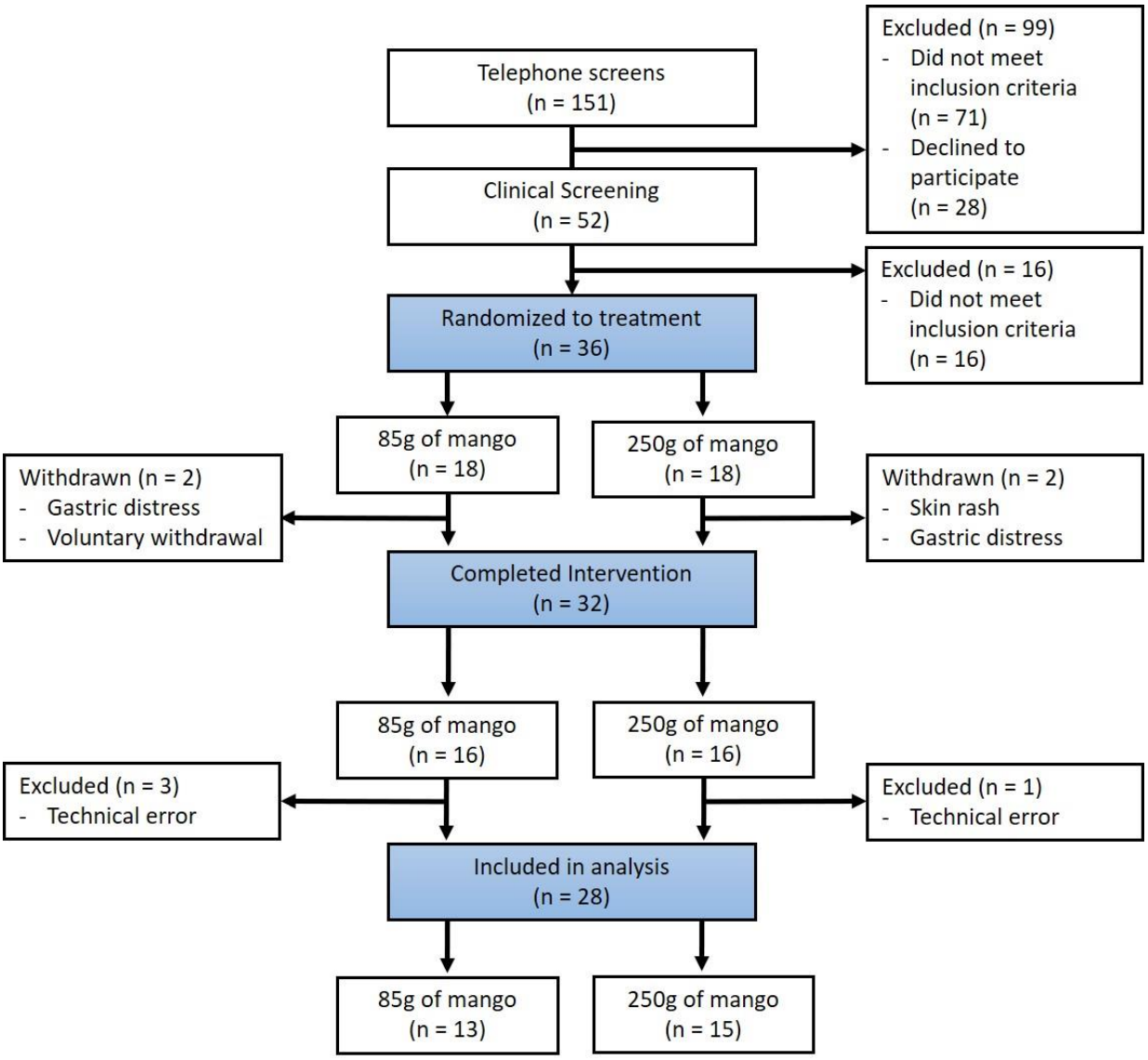


Figure 2. CONSORT flow diagram.

At the start of the study, the two groups were similar in age, blood pressure, lipids, and fasting blood glucose (Table 1). Participants had either Fitzpatrick Skin Type II or III (*n* = 9 and 19, respectively). Baseline BMI values were significantly different, with the mean for those in the 85 g group were classified as overweight, while the average for the 250 g group was in the normal range. Left side measurements were significantly different between the two groups at baseline for fine (FL) and emerging wrinkles length (EL).

Table 1. Baseline characteristics of participants.

	85 g Group (n = 13)	**250 g Group (n = 15)**	p
	Mean ± SD	**Mean ± SD**	
Age (years)	61 ± 5.1	60 ± 5.3	0.58
BMI (kg/m^2)	26.4 ± 4.0	22.9 ± 2.6	0.01 *
WC (cm)	89 ± 12.0	83 ± 9.4	0.13
SBP (mmHg)	115 ± 10.7	113 ± 6.9	0.96 †
DBP (mmHg)	75 ± 6.5	74 ± 4.0	0.61
HR (BPM)	63 ± 6.2	61 ± 7.2	0.53
RS Carotenoid (nm wavelength)	363 ± 78	432 ± 105	0.06
Glucose (mg/dL)	97.5 ± 6.5	95.1 ± 7.5	0.46 ‡
Cholesterol (mg/dL)	220 ± 31.9	225 ± 44.2	0.83 ‡
LDL (mg/dL)	138 ± 25.6	127 ± 27.7	0.42 ‡
Non-HDL (mg/dL)	152 ± 24.7	143 ± 27.1	0.49 ‡
Triglycerides (mg/dL)	71 ± 21.6	83 ± 69.3	0.16 ‡,†
Left lateral canthus			
Deep Wrinkle Severity	7575 ± 1063.4	8037 ± 1403.2	0.46 ₸
Deep Wrinkle Length (mm)	17.61 ± 5.05	17.68 ± 3.77	0.98 ₸
Deep Wrinkle Width (mm)	1.63 ± 0.25	1.84 ± 0.24	0.09 ₸
Fine Wrinkle Severity	5866 ± 680.4	5148 ± 1510.4	0.08 †
Fine Wrinkle Length (mm)	5.49 ± 1.05	4.01 ± 1.40	0.004 *
Fine Wrinkle Width (mm)	1.46 ± 0.18	1.29 ± 0.40	0.24
Emerging Wrinkle Severity	4279 ± 123.8	4249 ± 110.2	0.51
Emerging Wrinkle Length (mm)	5.06 ± 2.92	3.64 ± 0.61	0.02 *,†
Emerging Wrinkle Width (mm)	1.20 ± 0.13	1.21 ± 0.13	0.90
Average Wrinkle Severity	5166 ± 379.1	5329 ± 639.3	0.54 ₸
Average Wrinkle Length (mm)	5.71 ± 1.18	5.57 ± 1.08	0.80 ₸
Average Wrinkle Width (mm)	1.28 ± 0.09	1.35 ± 0.18	0.47 ₸,†
Right lateral canthus			
Deep Wrinkle Severity	7487 ± 922.1	7537 ± 1338.0	0.93 ₸
Deep Wrinkle Length (mm)	16.62 ± 5.53	17.41 ± 6.27	0.78 ₸
Deep Wrinkle Width (mm)	1.70 ± 0.20	1.71 ± 0.27	0.93 ₸
Fine Wrinkle Severity	5730 ± 330.4	5778 ± 485.1	0.76
Fine Wrinkle Length (mm)	5.75 ± 3.07	4.91 ± 0.85	0.85 †
Fine Wrinkle Width (mm)	1.52 ± 0.23	1.38 ± 0.15	0.08
Emerging Wrinkle Severity	4240 ± 160.6	4178 ± 186.9	0.18 †
Emerging Wrinkle Length (mm)	4.00 ± 0.61	3.62 ± 0.52	0.09
Emerging Wrinkle Width (mm)	1.22 ± 0.12	1.26 ± 0.16	0.47
Average Wrinkle Severity	5169 ± 256.9	5336 ± 541.7	0.40 ₸
Average Wrinkle Length (mm)	6.10 ± 1.84	6.12 ± 1.44	0.82 ₸,†
Average Wrinkle Width (mm)	1.36 ± 0.13	1.38 ± 0.16	0.81 ₸
Erythema			
Left Cheek	14.73 ± 6.90	16.18 ± 4.96	0.55
Right Cheek	18.02 ± 13.08	14.00 ± 8.85	0.43 †

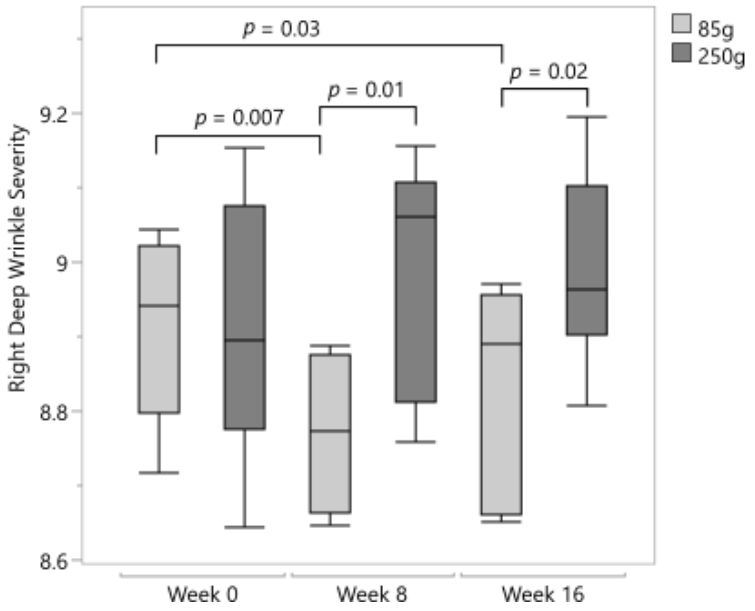
* Significantly different between 85 g and 250 g groups ($p < 0.05$). Statistical analysis by t test or † Wilcoxon's signed-rank test. ₸ Average and deep wrinkles measurements had an n of 8 and 9 in the left, and an n of 10 and 8 in the right, in the 85 g and 250 g group, respectively. ‡ Glucose measurement had an n of 12 and 9 participants for 85 g and 250 g group, respectively. BMI = Body Mass Index; WC = Waist Circumference; SBP = Systolic Blood Pressure; DBP = Diastolic Blood Pressure; HR = Heart Rate; RS = Reflection Spectroscopy; LDL = Low-density Lipoprotein; HDL = High-density Lipoprotein.

3.2. Dietary Intake

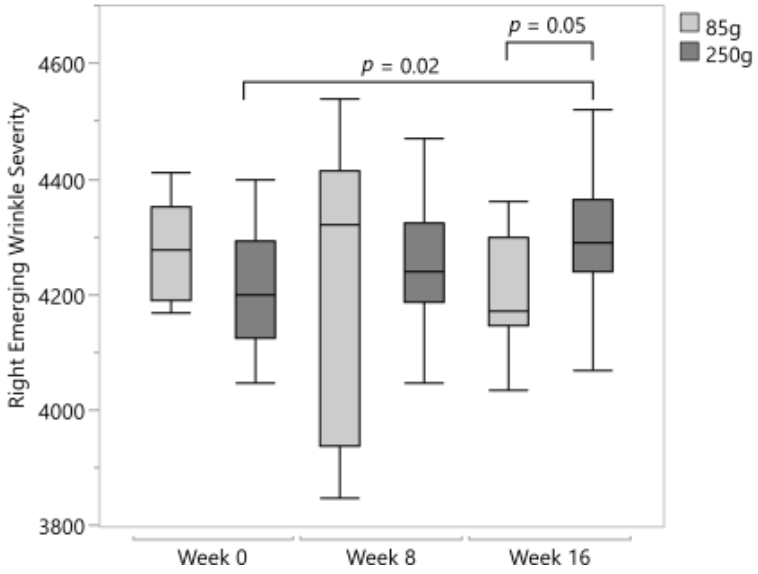
Both groups consumed approximately 1700 kcals at the baseline, with few significant differences in macronutrients (Table S1); the 85 g group had higher intakes of fiber, folate, and lutein plus zeaxanthin. After 16 weeks, the 85 g group reported increases in dietary potassium and cholesterol, while the 250 g group had a significant increase in total sugars, as well as potassium and folate.

3.3. Facial Wrinkles and Erythema

At baseline, no differences were noted in right lateral canthi measures, while left FL and EL were significantly lower in the 250 g compared to the 85 g group (Table 1). In the 85 g group, right deep wrinkle severity decreased by 23% after eight weeks and 20% after 16 weeks (both of which were significant ($p = 0.007$ and $p = 0.03$, respectively; Figure 3a). A trend for reduced left deep wrinkle severity (DS) was noted in the 85 g group (severity scores at baseline: 7575 ± 1063 vs. 16 weeks: 5339 ± 3153, $p = 0.10$). In contrast, an increasing trend in right DS was observed in the 250 g group (severity scores at baseline: 7537 ± 1338 vs. 16 weeks: 8056 ± 982, $p = 0.07$). Comparison of the two groups showed a trend for lower left DS after 16 weeks in the 85 g group relative to the 250 g group (severity scores for 85 g: 5339 ± 3153 vs. 250 g: 8105 ± 1840, $p = 0.08$). For the right DS, the 85 g mango group was significantly lower compared to 250 g at both week eight ($p = 0.01$) and week 16 ($p = 0.02$; Figure 3a).



(a)



(b)

Figure 3. (**a**) Right deep wrinkle severity was significantly different between groups at week 8 ($p = 0.01$) and 16 ($p = 0.02$) and significantly decreased by week 8 ($p = 0.007$) and 16 ($p = 0.03$) in the 85 g group. Data transformed by natural log. (**b**) Right deep wrinkle severity was significantly different between groups at week 8 ($p = 0.01$) and 16 ($p = 0.02$) and significantly decreased by week 8 ($p = 0.007$) and 16 ($p = 0.03$) in the 85 g group. Data transformed by natural log.

Deep wrinkle length (DL) in the 85 g group was reduced by 32.7% after 16 weeks compared to baseline (baseline: 17.61 ± 5.05 mm vs. 16 weeks: 11.85 ± 7.56 mm, $p = 0.07$) that was significantly lower compared to the 250 g group (85 g: 11.85 ± 7.56 mm vs. 250 g: 18.21 ± 5.91 mm, $p = 0.02$). A between-group trend for increased right deep wrinkle width (DW) (85 g: 1.44 ± 0.63 mm vs. 250 g: 1.74 ± 0.20 mm, $p = 0.08$) and right emerging wrinkle severity (ES) (severity scores at 85 g: 4203 ± 94 vs. 250 g: 4299 ± 108, $p = 0.05$) was observed after 16 weeks in the 250 g. These between-group differences in right ES may be attributed to a 2.9% increase from baseline in the 250 g group ($p = 0.02$; Figure 3b).

A number of left side wrinkle measures significantly increased in the 250 g group, with no significant changes from baseline for the 85 g group. Compared to baseline, left average wrinkle severity (AS) significantly increased by week 8 ($p = 0.03$), which persisted to week 16 ($p = 0.049$; Figure 4a). A 25% increase from baseline in left average wrinkle length (AL) was observed ($p = 0.007$), which was also significantly higher than the value for the 85 g group at week 16 ($p = 0.01$; Figure 4b). Left fine wrinkle severity (FS) increased from baseline after 8 weeks ($p = 0.048$) and after 16 weeks ($p = 0.02$; Figure 4c).

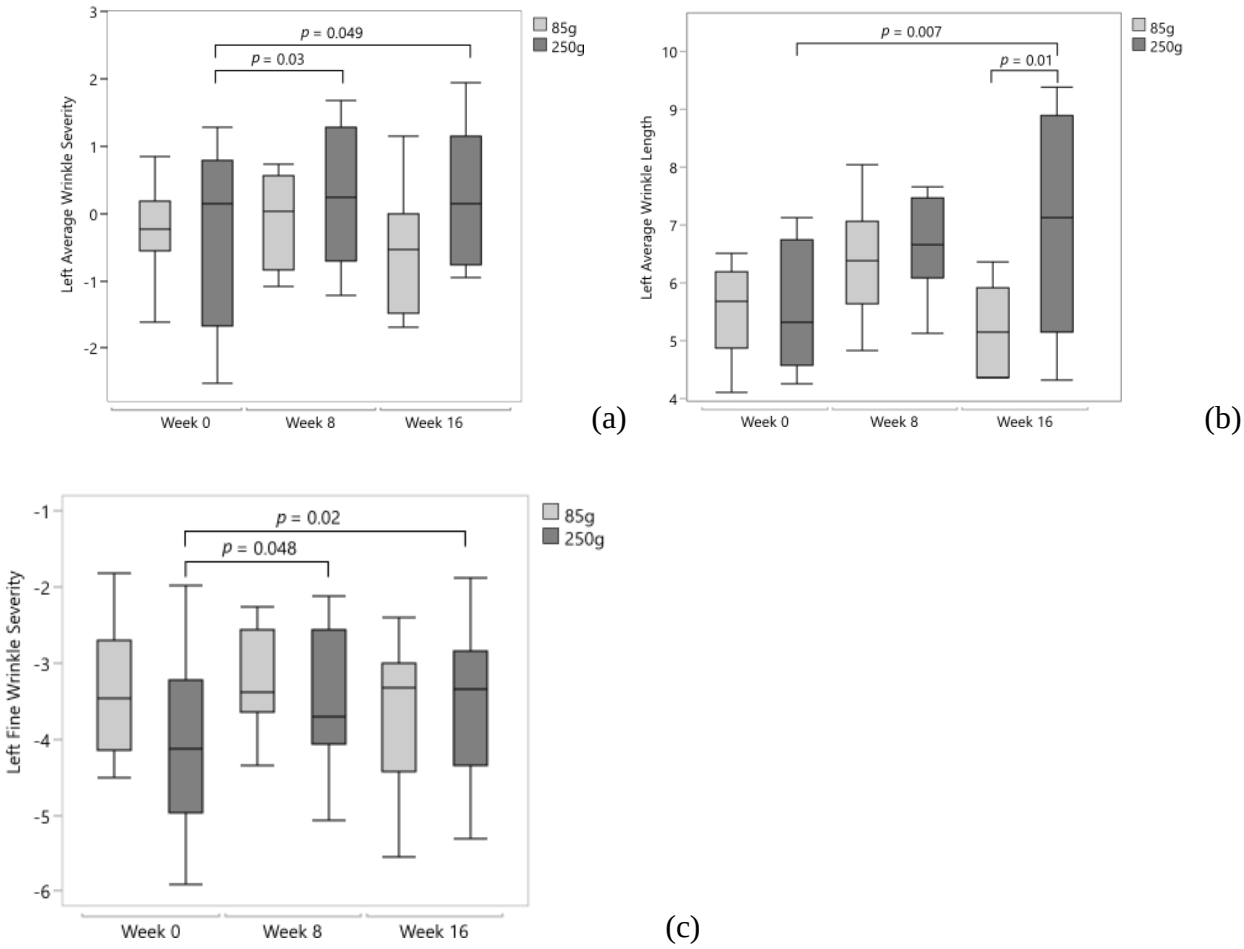


Figure 4. (**a**) Left average wrinkle severity significantly increased at week 8 (p = 0.03) and week 16 (p = 0.049) after 250 g of mango intake. Data transformed by Johnson transformation. (**b**) Left average wrinkle length significantly increased at week 16 (p = 0.007) after 250 g of mango intake and is significantly higher than the 85 g group (p = 0.01). (**c**) Left fine wrinkle severity significantly increased at week 8 (p = 0.048) and 16 (p = 0.02) after 250 g of mango intake. Data transformed by Johnson transformation.

For erythema measures, left cheek erythema was significantly increased after 16 weeks in the 85 g group (degree of intensity % at baseline: 21.2 ± 18.0 vs. 16 weeks: 27.2 ± 16.0, p = 0.04), while no changes were observed in the 250 g group.

3.4. Skin Carotenoids

At baseline, SCs were generally lower in the 85 g compared to the 250 g group (85 g: 363 ± 78 mm wavelength vs. 250 g: 432 ± 105 mm wavelength, $p = 0.06$). Mango intake did not result in a significant change within or between groups over time. While no significant changes in SCs were observed for those who were normal weight in either group, those who were overweight or obese showed a significant increase from baseline after eight (348 ± 59 mm wavelength, $p = 0.01$) and 16 (352 ± 76 mm wavelength, $p = 0.03$) weeks, regardless of group assignment. These findings may be due, in part, to the fact that participants who were overweight or obese had significantly lower SCs at baseline compared to those who were of normal weight (329 ± 77 mm wavelength vs. 452 ± 104 mm wavelength, respectively, $p = 0.008$).

3.5. Blood Pressure and Plasma Lipids

A significant decrease in SBP was observed in the 250 g group after eight and 16 weeks of intake, compared to baseline measures (baseline: 113 ± 2.28 mm Hg vs. 8 weeks: 108.5 ± 2.28 mm Hg, $p = 0.02$ and baseline vs. 16 weeks: 108.2 ± 2.28 mm Hg, $p = 0.01$). Similar declines were observed for DBP (baseline: 74.1 ± 1.41 mm Hg vs. 8 weeks: 71.2 ± 1.41 mm Hg, $p = 0.01$ and baseline vs. 16 weeks: 71.0 ± 1.41 mm Hg, $p = 0.009$), and mean arterial pressure (MAP) (baseline: 87.1 ± 1.65 mm Hg vs. 8 weeks: 83.6 ± 1.65 mm Hg, $p = 0.01$ and baseline vs. 16 weeks: 83.4 ± 1.65 mm Hg, $p = 0.008$). No significant interactive effects (treatment x time) for any of the blood pressure or plasma lipid measures were noted. No significant changes in blood pressure were observed for the 85 g group.

Serum cholesterol decreased significantly by 7.4% in the 250 g group at the end of the study (baseline: 216 ± 9.7 mg/dL vs. 16 weeks: 200 ± 9.7 mg/dL, $p = 0.02$), while trends were

observed for decreasing LDL (baseline: 122 ± 7.6 mg/dL vs. 16 weeks: 108 ± 7.3 mg/dL, p = 0.06) and non-HDL (baseline: 138 ± 7.9 mg/dL vs. 16 weeks: 127 ± 7.9 mg/dL, p = 0.07). In the 85 g group, triglycerides were lower compared to baseline after 16 weeks (baseline: 70.6 ± 20.7 mg/dL vs. 16 weeks: 63.3 ± 36.1 mg/dL, p = 0.06).

No significant changes were observed in blood glucose with either 85 g or 250 g of mango intake after 16 weeks.

3.6. Correlations

Reported intake of dietary β-carotene was positively correlated with SCs (r = 0.22, p = 0.048) and with erythema in the right (r = 0.39, p = 0.0006) and combined cheek measurements (r = 0.39, p = 0.0009). In addition, dietary β-carotene intake was negatively correlated with blood pressure: SBP (r = −0.28, p =0.01), DBP (r = −0.29, p = 0.008), and MAP (r = −0.29, p = 0.008). Skin carotenoids were positively correlated with erythema on the left (r = 0.24, p = 0.04) and right cheek (r = 0.40, p = 0.0005). Skin carotenoids were also negatively correlated with SBP (r = −0.37, p = 0.0006), DBP (r = −0.33, p = 0.002), and MAP (r = −0.34, p = 0.002).

4. DISCUSSION

In this exploratory trial, we observed that 85 g (0.5 cup) of mango intake for two to four months reduced facial wrinkles while 250 g (1.5 cups) increased them. We report here a significant decrease in right DS and a trend in the reduction for left DS and DL in the 85 g group. In contrast, the 250 g group showed a significant increase in left AS, AL, FS, and right ES and trended towards an increase in right DS and DW. While further research is needed to explore the

mechanisms behind these findings, the reduction in wrinkles with 85 g of mango intake may be due to the beneficial effects of carotenoids, flavonoids, and mangiferin, all of which, as part of a whole food complex, could lead to improvements in collagen bundles and a reduction in epidermal thickening as seen with the mouse study noted above [18]. The increase in wrinkles in the 250 g group was notable and unexpected. Since a significant increase in total sugar intake was noted in this group after eight and sixteen weeks, this increased sugar intake may have led to glycation of collagen fibers, thereby disrupting the collagen structure [22]. The effects of whole food intake on skin health in humans is a relatively new area of research. A recent study reported a significant reduction in facial wrinkles in postmenopausal women after regular intake of almonds [23]. Although dietary and skin carotenoids were positively correlated with erythema, a significant increase in erythema was only observed in the 85 g mango group. Therefore, it is unlikely that erythema was caused by the amount of β-carotene consumed from the fruit. Furthermore, β-carotene has been shown to decrease erythema in other studies [24,25].

Favorable changes in markers of cardiovascular risk were noted in the 250 g group. Serum cholesterol significantly decreased by 7.4%, while a trend for reduced LDL and non-HDL cholesterol was observed. This may possibly be attributed to plant sterols that are abundant in mangos (24.4 mg/100 g fruit), as these compounds have been associated with a reduction in LDL cholesterol [26–28]. The 250 g of mango intake may also have improved cholesterol levels by providing a significant source of both soluble (28.2 g/100 g dry matter) and insoluble (41.5 g/100 g dry matter) fiber [29,30]. Our data is consistent with the observation of reduced total and LDL cholesterol levels after Ataulfo mango pulp intake in a rat model [31].

We also observed a 4% reduction in blood pressure with higher levels of mango intake. The inverse correlations observed between dietary and skin carotenoids with blood pressure are

consistent with findings from other studies that report negative correlations between β-carotene and SBP, as well as with cardiovascular mortality [32,33]. Taken together, these results suggest a potential role of mango intake on cardiometabolic health but require confirmation through future trials powered explicitly for these outcomes.

Limitations

Two levels of mango intake were used in this exploratory study to determine whether either amount would produce a change in facial wrinkles. Thus, a control group consuming no mangos was not employed. However, a strength of the study is that the design allowed for the assessment of an amount-based response and allowed for better control of any placebo effect that could be present as women in both groups received mangos. Finally, the study was limited to healthy postmenopausal women with Fitzpatrick skin type II and III; therefore, the findings reported here may not be generalizable to other groups.

5. CONCLUSIONS

Results from this pilot study support the concept that regular intake of modest amounts of mangos may improve facial wrinkles. The apparent beneficial effects of mangos on skin health may be lost if the intake of mangos is particularly high. The effects of whole food intake on skin health are limited but promising. Further prospective studies are warranted.

Supplementary Materials:

Table S1. 24-hour reported dietary intake

	85g group (n = 16)					250g group (n = 16)					Between groups		
	Week 0	Week 8	Week 16	*p* Week 8 vs 0	*p* Week 16 vs 0	Week 0	Week 8	Week 16	*p* Week 8 vs 0	*p* Week 16 vs 0	*p* Week 0	*p* Week 8	*p* Week 16
	Mean ± SD	Mean ± SD	Mean ± SD			Mean ± SD	Mean ± SD	Mean ± SD					
Energy (kcal)	1698 ± 555	1865 ± 575	1904 ± 801	0.21	0.37	1713 ± 440	2037 ± 549	1820 ± 827	0.12	0.75	0.76	0.93	0.21
Protein (g)	70 ± 37	83 ± 30	79 ± 30	0.09	0.29	74 ± 42	84 ± 33	69 ± 39	0.3	0.16	0.71	0.83	0.08
Total Fat (g)	70 ± 35	75 ± 35	90 ± 51	0.48	0.26	76 ± 25	95 ± 25	77 ± 50	0.21	0.17	0.69	0.37	0.07
Carbohydrates (g)	201 ± 45	208 ± 85	194 ± 81	0.81	0.58	180 ± 56	214 ± 71	220 ± 89	0.09	0.06	0.06	0.39	0.86
Sugars, total (g)	90 ± 30	111 ± 56	74 ± 38	0.22	0.13	78 ± 31	97 ± 31	107 ± 51	0.04*	0.01*	0.06	0.25	0.15
Fiber, total dietary (g)	26 ± 18	20 ± 7	22 ± 8	0.68	0.79	18 ± 6	26 ± 11	24 ± 11	0.03	0.13	0.02*	0.87	0.41
Potassium (mg)	2667 ± 770	3183 ± 1182	2718 ± 857	0.01*	0.99	2441 ± 846	3233 ± 746	3074 ± 1267	0.008*	0.049*	0.17	0.2	0.8
Vitamin C (mg)	99 ± 77	201 ± 276	171 ± 293	0.15	0.7	88 ± 67	140 ± 79	135 ± 97	0.02*	0.15	0.23	0.64	0.78
Folate, total (mcg)	401 ± 147	419 ± 243	466 ± 177	0.85	0.4	316 ± 118	467 ± 205	439 ± 171	0.005*	0.03*	0.02*	0.96	0.24
Vitamin A, RAE (mcg_RAE)	826 ± 438	996 ± 741	1046 ± 641	0.59	0.8	673 ± 362	1483 ± 1401	806 ± 540	0.04	0.69	0.29	0.63	0.36
Retinol (mcg)	315 ± 216	373 ± 206	397 ± 235	0.38	0.18	327 ± 122	386 ± 227	373 ± 265	0.59	0.48	0.51	0.74	0.2
Carotene, beta (mcg)	5614 ± 5746	6957 ± 8452	6935 ± 5539	0.61	0.89	3504 ± 3594	11154 ± 14647	4865 ± 6507	0.01	0.52	0.11	0.93	0.24
Carotene, alpha (mcg)	619 ± 740	935 ± 1149	1584 ± 2010	0.24	0.16	893 ± 1511	2661 ± 6638	496 ± 1143	0.24	0.38	0.56	0.53	0.12
Lutein + zeaxanthin (mcg)	5692 ± 8191	6934 ± 11437	5785 ± 6129	0.71	0.69	2289 ± 2418	5846 ± 12381	4000 ± 5678	0.61	0.74	0.03*	0.17	0.13
Vitamin E, alpha-tocopherol (mg)	10.8 ± 7	10.2 ± 4.5	11.2 ± 5.3	0.6	0.93	9.5 ± 3.8	17.4 ± 10.4	13.4 ± 10.1	0.008	0.27	0.23	0.44	0.88
Cholesterol (mg)	314 ± 283	330 ± 211	414 ± 276	0.51	0.04*	282 ± 218	264 ± 163	212 ± 193	0.82	0.046*	0.29	0.77	0.01*
Fatty acids, total saturated (g)	19 ± 12	22 ± 12	27 ± 22	0.38	0.29	25 ± 9	27 ± 9	23 ± 15	0.53	0.07	0.15	0.22	0.28
Fatty acids, total monounsaturated (g)	26 ± 14	27 ± 12	34 ± 18	0.45	0.25	26 ± 11	36 ± 11	29 ± 21	0.08	0.5	0.91	0.43	0.08
Fatty acids, total polyunsaturated (g)	20 ± 9	19.5 ± 10.7	23 ± 10	0.96	0.49	20 ± 8.5	26 ± 12	19 ± 13	0.34	0.17	0.68	0.59	0.02*

*Significant at the $p < 0.05$ level. Analyzed with Two Way ANCOVA and BMI as a covariate.

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CHAPTER III

Plant-based Foods for Skin Health: A Narrative Review

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Keywords: Skin, Photoprotection, Carotenoids, Phenolics, Antioxidants, Inflammation

Abbreviations: ALA = alpha-linolenic acid, CPP = coffee polyphenol, DHA = docosahexaenoic acid, EFA = essential fatty acids, EGCG = epigallocatechin gallate, EPA = eicosapentaenoic acid, FDC = Food Data Central, FSPT = Fitzpatrick skin phototype, GPx = glutathione peroxidase, GSE = grape seed extract, GSH = glutathione, GSSG = oxidized glutathione, HA = hyaluronan, MED = minimal erythema dose, MMP = metalloproteinase, MPJC = melon pulp juice concentrate, MUFA = monounsaturated fatty acids, NF-kB2 = nuclear factor-kappa B subunit 2, PFSE = passion fruit seed extract, PUFA = polyunsaturated fatty acids, RDA = recommended daily allowance, ROS = reactive oxygen species, SAAID = second harmonic generation to autofluorescence aging index of dermis, SC = stratum corneum, SOD = superoxide dismutase, TEWL = transepidermal water loss, TNC = tomato nutrient complex, UV = ultraviolet, USDA = United States Department of Agriculture, UVR = ultraviolet radiation, Vit = Vitamin

Abstract

The potential role of plant-based foods in the promotion of skin health is an emerging area of nutrition research. Plant-based foods are rich in bioactive compounds, including vitamin C, alpha-tocopherol, beta-carotene, polyphenols, and phenolic acids that can act to provide oxidant defense, lower inflammation, and promote structural support of the skin. In addition, epidemiological studies have associated higher intakes of fruits and vegetables with greater skin health. Beneficial effects of select fruits, vegetables, nuts, legumes, and polyphenolic-rich beverages on the skin have been reported, with some nutrients in common and others being unique to the individual food. Collectively, the evidence to date suggests a promising future for plant-based dietary interventions to address certain skin conditions pertaining to collagen, elasticity, erythema, hydration, roughness, and wrinkles. Most studies have been conducted using extracts, with a small number investigating whole foods and minimally processed products. While the evidence to date is suggestive, replication and extension of existing studies are needed to address issues such as study design and the difference between a concentrated food extract and whole food intake will help provide targeted dietary recommendations.

Introduction

Skin, the largest organ in the human body, acts as a barrier to protect internal organs and cells from external elements, helps regulate body temperature, permits sensations of touch and produces Vitamin D, a key regulator of bone, immune and vascular health. Both intrinsic and extrinsic factors affect skin health and aging[1]. An individual's genetic background influences intrinsic factors, such as skin pigmentation and thickness, microvasculature structure, and sex hormones that, with aging, can impact skin dryness, thinning, and wrinkles[1]. Extrinsic factors such as smoking, diet, sleep, exercise, chronic diseases, environmental temperature and pollution, humidity, and ultraviolet radiation (UVR) can further accelerate the skin aging process, in part, through an increase in inflammation and oxidative stress[1–3]. Indeed, repeated exposure to UVR can increase pro-inflammatory cytokines that contribute to the appearance of wrinkles and pigmentation in the skin[4]. Moreover, age- or obesity-related induction of protein glycation, and subsequent inflammation, increases skin rigidity and impairs skin repair[5].

Adequate intakes of a number of essential micronutrients are vital for the maintenance of skin health as they provide structural integrity and oxidant defense[6]. Deficiencies of Vitamin A (VitA) and Vitamin C (VitC) can lead to hyperkeratosis, or thickening of the skin[6]. Poor wound healing has been observed with deficiencies of VitC and essential fatty acids (EFA)[6–8], while petechiae can be the result of Vitamin E (VitE) or Vitamin K (VitK) deficiencies[6]. Inadequate intakes of riboflavin, niacin, pyridoxine, biotin, zinc, or EFA can lead to various forms of dermatitis[6,9]. Classic studies on pellagra[10] and acrodermatitis enteropathica[11] identified niacin or zinc deficiencies, respectively, to be causative factors.

Current consumer trends in dermatology include the potential role of diet for skin health and esthetics. Beyond deficiencies, epidemiology studies suggest that high dietary intakes of

specific plant-based foods are key in the maintenance of skin health. A robust intake of vegetables, olive oil, and legumes was correlated with lower actinic skin damage caused by long-term UVR exposure among 2,000 people aged 70 and older in Australia, Greece, China, Japan, and Sweden[12]. Better adherence to the Dutch Healthy Diet Index guidelines and a diet pattern rich in fruits, supplemented with yogurt, milk, and vegetables was significantly associated with fewer wrinkles in women[13]; while among Japanese women, a significant inverse association was observed between wrinkling and green and yellow vegetable intake[14]. In contrast, an unhealthy eating pattern consisting mainly of meat, grains, snacks, soft drinks, coffee, and alcoholic beverages was associated with more wrinkling in women[13].

Moreover, plant-based foods are rich in polyphenols and carotenoids that are typically not found in appreciable amounts in other categories of food. However, each food has a unique nutrient profile that provides an array of bioactive compounds that either alone or synergistically may afford protection for the aged skin. Therefore, the current review focus on exploring the clinical evidence to quantify the amount needed for select foods that may benefit the skin. Studies discussed provide data on outcomes of skin aging, including measurements of collagen, elasticity, erythema, hydration, roughness, and wrinkles.

Methods

Articles were identified by searching PubMed and Google Scholar using the key terms or combinations of: *fruit, vegetable, nut, legume, bean, food, skin, wrinkle, erythema, hydration, elasticity, aging, photoaging*. All studies available in English were reviewed. Eligibility criteria for clinical trials included dietary interventions and skin parameter measurements relevant to

wrinkles, erythema, hydration, and elasticity. Dietary interventions that used foods and beverages that are whole, processed, or made into extracts were considered. Extracts were also equated to quantities as whole foods or beverages to deduce feasibility of consumption. Isolated compounds given at pharmacological levels were not considered. Dermatological pathologies were not considered. Animal or *in vitro* studies were included for select plant-based foods or relevant bioactive compounds that supported potential mechanisms of action for the clinical trials.

Results

Twenty-one studies with 14 plant-based foods were identified, including nine fruits and vegetables, two nuts and legumes, and three polyphenolic-rich beverages. Products used in dietary interventions included: whole foods, pastes, beverages, juice, and extracts (Table 1). All participants were adults between 18 – 86 years old. Most studies reported the Fitzpatrick skin phototype (FSPT), a standard tool used to categorize individual reactions to UVR exposure. FSPT I and II individuals have less melanin pigmentation and increased sensitivity to harmful effects of ultraviolet (UV) radiation such as sunburn and premature aging, while III and IV tend to tan[15].

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

Plant-based item		Country / First Author (Year)	Study Design	Subjects	Smokers	BMI (kg/m^2)	Age (Years)	Intervention	Form and intake of product	Location of skin	Fitzpatrick Skin Phototype (FSPT)	Effects
FRUITS and VEGETABLES	Grape	Japan / Yamako-shi (2004)[70]	Open-label Total 11 months: 1st period – 6 months, 1 month break, 2nd period – 5 months	12 Japanese women with chloasma	Not specified	Not specified	All range: 34 to 58 45.4 ± 6.1	1) Grape seed extract Per capsule: 81%, 54 mg PAC	Extract in a capsule 67 mg, 3 times/ day	Cheeks	Not specified	**L*:** ↑ **Diameter of chloasma:** ↓ **Melanin index:** ↓
		Japan / Tsuchiya (2020)[72]	RCT, double-blind, parallel arm 12 weeks	97 women with lentigo spots on cheeks	Not specified	21.07 ± 1.78	All range: 30 to 60 44.28 ± 6.50	1) 200 mg dealcoholized red wine oligomeric PACs Per bottle: 208 mg	Extract in beverage 200 mL/ day	Cheeks	Not specified	**Lentigo scores:** ↓ **SC water content:** ↑
						21.08 ± 2.59	44.66 ± 5.93	2) Control: calories, color, and taste matched				
	Mango	USA / Fam (2020)[27]	Randomized parallel arm 16 weeks	32 post-menopausal women	Excluded	26.4 ± 4.0	61 ± 5.1	1) 85g (0.5 cup) fresh-frozen mangos	Fresh-frozen mangos 4 times/ week	Lateral canthi, cheeks	II & III	<u>Lateral canthi:</u> 85g **Deep wrinkle severity:** ↓ 250g **Emerging wrinkle severity:** ↑ **Average wrinkle severity:** ↑ **Average wrinkle length:** ↑
						22.9 ± 2.6	60 ± 5.3	2) 250g (1.5 cups) fresh-frozen mangos				

												Fine wrinkle severity: ↑ <u>Cheeks:</u> **Erythema: ↑ 85g,** ↔ 250g
	Melon	France / Egoume-nides (2018)[35]	RCT, double blind, parallel arm 34 days	44 Caucasian adults, men (15.9%) and female (84.1%)	Not specified	Not specified	All range: 18 to 50 mean 37.2	1) 20mg dried melon concentrate 2) Control pill	Extract in a capsule 20 mg/day	Buttock, back, or arms	II & III	**MED:** ↑
	Orange	Italy / Puglia (2014)[40]	Crossover 15 days	20 Caucasian adults	Excluded	Not specified	UV irradiation: 26 to 47 Sunlamp exposure: 45 to 70	Blood orange (Moro, Tarocco and Sanguinello) extract Per capsule: ANC 2.8-3.2%, hydroxycinnamic acids (caffeic, cumaric, ferulic, sinapic acid) 1.8-2.2%, flavone glycosides (narirutin, hesperidin) 8.5-9.5%, ascorbic acid 5.5-6.5%	Extract in a capsule 100 mg/ day	Forearm and dorsal hand	UV irradiation: II & III Sunlamp exposure: II & IV	<u>Forearm:</u> **Skin erythema index:** ↓ <u>Dorsal hand:</u> **Melanin index:** ↓
	Passion fruit	Japan / Maruki-Uchida (2018)[63]	RCT, double blind, parallel arm	32 women with dry skin	Not specified	Not specified	All range: 35 to 54	1) Passion fruit seed extract containing Per capsule: 5mg piceatannol	Extract 2 capsules /day	Cheeks	Not specified	**Moisture content:** ↑ TEWL: ↓ Skin elasticity: ↓ L* a* b*: ↓

			8 weeks,					2) Control: dextrin				
	Pome-granate	USA / Henning (2019)[59]	RCT, parallel, 3-arm, open-label 12 weeks	74 women	Not specified	26.6 ± 5.0	All range: 30 to 40 35.1 ± 4.3	1) Pomegranate juice Per cup: 100 mg punicalagin, 23 mg ellagic acid	Juice 8 oz/day or Extract in a capsule 1000 mg /day	Inner arm	II, III, & IV	**MED:** ↑ Melanin index: ↓
						27.1 ± 5.1	35.9 ± 4.1	2) Pomegranate extract Per capsule: 100 mg punicalagin, 44 mg ellagic acid				
						29.9 ± 6.7	37.9 ± 4.2	3) Control: dextran				
	Kale	Germany / Meinke (2017)[55]	RCT, parallel arm 10 months	29 women	10 smokers (34.5%)	Not specified	All range: 40 - 56 mean 49.2	1) Curly kale extract Per capsule: Total 550 mcg carotenoids: 430 mcg lutein, 70 mcg beta-carotene, 30 mcg lycopene, 20 mcg zeaxanthin	Extract 3 capsules /day	Inner forearm, and cheeks	II	**Collagen I/elastin ratio (SAAID):** ↑
								2) Control: olive oil				
	Paprika	Japan / Nishino (2018)[77]	RCT, double blind, parallel arm 5 weeks	43 adults. men (19%) and women (81%)	Not specified	Not specified	All range: 30 to 50 40.7 ± 4.3	1) paprika-xanthophyll extract Per capsule: 9 mg total xanthophylls, 5 mg capsanthin,	Extract 1 capsule /day	Back and cheeks	II	<u>Back:</u> **MED:** ↑ **MTD:** ↑ **L*:** ↑ a*: ↓ TEWL: ↔ compared to control, ↑

								0.5 mg β-cryptoxanthin				**compared to baseline** SC hydration: ↔ <u>Cheek:</u> L*, a*, TEWL, SC hydration: ↔
							41.4 ± 4.7	2) Control: vegetable oil				
	Tomato	Germany / Groten (2019)[51]	RCT, double blind, parallel arm, multi-center 12 weeks	145 adults. men (23%) women (77%)	8 smokers (5.5%)	≤ 30	All range: 20 to 50 mean 40.9	1) Tomato nutrient complex Per capsule: Tomato – 7.5 mg lycopene, 2.9 mg phytoene and phytofluene, 0.4 mg β-carotene, 2.8 mg tocopherols Rosemary – 2 mg carnosic acid	Extract 2 capsules /day	Buttock	I & II	MED: ↔ **a*:** ↓ **L*:** ↔
							mean 40.9	2) Control: medium-chain triglycerides				
		UK / Rizwan (2011)[50]	RCT, single blind, parallel arm 12 weeks	17 Caucasian women	Excluded	Not specified	All range: 21 to 47 median 33	1) Tomato paste with olive oil, 55g Per serving: 16 mg lycopene	Paste Daily	Upper buttock	I & II	MED: ↑ **Erythema index:** ↑ **Procollagen I:** ↑ **Fibrillin-1:** ↓
								2) Control: Olive oil, 10g				
		Germany / Stahl (2001)[49]	RCT, parallel arm 10 weeks	22 adults men (36%) women (64%)	Included with a limit of ≤ 3 cigarettes/day	Not specified	All range: 26 to 67	1) Tomato paste (40g) with olive oil (10g) Per serving: 16 mg lycopene,	Paste Daily	Scapular region	II	**a*:** ↓

								0.5 mg β-carotene, 0.1 mg lutein 2) Control: Olive oil, 10g				
NUTS and LEGUMES	Almond	USA / Foolad (2019)[122]	RCT, parallel arm 16 weeks	28 post-menopausal women	Excluded	30.7 ± 7.31 29.7 ± 7.66	All range: 53 to 80 63.63 ± 7.09 58.93 ± 6.10	1) 20% of daily kcals consumed as almonds Average 2.1 oz/day 2) Control: 20% of daily kcals consumed as calorie-matched nut-free snack	Whole raw almonds Daily	Lateral canthi	I & II	**overall wrinkle severity** ↓ **overall wrinkle width** ↓ TEWL: ↔ sebum production: ↔
	Soy-bean	Japan / Izumi (2006)[95]	RCT, double blind, parallel arm 12 weeks	26 women	Not specified	Not specified	All range: 35 to 48 40.1 ± 1 40.5 ± 0.95	1) 25mg fermented soybean extract containing 10 mg (40%) isoflavone aglycones 2) Control: color-matched, no extract	Extract in a 250 mg capsule 4 capsules /day	Lateral canthi, Cheeks	Not specified	<u>Lateral canthus:</u> **Fine wrinkles:** ↓ Linear wrinkles: ↔ **Skin microrelief:** ↑ <u>Cheeks:</u> **Skin elasticity:** ↑
		Korea / Lee (2015)[96]	RCT, parallel arm 8 weeks	65 women with dry and dark skin	Not specified	21.99 ± 1.7 21.43 ± 1.95	All range: 25 to 60 42.58 ± 4.60 43.41 ± 4.68	1) Barley and soybean formula Per 100 mL: 3 g 2) Control: no formula	Extract in beverage 100 mL/day	Forearm and front cheeks	Not specified	**Skin hydration:** ↑ **SC thickness:** ↓

POLYPHENOL-RICH BEVERAGES	Cocoa	S. Korea / Yoon (2015)[102]	RCT, double blind, parallel arm 24 weeks	64 women with visible wrinkles ≥ grade 2	Not specified	Not specified	All range: 48 to 86 63.3 ± 13.9	1) Cocoa powder Per day: 320 mg total cocoa flavanols	Cocoa powder dissolved in 150 – 200mL hot water 4g/day	Lateral canthi, cheeks, buttock	Not specified	<u>Lateral canthi</u> **Wrinkle depth: ↓** <u>Cheek:</u> **Skin elasticity: ↑** Skin hydration: ↔ <u>Buttock:</u> **MED: ↑**
							60.0 ± 12.6	2) Control: Nutrient-matched cocoa-flavored beverage without flavanols				
		Germany / Heinrich (2006)[103]	RCT, double blind, parallel arm 12 weeks	24 women	Excluded	Not specified	All range: 18 to 65	1) High flavanol (HF) Per day: 329mg total cocoa flavanols (61.1 mg epicatechin, 20.4 mg catechin)	Cocoa powder dissolved in 100mL hot water 18 g/day	Dorsal skin (back and scapular region)	II	**a*: ↓** **Cutaneous blood flow: ↑** **Skin density: ↑** **Skin thickness: ↑** **Skin roughness: ↓** **Scaling: ↓** **Skin hydration: ↑** **TEWL: ↓**
								2) Low flavanol (LF) Per day: 27mg total cocoa flavanols (6.6 mg epicatechin, 1.6 mg catechin)				
	Coffee	Japan / Ueda (2017)[108]	RCT, double blind, parallel arm 4 weeks	31 women with reported skin dryness	Excluded	20.7 ± 2.1	All range: 25 to 35 31.3 ± 3.7	1) Coffee polyphenol (CPPs), caffeine-free Per 100mL: 297.8 mg CPP	Extract in beverage 100 mL/ day	Cheeks, Perioral	Not specified	**Skin scaliness: ↓**
						21.3 ± 1.7	29.9 ± 3.4	2) Control: taste-matched, no CPP				
		Japan / Fukaga-	RCT, double blind,	49 women with xerotic skin	Excluded	Range: 18.5 to 25.0	All range: 25 to 40	1) CPP, caffeine-free	Extract in beverage	Lower cheeks, hands	Not specified	<u>Lower cheek</u> **Skin dryness: ↓** **TEWL: ↓**

		wa (2017)[109]	parallel arm 8 weeks					Per 100mL: 270 mg CPP 2) Control: taste-matched, no CPP	100 mL/ day			**SC hydration:** ↑ **Skin surface pH:** ↓ **SC lipids:** ↑ **SC lactic acid:** ↑ <u>Hands</u> **Skin dryness:** ↓ **TEWL:** ↓ **SC hydration:** ↑ **Skin surface pH:** ↓
	Green Tea	Germany / Heinrich (2011)[116]	RCT, double blind, parallel arm 12 weeks	60 women	Excluded	Range: 18 - 25	All range: 40 to 65	1) Green tea beverage Per 1L: 1402 mg total tea catechins 2) Control: taste-matched, no catechins	Extract in beverage 1 L/day	Back Scapular region, Inner forearm	II	<u>Back and scapular region:</u> **a*:** ↓ <u>Inner forearm:</u> **Viscoelasticity:** ↓ **Biological elasticity:** ↑ **Skin density:** ↑ Skin thickness: ↔ **Skin roughness:** ↓ **TEWL:** ↓ **Skin volume:** ↓ **Skin Scaling:** ↓ **Skin hydration:** ↑
		Farrar / United Kingdom (2015)[123]	RCT, double-blind, parallel arm 12 weeks	50 adults, men (26%) and women (74%)	Not specified	36 ± 13.6 35 ± 12.3	All range: 18 to 65 27.9 ± 5.4 25.5 ± 3.8	1) Green tea extract & vitamin C Per 450 mg green tea capsule: 180 mg green tea catechins Per vitamin C capsule: 25 mg vitamin C 2) Control: maltodextrin	Extract 3 green tea & 2 vitamin C capsules, twice/day	Upper buttocks	I & II	MED: ↔ Erythema index: ↔
	ANC: anthocyanin; a*: skin redness; b*: skin pigment; L*: skin lightness; RCT: randomized controlled trial; MED: minimal erythema dose; MTD: minimal tanning doses; SAAID: second harmonic generation to autofluorescence aging index of dermis; SC: stratum corneum; TEWL: transepidermal water loss; ↑: increase; ↓: decrease; ↔: no changes; **significant changes ($p < 0.05$) are bolded.**											

1. Fruits and Vegetables

Fruits and vegetables are rich in bioactive compounds such as carotenoids[16], vitamins, and polyphenols[17] that are distributed to all tissues, including the skin, and provide photoprotection through improved oxidant defense and promotion of structural integrity[17,18]. Certain fruits and vegetables, many described below, are an excellent source of VitC, with reduced intakes associated with increased dry or wrinkled skin in women[19]. VitC is a cofactor for prolyl and lysyl hydroxylases that are important for collagen synthesis, and as a major circulating antioxidant, can combat the effects of ROS derived from UVR[20]. In rodent models, ozone exposure decreases skin VitC content while increasing malondialdehyde, a marker of oxidative stress[21]. A number of the fruits and vegetables in this report are substantial sources of carotenoids and flavonoids. Carotenoid supplementation studies, in the range of 24 – 25 mg per day from 12 weeks, significantly reduced UV-induced erythema[22,23], increased melanin density, while preventing both a decrease in Langerhans cells and CD45+ dermal inflammatory cells. In addition, the provision of young adults 13.1 mg of carotenoids significantly reduced oxidative stress induced DNA damage in young adults after 26 days[24]. A number of phenolic and polyphenolic, such as the flavonoids, are plant metabolites known for their beneficial effects on cardiometabolic function and immune support[25].

In general, yellow, orange, and red fruits such as mango, melon, citrus, tomatoes, and red bell peppers, as well as dark-green leafy kale, are a substantial source of vitamins as well as carotenoids that gives them the color. Fruits with a deep red or purple color such as grapes, pomegranate, and passion fruit are mainly rich in anthocyanins and polyphenols. Collectively, while there is considerable evidence to demonstrate that the intake of certain micronutrients and bioactive compounds from fruit and vegetables are beneficial to skin health in isolation, data on

the potential interactive effects of these phytochemicals within a specific food are limited to the following nine.

1.1 Mangos

Mangos (*Mangifera indica* L.) are rich in carotenoids, primarily beta-carotene, VitC, and the phenolics gallic acid and the polyphenol mangiferin[26]. A significant decrease in deep facial wrinkles following 16 weeks of mango intake was recently reported in healthy postmenopausal females aged 50 – 70 with FSPT II or III[27]. A serving size of mango is 0.75 cups, with study participants consuming either 85 g (0.5 cup) or 250 g (1.5 cups) of fresh-frozen Ataulfo mangos four times per week. Overall, compared to baseline values, wrinkle measurements significantly decreased or trended less in the 85 g group. Ataulfo mangos have the highest VitC content of the mango cultivars commonly found in the US[28], with 85 g providing an estimate of 107 mg of VitC (143% of the RDA for postmenopausal women) and 2,219 mcg of beta-carotene or 185 mcg RAE (12 mcg dietary beta-carotene/mcg RAE[29]) of Vitamin A (16% of the RDA for women). It is important to stress the differences in micronutrient levels between cultivars, as the level of beta-carotene in Ataulfo mangos is four times higher than the reference level reported by the United States Department of Agriculture (USDA) Food Data Central (FDC) #169910 (640 mcg/100 g of raw mango)[30]. Ultraviolet-irradiated mice fed a mango extract that provided 13.5% of mangiferin in the diet for 12 weeks, had a significant decrease in the length of wrinkles, along with an increase in collagen bundles and inhibition of collagen fiber damage[31]. The mechanism of these effects with mangiferin intake may be through inhibition of inflammation, as supplementation studies in mouse models report a decrease in pro-inflammatory indices, such as iNOS, IL-1β, and IL-6, and inhibition of inflammation-induced cellular signaling pathways to include nuclear factor-kappa B subunit 2 (NF-kB2) and IkB phosphorylation[32].

With regards to higher levels of mango intake, individuals in this trial who consumed 250 g of mango per day had an increase in wrinkles. The reasons for the discrepancy are currently unexplored; however, 250g of mangos provide a substantial amount of dietary sugar with a total of 34 g, including glucose 5 g and fructose 12 g. While speculative, the type of sugars present in mangos, glucose, and fructose, are known to increase glycation of collagen and elastin fibers that may disrupt the skin protein structure[33].

1.2 Melons

Melons (*Cucumis melo* L.) are a good source of VitC and beta-carotene, with cantaloupes containing higher amounts than honeydews[34]. Photoprotection was observed with the consumption of a dried melon pulp juice concentrate (MPJC) in 44 Caucasian adults aged 18 – 50 with FSPT II and III. Participants consumed either the MPJC or a color-matched control capsule daily along with a control topical cream for 30 days[35]. Participants' buttocks were irradiated at baseline and post-supplementation, and a significant increase in the minimal erythema dose (MED) was observed in the MPJC group compared to controls. The MED is the amount of UVR required to produce minimal sunburn or redness due to dilation of capillaries in the skin, with an increase in this index correlated to enhanced photoprotection[36]. In another study, females aged 40 – 70 years with FSPT II – IV consumed a capsule containing the MPJC, plus grape seed extract, VitC, and zinc for eight weeks, and significant improvements in skin color, luminosity, dark circles under the eyes, erythema, and overall subjective satisfaction was noted[37]. Although the authors of both studies suggested that superoxide dismutase (SOD) was a primary driver contributing to the observed effects, this mechanism is unlikely since SOD is a protein that is catabolized during digestion and not absorbed intact[38]. Regrettably, the studies did not provide adequate information to estimate the amount of melons consumed. One cup of

cantaloupe melon cubes (USDA FDC #169092) provides about 58.7 mg of VitC (65 – 90% of RDA for adults) and 3,230 mcg of beta-carotene[30].

1.3 Oranges

Oranges (*Citrus sinensis*) are widely recognized as a fruit rich in VitC, with varying nutrient and polyphenolic profiles depending on the cultivar and environmental factors[39]. Flavonoids are the most common polyphenols in citrus, including quercetin, hesperedin, and narirutin. Anthocyanins are found in blood oranges, which give the pulp and juice its red color. Inhibition of UV-induced erythema was observed in 20 Caucasian participants aged 26 – 47 years with FSPT II and III when supplemented with 100 mg/day of a powdered extract, derived from a combination of three blood orange cultivars, containing 2.8 – 3.2% anthocyanins, 1.8 – 2.2% hydroxycinnamic acids, 8.5 – 9.5% flavone glycosides, and 5.5 – 6.5% VitC all on a weight to weight basis[40]. The same study also found a significant inhibition of UV-induced melanogenesis in 25 participants with FSPT II and IV, aged 40 – 70 years, as assessed in three locations of solar lentigo (spots) along with one spotless (control) region per hand. Melanin, an essential molecule for skin photoprotection, absorbs a broad range of UV rays and dissipates the energies as heat, thereby protecting against DNA damage[41]. However, chronic UV exposure, particularly UVB, could exceed the melanin absorbance threshold, resulting in increased photosensitization and generation of ROS. Increased melanogenesis is also physically observed as darkening of the skin and hyperpigmentation. A reduction in DNA damage and an increased in the concentrations of plasma anthocyanin (cyanidin 3-glucoside), VitC, and carotenoids that provide oxidant defense was noted with 600 mL of blood orange juice intake for 21 days in adults aged 20 – 27 years old[42]. In mice, the intake of a citrus extract rich in hesperidin and narirutin for seven weeks resulted in a significant inhibition of UV-induced transepidermal water

loss (TEWL), an important measure of skin barrier function that assesses water diffused from the dermis and epidermis through the stratum corneum to the skin surface[43]), improved skin hydration, and reduced epidermal thickening[44]. In addition, consumption of unripened Jeju mandarin orange extract for ten months reduced UV-induced TEWL, wrinkle depth, epidermal thickness, and collagen degradation in mice[45]. The amount of anthocyanins and VitC in the extract[40] can be met through 300 mL or 1.3 cups of blood orange juice, containing 225 mg VitC and 10.5 mg cyanidin-3-glucoside[42].

1.4 Tomatoes

Tomatoes (*Lycopersicon esculentum*) are rich in lycopene, another carotenoid with strong oxidant defense capabilities[46,47]. The skin and plasma contain the highest amounts of lycopene compared to other human body tissues[48]. Among men and women aged 26 – 67 with FSPT II, a significant decrease in UV-induced erythema, along with an increase in serum lycopene levels, was observed after ten weeks daily intake of 40 g of tomato paste (providing approximately 16 mg lycopene) compared to an olive oil control group[49]. Twelve-week consumption of 55 g of tomato paste combined with olive oil, containing 16 mg of lycopene, also provided photoprotection in women aged 21 – 74 with FSPT I or II by significantly increasing the erythemal index and trending favorably for the MED post-irradiation compared to the intake of the olive oil alone[50]. Additionally, those consuming the tomato paste showed a significant increase in procollagen I and inhibition of collagenase metalloproteinase (MMP)-1 expression, mitochondrial DNA damage, and a reduction in fibrillin-1 in response to UVR-induced tissue injury. Similarly, 12-week supplementation with a carotenoid-rich tomato nutrient complex (TNC) that included rosemary extract significantly decrease UV-induced erythema in adults aged 20 – 50 with FSPT I or II compared to a control containing medium-chain triglycerides[51]. A

significant increase in serum lycopene and the carotenoid precursors' phytofluene and phytoene was also observed in the TNC, but not the control group. Tomato is the second most consumed vegetable in the US, with pizza sauce contributing to the highest form of consumption[52]. In the studies above, 40 – 55 g of tomato paste provided 16 mg of lycopene. However, a higher consumption of raw tomatoes (USDA FDC #321360), 390 g or 2.5 cups, is needed to achieve the same amount of lycopene[30]. While the consumption of fresh fruits and vegetables is encouraged, seasonal changes may affect the availability of these foods. Minimally processed foods have a consistent availability due to longer shelve-life and can be nutrient-dense in a smaller quantity which provides another effective alternative to obtaining optimal nutrition.

1.5 Kale

Kale (*Brassica oleracea)* is rich in carotenoids, VitC, and glucoraphanin, a glucosinolate that is converted to sulforaphane, which can decrease inflammation and oxidative stress mediated by the Nrf2 signaling pathway[53,54]. Consumption of carotenoid-rich curly kale extract (2,200 mcg lutein, 1000 mcg beta-carotene, 50 mcg alpha-carotene, 400 mcg lycopene, 700 mcg zeaxanthin, 100 mcg cryptoxanthin) daily for ten months improved collagen I and elastin levels in 29 females (10 smokers, 19 non-smokers) aged 40 – 56 with FSPT II compared to an olive oil control[55]. The second harmonic generation to autofluorescence aging index of dermis (SAAID; a measure of the collagen I to elastin ratio) was taken at the cheek and inner forearm, and values increased significantly in both groups after five months, but only the kale group remained significantly increased after ten months. In addition, a significant increase in epidermal and dermal thickness was observed when mice prone to accelerated skin aging consumed spray-dried kale or a glucoraphanin-enriched kale extract daily for 43 weeks, compared to controls[56]. The beneficial effects were more prominent in the glucoraphanin-enriched compared to the spray-

dried kale group. The amount of carotenoids in the extract closely matches one cup (118 g) of boiled kale (USDA FDC #169238), which contains about 5,880 mcg lutein and zeaxanthin, 2,040 mcg beta-carotene, 11.8 mcg alpha-carotene, and 30.7 mcg cryptoxanthin[30]. Furthermore, other cooking techniques of stir-frying and steaming were reported to preserve more glucosinolates in cooked kale, compared to boiling[57]. It is probable that consuming a cup of cooked kale may elicit similar skin benefits, but this speculation needs to be confirmed in a study.

1.6 Pomegranate

Pomegranate (*Punica granatum* L.) is rich in polyphenols, mainly anthocyanins, punicalagin, and ellagic acid[58]. Photoprotection, as observed by a significant increase in the MED, was observed with a daily intake of either 12 weeks of pomegranate juice (8 oz; 237 mL) or its extract in 74 women aged 30 – 40 years with FSPT II – IV[59]. The pomegranate juice and extract provided similar amounts of punicalagin and ellagic acid polyphenols. Furthermore, the intake of pomegranate juice concentrate powder significantly decreased wrinkle formation, inhibited reduction in collagen type I and hyaluronan concentrations, and increased skin water content in UVB-treated mice compared to the group not receiving the powder[60]. Supplementation also inhibited pro-inflammatory cytokine IL-1β, and myeloperoxidase activity that promotes the formation of ROS, while increasing anti-inflammatory cytokine IL-10. Although whole fruits are encouraged for consumption due to the loss of fiber in juice, an easy and convenient way to consume fruits may be to drink its juice. One cup of pomegranate juice is equivalent to a serving of fruit and counts towards the daily recommendation.

1.7 Passion fruit

Passion fruit (*Passiflora edulis*) is filled with edible seeds that contain minerals and flavonoids beneficial for the skin: zinc, iron, copper, and anthocyanins[61]. Passion fruit seeds have more polyphenols than the pulp or rind, with piceatannol only present in the seeds[62]. Improved skin barrier function, as evidenced by a significantly increased moisture content and decreased TEWL, was observed in 32 Japanese females aged 35 – 55 with dry skin complaints who consumed passion fruit seed extract (PFSE) containing 5 mg piceatannol for eight weeks compared to controls[63]. Increased facial water content and viscoelasticity were also observed in adults who consumed piceatannol-rich beverages for eight weeks[64]. Piceatannol has been shown to increase oxidant defense, as evidenced by a reduction in amine-induced hydrogen peroxide generation in rats after six weeks of daily supplementation[65]. Approximately 2.3 g of raw passion fruit seeds are needed to obtain 5 mg (2.2mg/g) piceatannol[62]. This may be consumed through one passion fruit which is estimated to contain about 1 to 2 tablespoons of pulp and seeds providing at least 2.3 g of seeds.

1.8 Grape

Grape (*Vitis vinifera*) contains significant amounts of polyphenols, including anthocyanins[66], flavan-3-ols, resveratrol[67], and proanthocyanidins[68] as well as Vit C[69]. Six months of daily supplementation with a grape seed extract (GSE) providing 162 mg of proanthocyanidins to 12 Japanese women who had chloasma (brown patches on the skin) significantly reduced the melanin index and chloasma size and improved skin lightening[70]. The same group had previously supplemented guinea pigs with the GSE for eight weeks and observed an inhibition in melanin synthesis and increased lightening of the skin[71]. Another study observed a significant decrease in lentigo spots on the cheeks of women aged 30 – 60 years who

consumed 200 mL of a test beverage containing 200 mg of dealcoholized red wine oligomeric proanthocyanidins compared to a calorie matched control drink for 12 weeks[72]. Stratum corneum (SC) water content was significantly increased in the test group while it decreased in the controls. The melanin index and lightness improved in both the test and control groups. Taken together, the two clinical trials suggest reduced melanogenesis and skin lightening from the proanthocyanidins-rich grape extracts. Improved cellular oxidant and anti-inflammatory defenses, as seen with an inhibition of Nrf2-dependent antioxidant enzymes in the skin, along with a reduction in epidermal thickness, were observed with 14 days of GSE supplementation (2 mg/kg body weight) in UV-irradiated mice[73]. The studies provided about three to four times higher proanthocyanidins than the mean daily intake of 57.7 mg in the US[74]. Generally, grapes are the third major dietary source of proanthocyanidins, and their juice contains about 524 mg/L[74]. In dietary amounts, approximately 300 – 382 mL or 1.3 – 1.6 cups of grape juice may provide 162 – 200 mg of proanthocyanidins that was shown to benefit the skin.

1.9 Red bell peppers

Paprika (*Capsicum annuum* L.) and its essential oil and resin are rich in capsanthin and other carotenoids, including beta-carotene, beta-cryptoxanthin, and zeaxanthin[75]. Capsanthin has a keto-extended polyene chain, enabling it to be a more effective singlet oxygen quencher and peroxyl radical scavenger than beta-carotene[75,76]. Enhanced photoprotection was observed with paprika oil supplementation containing 9 mg xanthophylls, 5 mg capsanthin, and 0.5 mg beta-cryptoxanthin in 44 Japanese adults aged 30 – 50 with FSPT II for five weeks compared to a vegetable oil control[77]. Measurements from the skin on the back showed that supplementation with the paprika extract significantly minimized a decrease in skin lightness following UV-irradiation. A significant reduction in TEWL was also seen in the extract group when compared

to their baseline values, while no changes were noted for this or any other indices in the control group or in the face. Although the results are suggestive of bioactivity for the paprika extract, the authors also note that decreased humidity during the months of the intervention could have affected the facial skin since it was exposed to the environment, while the skin on the back was not. Although paprika is a ground spice, it can be made from dried red bell peppers (USDA FDC #170108), which contains 0.45 mg beta-cryptoxanthin per cup (92 g)[30], a similar amount to the paprika oil. Xanthophylls and capsanthin were not reported in the database, but these compounds are known to be abundant in red bell peppers[75].

The 2020-2025 Dietary Guidelines for Americans recommend two cups of fruits, especially whole, and 2.5 cups of vegetables with specific suggested amounts for dark-green, red and orange ones[78]. The studies above illustrate that the estimated amount of select fruits and vegetables generally aligns with the guideline, but each has a unique amount associated or could potentially benefit the skin. While estimations were performed to equate extracts to whole food or juice quantities, studies are needed to confirm these approximations. Clearly, more studies on whole fruits and vegetables are needed to provide practical and targeted dietary recommendations for different skin conditions.

2. Nuts and Legumes

Nuts and legumes are known for their abundance of beneficial fats and are a good source of plant-based protein and some vitamins. A higher intake of omega-3 polyunsaturated fatty acids (n-3 PUFAs) alpha-linolenic acid (ALA) from plant-based sources has been associated with a lower risk of severe photoaging[79]. Furthermore, women with the highest intake of n-3

PUFAs, especially eicosapentaenoic acid (EPA), were noted to be less prone to severe photoaging. Alpha-linolenic acid and its metabolites EPA, and docosahexaenoic acid (DHA) are not present in a normal epidermis[80]. However, enzymes in the skin can metabolize EPA and DHA, and the metabolites have been observed to accumulate in the epidermis. Omega-3 PUFAs, EPA, and DHA have been associated with healthy aging, in part by their ability to diminish inflammatory processes and ameliorate chronic inflammatory diseases[81]. The 2020-2025 Dietary Guidelines for Americans recommend an intake of 5 oz per week of nuts, seeds, and soy products[78]. However, higher amounts may be required to promote specific health benefits, as discussed in the nut and legume below.

2.1 Almonds

Almonds (*Prunus dulcis*) are rich in alpha-tocopherol, monounsaturated fatty acids (MUFAs), and polyphenols, all of which provide oxidant defense[82,83]. One ounce of almonds (USDA FDC #170567) contains 8.94 g of MUFAs, 3.5 g of PUFAs, 6 g protein, and 7.27 mg of alpha-tocopherol[30]. Alpha-tocopherol is the most abundant VitE in human tissues and functions in part by quenching lipid peroxidation[84] and increasing the levels of plasma glutathione (GSH), an endogenous antioxidant[85]. A significant decrease in overall wrinkle severity and width was observed in postmenopausal females aged 55 – 80 with FSPT I or II who consumed almonds providing 20% of total daily caloric needs for 16 weeks, compared to energy-matched nut-free snacks[86]. A follow-up study using the same dietary intervention found a significant decrease in average wrinkle severity in postmenopausal females aged 47 – 84 with FSPT I or II after 24 weeks of daily intake. In addition, facial pigment intensity decreased significantly with almond intake, but no changes were observed in the snack group[87].

The beneficial effects are partly attributed to almond's oxidative defense capability. At 10% or 20% of total calories, almonds have been found to increase both plasma and red blood cells alpha-tocopherol and significantly decrease serum cholesterol[88] and pro-inflammatory hsCRP[89]. An increase in glutathione peroxidase (GPx) activity has also been noted from daily intake of 0.7 oz (20 g) of almonds for eight weeks in overweight and obese women[90]. Additionally, the consumption of almond skin powder was demonstrated to decrease oxidized glutathione (GSSG), increase GSH and the GSH/GSSG ratio, and upregulate glutathione peroxidase (GPx) activity in healthy adults[91]. Glutathione neutralizes free radicals and is also a co-substrate for GPx, an important mechanism for the reduction of hydrogen peroxide and lipid hydroperoxides[92]. Based on a 2,000 kcal diet, 20% of calories equates to 400 kcals, which can be obtained from approximately 2.4 oz (68 g) of almonds, providing 21.5 g of MUFAs, 8.4 g of PUFAs, and 17.5 mg of alpha-tocopherol (117% of RDA). Currently, 1.5 oz (42 g) of nuts per day is recommended by the American Heart Association, and an increase of 0.5 oz/day has been associated with a lower risk of cardiovascular disease[93]. The American Heart Association also recommends a daily intake of up to 10% of PUFAs and as much as 15% of MUFAs from total calories. While individual caloric intake varies, it is likely that 20% of total calories for most adults would exceed 1.5 oz of nuts.

2.2 Soybeans

Soybeans (*Glycine max*) are rich in isoflavones, particularly genistein and daidzein, that have structures similar to estrogen and may interact with this hormone's receptors[94]. One ounce of mature raw soybeans (USDA FDC #174270) contains 4.4 g of MUFAs, 11.3 g of PUFAs, and 36.5 g protein. A significant decrease in fine wrinkles and increased skin microrelief (a network of furrows and ridges) at the lateral canthi and elasticity in the cheeks were observed in Japanese

women aged 35 – 48 who consumed an isoflavone-rich (40 mg soy isoflavone aglycone) soybean extract for 12 weeks compared to a color-matched control[95]. Furthermore, a significant increase in hydration and a decrease in SC thickness was observed in adults aged 25 – 60 who consumed a soybean and barley beverage for eight weeks compared to controls[96]. The group also observed increased hyaluronan (HA) levels in dermal fibroblasts and decreased hyaluronidase-2 (an enzyme that degrades HA) mRNA and protein levels in the soy/barley group only, supporting the improvement seen in skin hydration. The amount of 40 mg isoflavone is close to data from current epidemiological and clinical studies suggesting an intake of 50 – 90 mg of isoflavones or 15 – 25 g of soy protein per day for women for general health[97].

3. Polyphenol-rich Beverages

Coffee, green tea, and cocoa (hot chocolate) are a few of the popular beverages that are known to be rich in polyphenols. However, the caffeine present in these beverages may be a concern for some consumers, and the U.S. Food and Drug Administration has cautioned against consuming more than 400 mg of caffeine a day[98]. Fortunately, decaffeination has little to no effect on polyphenols[99,100], which provides an alternative to consume polyphenol-rich beverages without excessive caffeine.

3.1 Cocoa

Cocoa (*Theobroma cacao*) is a rich source of flavan-3-ols (flavanols) that can inhibit lipid peroxidation, neutralize ROS, and chelate metals that enhance the production of ROS[101]. Furthermore, cocoa flavanols can inhibit enzymes that quench ROS and upregulate protective genes involved in cellular stress responses. Daily consumption of a cocoa beverage containing

320 mg of flavanols for 24 weeks significantly improved measures of elasticity and the MED, as well as skin roughness, suggesting an improvement in wrinkle depth in Korean women aged 43 – 86 compared to a nutrient-matched control drink[102]. In addition, intake of a cocoa beverage containing 329 mg flavanols for 12 weeks significantly decreased UV-induced erythema, skin roughness, scaling, and TEWL, as well as increased skin density, thickness, hydration, and blood flow to the cutaneous and subcutaneous tissues in women aged 18 – 65 with FSPT II compared to 27 mg flavanol drinks[103]. Although 4 g of cocoa powder delivered adequate flavanols to benefit the skin[102], the amount of flavanols had been enriched and is higher than natural powder. Furthermore, processing of natural cocoa powders to create products for drink mixes has been found to reduce the amount of flavanols[104]. Therefore, depending on the source of cocoa powder, the amount needed to derive 320 – 329 mg of flavanol may differ.

3.2 Coffee

Coffee (*Coffea* L.) is rich in polyphenols, particularly chlorogenic acid[105,106]. An observational study assessed the amount of chlorogenic acid consumed from coffee by 131 Japanese females aged 30 – 60, and noted a significant association between higher consumption of the coffee (≥450 mL/d) or polyphenol (≥900 mg/d) with lower hyperpigmentation[107]. Daily consumption of a decaffeinated beverage containing 297.8 mg CPPs for four weeks significantly improved scaly skin in the cheeks and around the mouth in Japanese females aged 25 – 35 with concerns about facial skin dryness compared to a control drink[108]. In another study, consumption of a 100 mL decaffeinated beverage with 270 mg CPPs daily for eight weeks containing mainly chlorogenic acid significantly improved skin permeability barrier function, as evidenced by a decrease in dryness, TEWL, and pH, as well as an increase in SC lipids, lactic acid, and hydration, in 49 females with dry, itchy, and cracked skin compared to controls[109]. Roasted

coffee beans contain 7.95 – 8.75 mg/g of total polyphenols and could decrease to 1.17 – 1.58 mg/g after 12 months of storage[110]. The Specialty Coffee Association recommends brewing coffee at a ratio of 1.63 g of beans per fluid ounce of water[111]. Using 8 mg total polyphenols per gram of beans, 270 – 300 mg CPPs would equate to about 20 – 23 oz or 2.5 – 2.9 cups of coffee which is an amount consumed by most American adults, with 29% and 36% drinking two cups and three or more cups per day, respectively[112].

3.3 Green tea

Green tea (GT; *Camellia sinensis*) provides catechins[113], particularly epigallocatechin gallate (EGCG), which can act as antioxidants[114]. Catechins also protect against UVR and have anti-allergenic properties that may be beneficial to skin[115]. Daily consumption of one liter of a GT drink providing 1402 mg total catechins (980 mg EGCG) for 12 weeks in 60 women aged 40 – 60 with FSPT II significantly decreased UV-induced erythema, roughness, TEWL, and viscoelasticity (resistance towards an applied vacuum), compared to a control beverage devoid of polyphenols[116]. Additionally, increased serum catechin levels, skin density, and biological elasticity (ability to return to original position) were observed. In both groups, skin volume, scaling, and hydration significantly increased, which likely reflected the large amount of fluid consumed. However, supplementation increased hydration more than controls. In another study, supplementation with GT extracts containing 540 mg total catechins for 12 weeks also significantly decreased UV-induced erythema in 16 adults with FSPT I or II at the maximum UVR dose compared to lower levels[117]. In humans, catechin metabolites have been found in the skin after daily intake of GT extract containing 450 – 540 mg catechins for 12 weeks[117,118]. An increase in the expression of genes related to skin moisturizing factors in response to EGCG was also observed *in vitro*[119]. Typically, 1.8 to 3 g of leaves are used to brew a cup of tea, but the

amount of EGCG varies drastically from 2.3 to 203 mg/100 g of infusion[120]. Therefore, depending on the quality of tea leaves, as little as one or more than 10 cups of tea may be needed to achieve at least 500 mg of catechins. The European Food Safety Authority has found that modest amounts of green tea infusions and similar beverages are safe but caution against taking more than 800 mg/day EGCG through extracts[120].

4. Conclusion

The studies above collectively provide evidence of the potential benefits of plant-based foods for skin health and esthetics (Figure 1). In the era of personalized nutrition, this review can help dietitians provide better dietary recommendations of select plant-based foods to consumers. Many of the foods and extracts discussed are rich in bioactive compounds such as VitC, alpha-tocopherol, beta-carotene, polyphenols, and phenolic acids that provide oxidant defense and support mechanisms to lower inflammation or promote structural support and UV protection in the skin[121]. A few of the clinical studies above explore the role of whole and minimally processed foods (juice, beverage, paste), while others use extracts as the dietary intervention. Some of the extracts were equated to amounts of whole foods and beverages to evaluate the feasibility of consumption, but further studies are clearly required to confirm the speculations. Evidence gathered in this review paper strongly supports the consumption of colored fruits and vegetables that are typically abundant in vitamins, carotenoids, anthocyanins, and polyphenols. Nut and legumes are also encouraged, along with cocoa, coffee, and tea that are rich in polyphenols, although decaffeinated options should be considered with large amounts of intake. While intake of an abundance of plant-based foods may seem desirable, overconsumption of a single food or extract can be an efficacy concern, as illustrated above by the intake of some, but

not a large amount of mangos[27]. It would be helpful for future research to include details about the nutrient composition and equivalent to whole food or beverage for simpler translation of research findings into practical recommendations. In conclusion, clinical studies in the field of nutrition and skin research are currently limited, but a growing body of literature points to future targeted dietary recommendations for dermatological concerns, and more investigations on whole foods and beverages fortified with plant-based extracts are needed to achieve this goal.

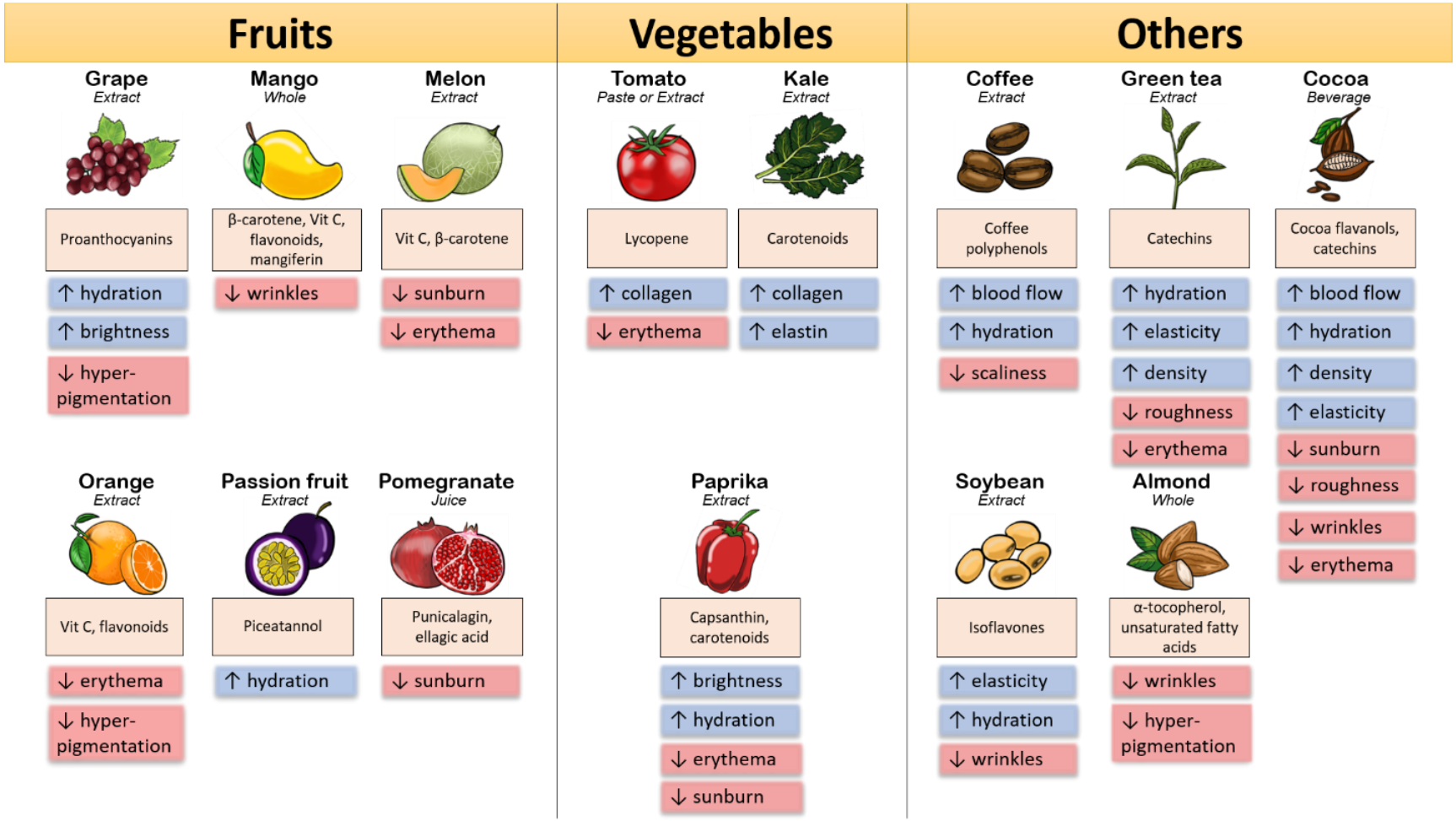

Figure 1. Reported effects of plant-based foods and extracts on the skin

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CHAPTER IV

Effects of Mango Intake on Vascular Function and Blood Inflammatory Markers in Postmenopausal Women

1. INTRODUCTION

A healthy vascular endothelium regulates the production of vaso-dilators and -constrictors, produces anti-inflammatory cytokines, and balances blood clotting factors(1). However, these processes may be disrupted by atherosclerosis, a buildup of plaque in the endothelium, which is the primary factor leading to cardiovascular disease(2). Endothelial dysfunction can be stimulated by various factors such as menopause causing estrogen deficiency, advanced glycation end products seen in aging(2), as well as increased oxidative stress and impaired nitric oxide balance(1). Peripheral arterial tonometry (PAT) is a non-invasive way to measure endothelial function(3). The measurements include reactive hyperemia index (RHI), augmentation index (AI), augmentation index corrected to 75 pulses/min (AI75), and Framingham reactive hyperemia index (fRHI). The RHI is a reliable measure of endothelial function, with a value under two non-officially categorized as endothelial dysfunction(4). The AI is an indirect measure of arterial stiffness, increases with age, and PAT measurements have been correlated with cardiac risk factors and coronary artery disease(5).

Aging and obesity are two factors that can induce low-grade, chronic inflammation(6,7). Pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor

necrosis factor (TNF)-α have been found to be significantly increased in postmenopausal women compared to those of reproductive age(8,9). These pro-inflammatory cytokines function in part to upregulate inflammatory reactions and stimulate acute phase reactants(10). Acute inflammatory responses are normal and essential, and with proper metabolic regulation, they are typically downregulated through a negative feedback loop. However, chronic inflammation is deleterious and increases the risk of many chronic diseases such as cardiovascular disease. High sensitivity C-reactive protein (hsCRP) is an acute-phase reactant induced by pro-inflammatory cytokines and is thought to be an independent predictor of future cardiovascular events(11). A higher hsCRP concentration (≥ 2.5 mg/L) has also been found to be associated with an increased breast cancer risk in overweight and obese (body mass index (BMI) ≥ 25 kg/m^2) postmenopausal women(12). Furthermore, postmenopausal status also significantly increases platelet aggregation(13). Activated platelets shed a soluble form of CD40 ligand (sCD40L), a costimulatory protein of the TNF family, which plays an important role in thrombosis and inflammation(14,15).

An improvement in endothelium-dependent vasodilation was observed in hypertensive adults who increased their fruit and vegetable intake(16). A meta-analysis also noted a significant improvement in endothelial function with vitamin C or E supplementation(17). Another meta-analysis found that most studies on fruit intake reported beneficial effects on select inflammatory biomarkers such as IL-1β, IL-6, TNF-α, and hsCRP(18). However, results may vary based on fruit type, the form of consumption, and the amount. Consumption of fruits rich in select polyphenols can reduce inflammation by inhibiting the expression and activity of enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2)(19). However, results from studies of specific fruit

polyphenols cannot be generalized to all fruits due to differences in digestion, absorption, metabolism, and bioactivity(20).

In 30 adults, consumption of 200 g of mango for 30 days significantly reduced plasma triglycerides and very-low-density lipoprotein, as well as increased plasma antioxidant capacity levels(21). Mango is a fruit abundant in vitamin C, carotenoids (β-carotene), fiber, and an array of polyphenols(22). The major phenolic acid in ripened Ataulfo mango variety has been reported to be chlorogenic acid, gallic acid, vanillic acid, and protocatechuic acid at a concentration of 301, 98.7, 24.4, and 1.1 mg/100 g dry weight, respectively(23), and interactions of these compounds in different combinations have been found to present both synergistic and antagonistic oxidant effects(24). Other polyphenols of importance in mango include gallotannins and mangiferin(22).

Mango extracts have been reported to decrease IL-1β, IL-6, and TNF-α protein levels and reduce mRNA expression of three pro-inflammatory cytokines, iNOS, and COX-2 in rats(25). Additionally, mangiferin supplementation has been demonstrated to inhibit stress-induced IL-1β, TNF-α, iNOS, and COX-2 expressions and increase antioxidant levels in the brain of rats(26). Mango juice intake with only pulp or an addition of peel both decreased TNF-α in mice compared to controls(27). Furthermore, a significant decrease in inflammatory cytokines was observed in obese adults who consumed 400 g of Ataulfo mango pulp daily for six weeks compared to lean individuals(28). Plasma vitamin C status has been inversely correlated with pro-inflammatory markers(29,30), and supplementation at 500 g twice daily for eight weeks in obese, hypertensive, and/or diabetic adults significantly reduced hsCRP and IL-6(31). Dietary fiber consumption reported through a food frequency questionnaire, which is able to capture dietary intake for the past three

months, has also been inversely associated with IL-6 and TNF-α in Caucasian postmenopausal women aged 50 to 79(32).

Increased vegetable and fruit, as well as total flavonoid intakes, have been inversely correlated with hsCRP(33). While direct evidence for the effect of mango intake on hsCRP and sCD40L is limited or non-existent, bioactive compounds of the fruit have demonstrated beneficial effects. Vitamin C supplementation at 1,000 mg/day significantly decreased elevated hsCRP (≥ 1.0 mg/L) levels in adults(34). In addition, intravenous infusion of vitamin C at 24 mg/min for 45 minutes significantly inhibited platelet CD40L expression and sCD40L formation(35). Fiber intake of 30 g/day from natural sources or supplementation has also been shown to reduce levels of hsCRP(36). Moreover, some clinical studies have reported significant sCD40L reductions after consuming different foods rich in polyphenols(37,38). Chlorogenic acid, specifically, has been found to significantly inhibit platelet aggregation and the release of sCD40L and IL-1β(39). Protocatechuic acid also inhibited platelet activation, although measurement of sCD40L was not assessed(40). While a clinical study demonstrated that consumption of mango in the form of a shake along with a high-fat meal did not change hsCRP levels(41), the intervention was acute and may not accurately reflect long-term intake of mangos. Furthermore, serum β-carotene has been demonstrated to have a strong inverse association with systemic markers of inflammation (hsCRP and white blood cells count)(42) and oxidative stress(30), and mango is a rich source of the red-orange pigment(22).

Overall, there is a large body of evidence that supports the idea that overweight and obese postmenopausal women are at increased risk of chronic inflammation that can lead to an elevation in plasma IL-1-β, IL-6, and TNF-α, as well as serum hsCRP and sCD40L,

which may affect their vascular function. The first objective of the probe study described in the current paper was to investigate the effects of acute and two weeks of daily mango intake on the vascular function measured with PAT. Secondary objectives were to investigate the effects of the same dietary intervention on the expression of pro-inflammatory cytokines, hsCRP, and sCD40L. We hypothesized that an acute intake and two weeks of 330 g of daily mango consumption would improve vascular function measures. We also hypothesized that two weeks of daily mango intake would decrease plasma IL-1-β, IL-6, TNF-α, serum hsCRP, and sCD40L.

2. METHODS

2.1 Participants

Postmenopausal women aged 50 to 70 years who were overweight or obese (BMI 25.0 to 40.0 kg/m^2), with a minimum weight of 110 pounds, and willingness to comply with all study procedures were eligible for the study. Exclusion criteria included high consumption of fruits (≥ 2 cups/day), vegetables (≥ 3 cups/day), fatty fish (≥ 3 times/week), dark chocolate (≥ 3 oz/day), coffee and/or tea (≥ 3 cups/day), and/or alcohol (≥ 3 drinks/week). Other dietary exclusion included consumption of a vegan, vegetarian, non-Western diet, and use of supplements a month prior to and during the study. Health exclusion criteria included a blood pressure of ≥ 140/90 mm Hg, having diabetes, renal, heart, liver, or gastrointestinal disease, cancer within the last five years, an abnormal liver panel, complete blood count (CBC), or other indices in a comprehensive metabolic panel (CMP). In addition, daily use of anticoagulation agents such as aspirin, an indication of substance or alcohol abuse within the last three years, and chronic or routine high-intensity exercises such as hiking, jogging, and sports that require equivalent energy were excluded.

The study was approved by the University of California (UC) Davis Institutional Review Board, and all volunteers gave their informed consent for inclusion before they participated in the study.

2.2 Mango variety and processing

The Ataulfo mango variety was chosen for this study as it has higher amounts of phenolic acids, β-carotene, and ascorbic acid than other varieties commonly found in the USA(43). All fruit was from a single shipment. The mangos were allowed to ripen under ambient conditions until they were a light yellow color with a medium-soft texture, after which they were washed, manually peeled and deseeded, cubed, weighed into daily portions (330 g; 2 cups), and frozen at –20°C until needed.

2.3 Study design

Volunteers were first screened by telephone to determine if they met the inclusion and exclusion criteria and, if qualified, were invited to the laboratory for a clinical screening visit in the morning after an overnight fast. If volunteers reported menses occurring within the past two years prior to the telephone screening, follicle-stimulating hormone (FSH) was measured to confirm postmenopausal status, and an FSH level of 23 - 116.3 mIU/mL was accepted as having reached menopause. Blood was collected for a CMP, CBC, and a lipid panel, and anthropometric measurements were taken, including body weight, height, waist circumference, blood pressure, and resting heart rate.

Participants who passed the screening visit were randomized into a four-week single-arm trial (Figure 1). Three study visits at weeks 0, 2, and 4 occurred in the morning after a 12-hour overnight fast. The study design consisted of two phases, which allowed each participant to be their own control. The control phase occurred between weeks 0 and 2, and participants did not consume any mangos during this period. The intervention phase occurred between weeks 2 and 4, and participants consumed 330 g of pre-packaged mangos daily (165 g before noon and the rest in the evening). Water was allowed *ad libitum*. Prior to each study visit, participants were instructed to refrain from strenuous exercise that would significantly increase their heartbeat 24 hours before arriving at the laboratory. Participants were asked to refrain from consuming additional mangos before the first study visit (run-in period) and throughout the entire study. Each study visit began with the collection of anthropometric measures (height, weight, and waist circumference), resting heart rate, and blood pressure (BP).

Thereafter, participants rested for 30 minutes in a supine position on a bed prior to PAT assessment at zero (0 hr) and two hours (2 hr) after water intake or a single 330 g of mango intake. The process began by inducing transient ischemia to the upper arm by inflating a pressure cuff above the systolic pressure for five minutes before deflating to stimulate reactive hyperemia. Procedures were performed at the same time of the day to minimize circadian effects.

The study was 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 (Identifier: NCT03203187).

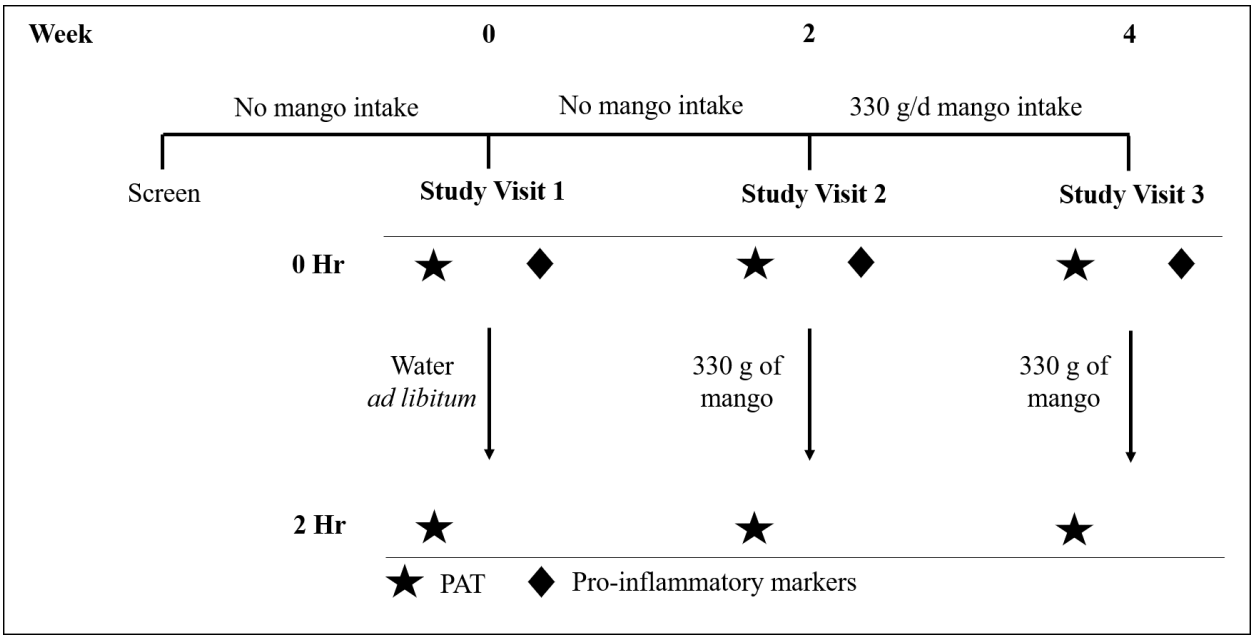

Figure 1. Study design and timeline

2.4 Collection of plasma and serum samples

Fasting blood samples for the measurement of pro-inflammatory markers were collected at 0 Hr for all study visits. A licensed phlebotomist drew samples into evacuated blood collection tubes (Becton Dickinson; Franklin Lakes, NJ). Five mL of whole blood was collected in a 10 mL clear blue-top vacutainer containing 3.2% trisodium citrate for plasma recovery. Another five mL of whole blood was collected in a serum separator tube (SST) for serum recovery. The tubes were inverted several times immediately after blood collection and placed in a rack at room temperature. Blood in the SST collection tube was allowed to clot in a vertical position at room temperature for one hour, then centrifuged at 1800 x g for 15 minutes at room temperature to obtain serum. The blue-top tube was centrifuged within two hours of blood collection at 2800 x g for 20 minutes at room

temperature to obtain platelet-poor plasma. Either 100 uL or 200 uL of serum or plasma was aliquoted into 0.6 mL clear microcentrifuge tubes (Fisher Scientific; Chicago, IL) and stored at -80℃ until further testing (Figure 2). A total of three aliquots were saved for each sample to avoid damage from freeze-thaw cycles during analysis.

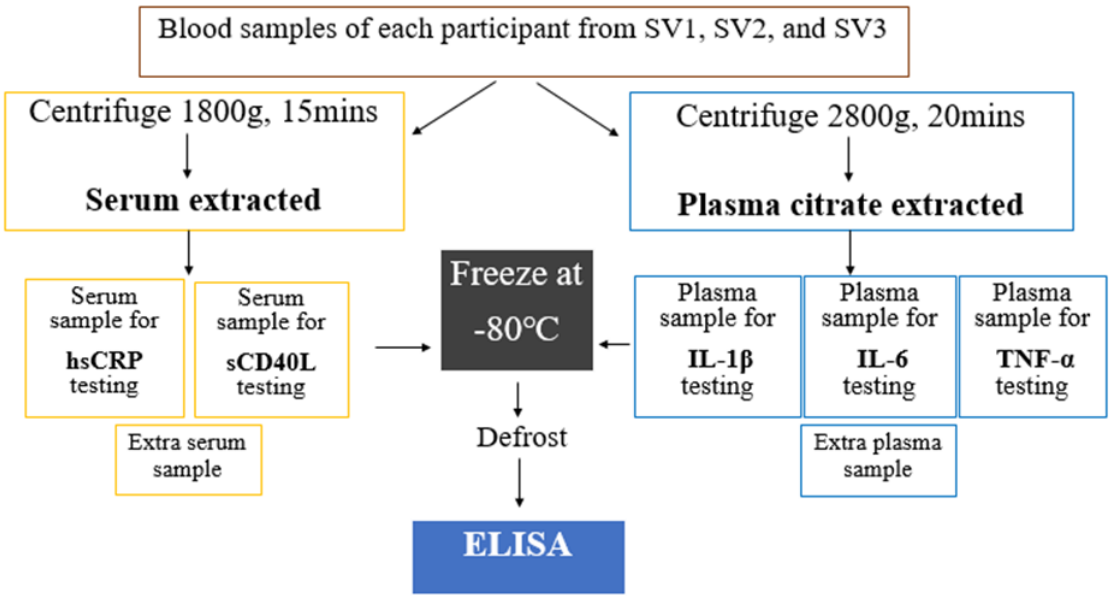

Figure 2. Blood sample collection, processing, and storage.

2.5 Pro-inflammatory biomarkers

Pro-inflammatory cytokines ((IL-1-β, IL-6, and TNF-α), high-sensitivity C-reactive protein (hsCRP), and soluble CD40 ligand (sCD40L) were assessed by pre-coated enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen™, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

2.6 Statistical analysis

Statistical analyses were performed with JMP version 15 (SAS Institute Inc., Cary, NC, USA). P values of 0.05 or less were considered statistically significant. Each parameter was assessed for normality prior to analysis. Pro-inflammatory markers, vascular function measures, and biochemistry labs were analyzed before and after two weeks of daily mango intake by comparing week 0 to 2, and 2 to 4. The effect of acute daily mango intake on vascular function measures was analyzed by comparing 0- to 2-hour with or without mango intake. All analyses were performed using paired *t* tests, or Wilcoxon signed ranked test. Data are presented as mean ± SD. Correlation between the pro-inflammatory and vascular function markers, as well as biochemistry measures, were performed with Pearson and Spearman correlations.

3. RESULTS

3.1 Participant characteristics and changes in biochemistry

The baseline (week 0) characteristics of the participants are shown in Table 1. A total of 23 postmenopausal women were included in this study, with a mean age of 60 ± 5 years and BMI of 29.1 ± 3.0 kg/m^2. Participants had borderline-high total cholesterol (202.7 ± 29.6 mmol/L), and borderline-low high-density lipoprotein (HDL) levels (55.2 ± 17.7 mmol/L). All other values were within normal ranges.

Participants' BMI, blood pressure, and biochemistry values were not significantly different with or without the mango intervention. A non-significant decrease was observed with cholesterol and platelets during the control phase. Serum cholesterol values also trended to decrease after the mango intervention.

Table 1: Participant characteristics and biochemistry

	Week 0 Mean ± SD	Week 2	Week 4	Week 0 vs 2 (no mango) *P*	Week 2 vs 4 (mango) *P*
Age (years)	60 ± 5	-	-	-	-
BMI (kg/m^2)	29.1 ± 3.0	29.1 ± 2.9	29.2 ± 3.0	0.92	0.43
SBP (mm Hg)	111.9 ± 10.5	112.2 ± 9.3	111.3 ± 9.8	0.77	0.55
DBP (mm Hg)	74.3 ± 6.0	75.0 ± 6.1	74.0 ± 6.5	0.33	0.29
HR (BPM)	66.4 ± 7.1	66.7 ± 8.4	65.4 ± 7.8	0.73	0.38
PP (mm Hg)	37.6 ± 5.2	37.1 ± 4.6	37.3 ± 4.5	0.61	0.72
MAP (mm Hg)	86.8 ± 7.5	87.4 ± 7.2	86.4 ± 7.6	0.48	0.37
Comprehensive metabolic panel					
Sodium (mmol/L)	139.6 ± 2.8	140 ± 2.2	139.6 ± 2.5	0.35[W]	0.33
Potassium (mmol/L)	4.2 ± 0.3	4.2 ± 0.3	4.3 ± 0.4	0.66	0.36
Chloride (mmol/L)	105.6 ± 2.7	105 ± 2.3	105 ± 2.4	0.28	0.94
CO2 (mmol/L)	27.1 ± 1.2	27.3 ± 0.4	27 ± 1.1	0.38[W]	0.30 [W]
BUN (mg/dL)	14.3 ± 8.0	13.0 ± 4.0	12.7 ± 2.6	0.72 [W]	0.66
Creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.29	0.98
Glucose (mg/dL)	98.1 ± 8.5	97.3 ± 8.5	99 ± 10.0	0.34	0.23
Calcium (mg/dL)	9.1 ± 0.3	9.1 ± 0.3	9.1 ± 0.3	1.00	0.91
Protein (g/dL)	6.8 ± 0.4	6.7 ± 0.5	6.6 ± 0.5	0.46	0.56
Lipid panel					
Cholesterol (mg/dL)	202.7 ± 29.6	195.8 ± 28.4	193.7 ± 25.4	0.06	0.07
HDL (mg/dL)	55.2 ± 17.7	53.7 ± 14.9	53.9 ± 14.9	0.24	0.82
LDL (mg/dL)	128.9 ± 21.1	123.8 ± 20.7	123.2 ± 18.0	0.11	0.23
Chol:HDL	4.0 ± 1.1	3.9 ± 1.0	3.85 ± 0.9	0.12	0.51
Triglycerides (mg/dL)	93.8 ± 52.3	89.1 ± 48.5	92.8 ± 47.7	0.21 [W]	0.92
Non-HDL (mg/dL)	143.9 ± 25.9	142 ± 26.1	140.1 ± 21.3	0.70	0.08
Complete blood count					
WBC (K/mm^3)	5.3 ± 1.3	5.2 ± 1.1	5.0 ± 1.1	0.37 [W]	0.83 [W]
RBC (M/mm^3)	4.5 ± 0.4	4.4 ± 0.4	4.9 ± 2.4	0.10 [W]	0.82 [W]
Hemoglobin (gm/dL)	13.2 ± 1.1	13.0 ± 1.3	13.0 ± 1.0	0.29 [W]	0.56 [W]
Hematocrit (%)	39.8 ± 3.1	39.0 ± 3.2	38.9 ± 2.7	0.11 [W]	0.43
MCV (fL)	88.7 ± 6.9	88.6 ± 7.1	89.1 ± 6.8	1.00 [W]	0.31 [W]
MCH (pg)	29.6 ± 2.6	29.7 ± 2.9	29.8 ± 2.7	0.31 [W]	0.99 [W]
MCHC (%)	33.3 ± 0.8	33.4 ± 1.0	33.4 ± 0.8	0.32 [W]	0.85 [W]
RDW (%)	13.6 ± 1.3	13.6 ± 1.4	13.4 ± 1.3	0.34 [W]	0.52 [W]
MPV	8.9 ± 1.2	8.9 ± 1.1	9.0 ± 1.2	0.27	0.69
Platelets (K/mm^3)	237.7 ± 49.6	234.6 ± 54.0	233.4 ± 49.3	0.09	0.12

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR = heart rate; PP = pulse pressure; MAP = mean arterial pressure; Alk Phos = alkaline phosphatase; AST = aspartate aminotransferase; ALT = alanine aminotransferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; Chol:HDL = cholesterol to HDL ratio; non-HDL = non-high-density lipoprotein; WBC = white blood cells; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume. Statistical analysis with two-tailed paired t-test. [W] Wilcoxon Signed Rank test. Significance $P \leq 0.05$.

3.2 Mango intake

According to the USDA Nutrient Database for standard reference 2018, 330 g of mangos will provide approximately 198 kcals, 5.28 g fiber, 45 g sugar, 120 mg vitamin C, and 2,120 mcg β-carotene, among other nutrients(44)(Table 2). However, values were based on analyses of Tommy Atkins, Keitt, Kent, and/or Haden cultivars, and the amount of vitamin C and β-carotene has been measured to be higher in Ataulfo compared to these varieties(43).

Table 2. Nutrient composition of mango pulp

Nutrients	**Amount per 330 g (2 cups)**
Water (g)	276
Energy (kcal)	198
Protein (g)	2.7
Fat (g)	1.25
Carbohydrate (g)	49.4
Dietary Fiber (g)	5.28
Total Sugars (g)	45
Vitamin C (mg)	120
β-carotene (mcg)	2120

3.3 Pro-inflammatory and vascular function markers

All plasma and serum samples were collected, frozen, and diluted according to the sample preparation guidelines for each ELISA kit. A total of three test sessions were performed for each of the pro-inflammatory cytokines: TNF-α, IL-1-β, and IL-6, and tested in replicates of two each time. In the first session, 80 to 90% of the samples developed into a darker color than the highest standard range, and the software failed to provide measurements. Therefore, further dilutions of each sample were performed for the second and third sessions, but most of them were still darker, and the software failed to provide any values again. Subsequently, no samples remained for further testing. Therefore, no valid data were collected for TNF-α, IL-1-β, and IL-6.

A significant decrease in sCD40L was observed during the control phase (no mango intake), followed by a non-significant increase near baseline values during the intervention phase (330g mango intake) (Table 3, Figure 3). No significant changes were observed in hsCRP (Table 3). As noted above, analyses for TNF-α, IL-1-β, and IL-6 were unsuccessful.

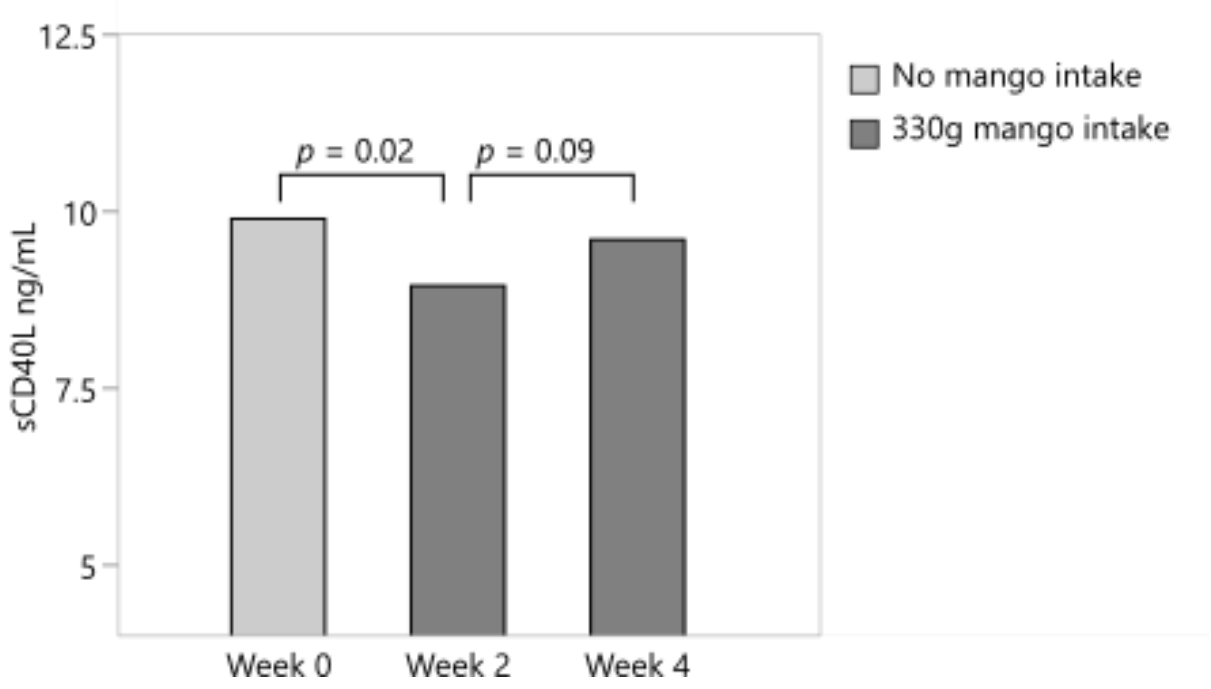

Figure 3. A significant decrease in sCD40L was observed from week 0 to 2 (no mango intake), followed by an increase from week 2 to 4 (330g mango intake).

Table 3. Values of sCD40L and hsCRP with and without mango intake.

	Week 0 Mean ± SD	Week 2	Week 4	Week 0 vs. 2 (no mango) *P*	Week 2 vs. 4 (daily mango) *P*
sCD40L (ng/mL)	9.9 ± 2.5	9.0 ± 2.1	9.6 ± 2.4	**0.02**	0.09
hsCRP (ug/mL)	42.4 ± 31.2	41.1 ± 31.8	43.9 ± 34.7	0.72	0.82

sCD40L = soluble CD40 ligand; hsCRP = high-sensitivity c-reactive protein. Statistical analysis with two-tailed paired t-test. [W] Wilcoxon Signed Rank test. Significance $P \leq 0.05$.

A significant decrease in AI was observed two hours after consuming one serving of 330 g of mango at both 2- ($p = 0.02$) and 4-week ($p = 0.04$; Table 4) time points. Mango intake also significantly decreased AI75 after two hours at 2- ($p = 0.04$) and 4-week ($p = 0.05$), while no differences were seen with RHI and fRHI (Table 4). In addition, no significant differences were observed in RHI, AI, AI75, and fRHI after two weeks of daily mango intake (Table 5).

Table 4. Peripheral artery tonometry (PAT) measures 2 hours after mango or no mango intake.

	Week 0 (no mango)			Week 2 (330 g mango)			Week 4 (330 g mango)		
	0 Hr	2 Hr	*P*	0 Hr	2 Hr	*P*	0 Hr	2 Hr	*P*
RHI	2.1±0.5	2.1±0.5	0.72	2.2±0.6	2.1±0.6	0.62	2.2±0.5	2.1±0.5	0.54
AI	22.8±16.2	18.3±14.9	0.19 [W]	20.5±15.1	14.9±11.6	**0.02**	19.8±14.1	14.2±10.9	**0.04**
AI75	14.7±15.0	10.7±13.0	0.26 [W]	13.3±14.4	8.4±10.6	**0.04**	11.9±11.0	7.4±9.6	**0.05** [W]
fRHI	0.5±0.3	0.5±0.3	0.80	0.6±0.3	0.5±0.4	0.32	0.5±0.4	0.5±0.3	0.38

AI = augmentation index; AI75 = augmentation index corrected to 75 pulses/min; fRHI = Framingham reactive hyperemia index; RHI = reactive hyperemia index. Statistical analysis with two-tailed paired t-test. [W] Wilcoxon Signed Rank test. Significance $P \leq 0.05$.

Table 5. Peripheral artery tonometry (PAT) measurements 2 weeks with and without mango intake.

	Week 0	Week 2	Week 4	Week 0 vs 2 (no mango) *P*	Week 2 vs 4 (mango) *P*
RHI	2.1 ± 0.5	2.2 ± 0.6	2.2 ± 0.5	0.58	0.93
AI	22.8 ± 16.2	20.5 ± 15.1	19.8 ± 14.1	0.57 [W]	0.79
AI75	14.7 ± 15.0	13.3 ± 14.4	11.9 ± 11.0	0.57 [W]	0.60
fRHI	0.5 ± 0.3	0.6 ± 0.3	0.5 ± 0.4	0.33	0.40

AI = augmentation index; AI75 = augmentation index corrected to 75 pulses/min; fRHI = Framingham reactive hyperemia index; RHI = reactive hyperemia index. Statistical analysis with two-tailed paired t-test. [W] Wilcoxon Signed Rank test. Significance $P \leq 0.05$.

The pro-inflammatory markers hsCRP and sCD40L were weakly correlated (r = -0.31, $p = 0.03$; Table 6). High-sensitivity CRP was also weakly correlated with SBP (r =

0.36, $p = 0.008$), DBP (r = 0.34, $p = 0.01$), and MPV (r = -0.28, $p = 0.049$; Table 6). While sCD40L was weakly correlated with platelets (r = 0.34, $p = 0.007$) and MPV (r = 0.42, $p = 0.008$; Table 6), no correlation was observed between sCD40L and hsCRP with any measures of vascular function.

Table 6. Correlations

		sCD40L	hsCRP†
hsCRP	r	-0.31	
	p	**0.03†**	-
SBP	r		0.36
	p	NS	**0.008**
DBP	r		0.34
	p	NS	**0.01**
Platelets	r	0.34	
	p	**0.007**	NS
MPV	r	0.42	-0.28
	p	**0.008†**	**0.049**
fRHI	r		
	p	NS	NS
AI	r		
	p	NS	NS
AI75	r		
	p	NS	NS
RHI	r		
	p	NS	NS

†Pearson's correlation. Significant correlations ($p \leq 0.05$) are bolded.

4. DISCUSSION

This probe study assessed the effects of mango intake on vascular function and inflammatory markers in overweight and obese postmenopausal women. An intake of 330 g of mango was found to decrease AI and AI75 two hours post-consumption, indicating acute lowering of arterial stiffness. However, this effect did not persist as no significant changes in AI or AI75 were observed after two weeks of daily mango intake. Mango intake also did not change RHI or fRHI. Therefore, results obtained from this study suggest that mango intake may acutely improve endothelial function in postmenopausal women, however, an extended study would be needed to determine its effectiveness for longer-term intake.

The values of the inflammatory markers were hypothesized to remain constant during the control phase from week 0 to 2 since no mango was consumed during that time. Furthermore, participants reported minimal to no mango intake in their usual diet prior to the start of the study. However, a significant decrease in sCD40L was noted during the control phase. This could be due to a non-significant decrease in platelets during the same time frame since sCD40L is primarily derived from activated platelets(15). This is also supported by a positive correlation between sCD40L with platelets and MPV in this study. Following this, a trend towards a significant increase was observed in the intervention phase (week 2 to 4) for sCD40L with 330 g of mango intake, but values returned to near baseline (week 0). Therefore, there is no conclusive evidence that mango intake increased sCD40L. In addition, no changes were seen with hsCRP, as well as vascular function measures of RHI, AI, AI75, and fRHI as values remained constant throughout the study. However, a study that provided 400 g of Ataulfo mango daily to lean and obese adults found a non-significant decrease in hsCRP in both groups after six weeks(28). Additionally, plasma β-

and α-carotene were inversely correlated with hsCRP in nonsmoking men who increased their fruit and vegetable intake from two to eight servings per day for four weeks(45). The positive correlation observed between hsCRP with SBP and DBP aligns with a meta-analysis that demonstrated higher levels of circulating hsCRP with an increased risk of developing hypertension(46).

While overweight and obesity are factors associated with low-grade chronic inflammation, participants in the current study were generally healthy, which could explain the null findings. Although the amount of mango intake was high at 330 g/day, participants consumed them for only two weeks, which was a very short intervention. Another study that provided obese adults with 10 g of freeze-dried mango pulp powder, equivalent to 100 g of fresh fruit, daily for 12 weeks also did not find any correlations with inflammatory markers such as IL-6 and TNF-α(47). However, the nutrient profile may not be comparable to the fresh fruit due to processing. The current study was powered for the primary outcome measure, PAT. Therefore, the sample size might also be too small to accurately interpret the results obtained from the measurements of pro-inflammatory markers. Furthermore, the samples unexpectedly contained analyte concentrations higher than the highest standard point and attempts to further dilute the samples still resulted in invalid data.

A number of animal studies have reported favorable changes in a variety of inflammatory indices in response to the intake of mango or its bioactive compounds. Rats fed a mango beverage rich in gallic acid and gallotannins showed a reduction in their inflammation score by 47% compared to animals consuming a control beverage containing sugar, Vitamin C, and a modest amount of polyphenols(25). The mango beverage contained a total soluble phenolics of 475.8 mg gallic acid equivalent (GAE)/L, equivalent to 14.55

mg GAE/kg/day or 873 mg GAE/day for a 60 kg person(25). About 670g to 880g of Ataulfo mangos would be needed to obtain 873 mg GAE/day since the specific cultivar has been reported to contain a total phenolic content of 99 to 130 mg GAE/100 g(43). When rats enduring stress via a plastic rodent restrainer were fed mangiferin extracts at 15, 30, and 60 mg/kg amounts for seven days, a reduction in the pro-inflammatory cytokine IL-1β was observed, whereas the control group fed distilled water had elevated levels(26). The mangiferin extract-fed group also showed less depletion of brain antioxidant levels, such as catalase, compared to the controls(26). Stressed-induced elevation in lipid peroxidation and pro-inflammatory enzymes in the brain were also significantly decreased with mangiferin supplementation(26). Based on the human equivalent dose calculation, 15, 30, and 60 mg/kg of mangiferin extract for rats would equate to 2.4, 4.8, and 9.7 mg/kg for humans, which is 144 to 582 mg per 60 kg(48). Therefore, it is challenging to consume this amount solely from fresh mangos, which contain 4.4 mg of mangiferin per kilogram of pulp(49). A 12 weeks study that provided overweight adults with 150 mg/day of mangiferin found a significant increase in serum levels of the xanthone and a decrease in triglycerides, free fatty acid, and high-density lipoprotein levels(50).

Mice were injected, respectively, with lipopolysaccharide stimulant with or without 10 mg/kg of β-carotene intervention, and blood samples were collected after 12 hours(51). Intervention with β-carotene inhibited enzymes (iNOS and COX-2) that synthesize inflammatory mediators (nitric oxide and prostaglandin E_2), cytokines (IL-1β, TNF-α) and their mRNA levels(51). The amount provided to mice was equivalent to 0.81 mg/kg or 48.6 mg for a 60 kg human(48), which is high considering that the recommended dietary allowances (RDAs) are about 1.4 to 1.8 mg of supplemental or 8.4 to 10.8 mg of dietary

beta-carotene (approximately 700 to 900 mcg retinol acetate equivalent for vitamin A) for adults(52).

The dietary amounts used in some interventions are much higher than what humans would typically consume. However, it shows the potential for reducing inflammatory markers with mango consumption. Future studies could investigate the effects of mango intake at an amount normally consumed by humans, in a population with elevated baseline inflammatory levels, with a larger sample size or a longer intervention duration.

Although the current study was designed with a focus on inflammation, it is also relevant to skin health. Exposure to ultraviolet radiation (UVR) has been found to activate the epidermal growth factor receptor, which increased the expression of pro-inflammatory cytokines(53). Ultraviolet radiation has also been shown to increase hsCRP and is associated with mild erythema(54). Mangiferin has also demonstrated anti-inflammatory capabilities and protection against UVR(55). Therefore, consumption of mangos could also potentially reduce inflammatory markers associated with the actions of ROS(56).

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APPENDIX A

Gamified Nutrition Education with Mastery Learning and Spaced Repetition Theory – Can Improve Nutrition Knowledge

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Keywords: Nutrition education, nutrition behavior, virtual education, virtual game, healthy snacking.

Abbreviations: BMI=body mass index, HEI=healthy eating index, MetS=metabolic syndrome, S.N.A.C.K=Self-paced Nutrition Activity on Choices for Knowledge, TP=time point

Abstract

Background: Children often consume up to 30% of calories from snacks that are usually low in nutrient value. Over time, poor dietary choices can contribute to an increased risk of obesity and related health problems.

Purpose: Describe the development and utilization of the Self-paced Nutrition Activity on Choices for Knowledge (S.N.A.C.K) nutrition education game to elicit and reinforce healthier snack choices by children aged 9 to 13 years.

Methods: Children engaged in S.N.A.C.K on a provided tablet for 15 to 25 minutes, at week 0 and 4. The game was self-paced, providing education about the Nutrition Facts Label, and consisted of three assessments to evaluate knowledge and application.

Results: Data from 41 children, 59% normal weight, and 41% overweight/obese, were included in the analysis. Significant improvements in knowledge and utilization of the Nutrition Facts Label were observed immediately after education, with further advancement after a second session four weeks later.

Discussion: In this study, children immediately improved, and sustained, their newly acquired nutrition knowledge with a single 15 to 25 minute session with S.N.A.C.K.

Translation to Health Education Practice: Educators could incorporate this consistent and accurate education into their curriculum to reinforce a child's nutrition knowledge over time.

Background

In many populations, dietary habits that are developed during childhood persist in adulthood, where poor dietary food choices can contribute to an increased risk for obesity and diverse nutrition-related health problems.[1] A dietary pattern that was relatively low in added sugars, total fat, and saturated fatty acids was associated with lower systolic blood pressure, triglycerides, and abdominal obesity, and significantly decreased the risk of developing metabolic syndrome (MetS), a cluster of conditions that increases the risk of chronic disease, in children and adolescents.[2] Higher intakes of dietary fiber has also been found to be inversely associated with the prevalence of MetS in adolescents.[3] Furthermore, high total energy intake has been strongly associated with increased levels of insulin resistance in children, a marker of diabetes mellitus.[4] On average, children consume up to three snacks per day, and calories from snacks often account for up to 30% of total calories.[5] Frequently consumed snack options include salty snacks, high-fat desserts, and sugar-sweetened beverages.[6] Multiple factors such as children's preference, parental selection, socioeconomic, and availability of food, can influence the selection of snacks. However, nutrition knowledge is an important component of health literacy and is associated with better dietary intake and health outcomes.[7–9] The current study focused on equipping children with the ability to select and then preferentially consume nutrient-dense snack options.

A potential approach to helping children understand healthy snacks is to educate them on reading and processing the information on a Nutrition Facts Label. This will be particularly

helpful in the event that fresh foods, such as fruits and vegetables, are unavailable at the time of snack consumption. Based on the Mastery Learning Theory, assessments are used as part of the instructional process to diagnose individual learning difficulties and provide instruction or correction to improve an individual's learning.[10] Students learn at different rate often resulting in an achievement gap that could be improved upon if instructional methods and time matches students' individual learning needs.[10] The theory supports that the Nutrition Facts Label can be taught to children when appropriate teaching methods are used and adequate time is given to learn.[11] In addition, based on the Spaced Repetition Theory, breaks should be given between repeated exposures to improve learning.[12] Spaced repetition requires the children to recall past exposure to the education, which can enhance memory.[12] In contrast, continuous repetition without breaks effectively eliminates the recall process.[12]

In addition, utilizing the concept of gamification to promote health and prevent disease has shown promising results by increasing knowledge, affecting behavioral change, and influencing feelings by increasing positivity and reducing negativity.[13,14] A study compared gamified versus blackboard teaching of nutrition in children aged 12 to 14 years and found that both interventions significantly improved nutrition knowledge.[15] Gamification can be an effective way to impart nutritional knowledge and skills to children as it is interactive and allows the individual to master content taught within their own capacity[16]. However, it is important to combine gamification with the theories of mastery learning and spaced repetition for a long-term impact[17]. This is evident from a study that exposed children to two consecutive days of game intervention and saw an immediate increase in nutrition

knowledge after the game compared to the control, but long-term benefits were not seen as both groups performed similarly at two weeks follow-up.[17]

Using these theories as a framework, a tablet-based nutrition education game called the Self-paced Nutrition Activity on Choices for Knowledge (S.N.A.C.K) with the goal of increasing the children's ability to read and utilize the Nutrition Facts Label was developed.

Purpose

The objective of the current paper is to describe the development, utilization, and efficacy of the S.N.A.C.K. game for children aged 9 to 13 years. We hypothesized that S.N.A.C.K would improve children's knowledge and understanding of the nutrition facts label.

Methods

Development of S.N.A.C.K *(Self-paced Nutrition Activity on Choices for Knowledge)*

The S.N.A.C.K game was developed with the objective to provide children aged 9 to 13 years with the necessary nutrition education to allow them to make healthier packaged snack choices. Assessment scores were collected from the game and the Healthy Eating Index (HEI) scores were derived from 24-hour dietary recalls. The education and assessments in

the game were constructed with the Nutrition Facts Label as a reference tool. The game aimed to impart knowledge about nutrients of concern with packaged snack purchases (carbohydrates, protein, fat, added sugar, sodium) and for children to acquire knowledge and skills to use the Nutrition Facts Label. A design team, comprising experts in nutrition education for school-aged children including faculty and graduate students, and a team of technology experts from TupleoLife, was convened in January 2017. A graduate student, who is a registered dietitian, designed the game concept and the TupleoLife team executed the game for use on an Android tablet. The game was password protected and accessible through any device with the ability to access a webpage. An Android tablet was selected as the education portal for this study due to its portability and large screen size. The nutrition education experts reviewed for content validity after the initial development of the game. Prior to use, the program was tested for acceptability and user experience in nine children aged 9 to 13 years at a Child Development Center in Davis, California. Most students were able to complete it within 15 to 25 minutes and found it interactive. After minor language adjustments to the game, to make it easier for students to read and understand, a final version was available in Fall 2017.

Participants and study design

Participants were recruited to this pre-post study through convenience sampling of children that were initially recruited for a larger dietary intervention trial (NCT# 03175003).[18] As part of this study, children were recruited to consume legume-based snack foods providing

6g or 12g of protein with or without micronutrient fortification for four weeks. Children aged 9 to 13 years from the Greater Sacramento area were enrolled in the study after providing their assent and consent from their parents. Participating children visited the UC Davis Department of Nutrition's Ragle Center Human Health and Nutrition for test days, with their parents, at week 0 (baseline) and after 4 weeks of their assigned snack consumption. On both test days, the children engaged in the S.N.A.C.K educational game, which consisted of educational content and assessments, on a provided tablet during a 30-minute resting period before clinical testing measurements. Children completed the education session twice (week 0 and 4), taking 15 to 25 minutes each. Duration was dependent upon how quickly each participant interacted with the information and answered questions. There were three sets of assessments, referred to as assessments A, B, and C. These assessments each underwent review by the previously mentioned expert panel for content validity. The entire study is categorized into four time points (TP); TP 1 – pre-education at week 0, TP 2 – post-education at week 0, TP 3 – pre-education at week 4, and TP 4 – post-education at week 4 (Figure 1). Anthropometric measurements and 24-hour recalls were collected on test days. All procedures were approved by the UC Davis Institutional Review Board.

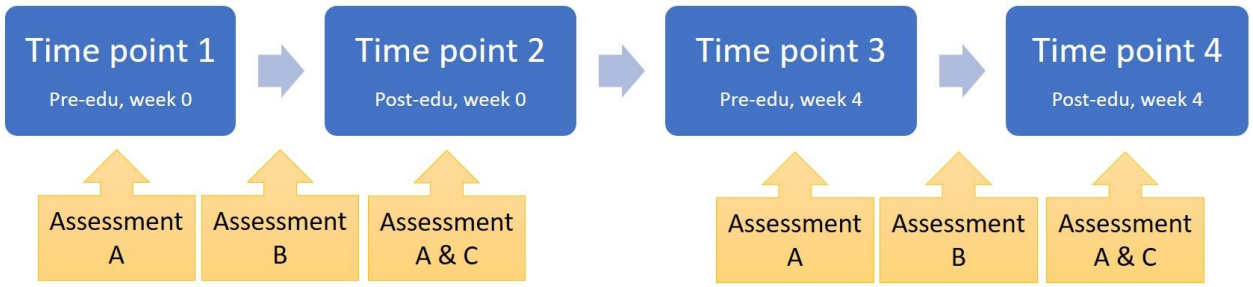

Figure 1. Flowchart for assessment A, B and C occurrence at different time points (TP).

Assessment A was performed twice at weeks 0 (TP 1 and 2) and 4 (TP 3 and 4).

Assessments B and C were performed at week 0 and 4.

Utilization of S.N.A.C.K

Throughout the educational game, a female avatar of ambiguous ethnicity/race was the "teacher" who guided the participants. The S.N.A.C.K game began with assessment A, a 10-question quiz. Items such as, "*What is the serving size of Snack A? Jade ate 1 cup of Snack A, how much saturated fat did she have? Lee needs 9g of protein, how much of Snack A should he eat?" assessed* knowledge and utilization of the Nutrition Facts Label (see Supplemental Material, Table S1). One point was awarded for each correct answer, for a maximum of ten points. Assessment A was administered at four time points: pre- and post-education at week 0 and 4, to assess whether the education effectively improved nutrition knowledge in the short-term and long-term.

Upon completion of assessment A, education began. The participants were shown a series of foods that were arranged on a continuous spectrum ranging from most processed to least processed (fresh) (see Supplemental Material, Figure S1-A). This part of the S.N.A.C.K. game encouraged the children to choose foods on the least processed side of the spectrum while acknowledging that it can be challenging to identify which foods are less processed. One such example is banana chips, which are made from fruit but can contain ~20 grams of saturated fat per cup. This activity provided an interactive way to educate children on the importance of using the Nutrition Facts Label to help make healthy snack choices.

The game continued with assessment B, which consisted of four questions. These questions allowed the children to apply the recently acquired knowledge about selecting snacks that are generally less processed. In each question, participating children were asked to compare pictures of two snacks e.g. “Apple Chips or Potato Chips” and were then asked to choose the “healthier” option (see Supplemental Material, Figure S1-B). All snacks in the assessment were shown previously in the spectrum of processed to fresh foods. Each question was worth one point, with a maximum of four. When a question was answered incorrectly, the avatar appeared to provide the correct answer and encouragement to the participant. When a question was answered correctly, the avatar would praise the participant. Following this, participants were taught how to read and interpret the Nutrition Facts Label in detail (see Supplemental Material, Figure S1-C). Through this, children were educated on the importance of each macronutrient interactively, and examples of foods rich in each macronutrient were also provided (see Supplemental Material, Figure S1-D). Following the section on macronutrients was a section focusing on nutrients of concern and nutrients to limit, such as saturated fat and added sugar.

Assessment C consisted of three questions, with two parts each, totaling six points. Firstly, the children were tasked to select the healthier option from two foods that each had a few pieces of nutrition information presented in Assessment A and B. The second part required them to indicate the specific nutrient that led to this decision. This assessment required them to apply comprehensive knowledge about the Nutrition Facts Label that they had just acquired. Once again, the avatar reinforced the correct answer if a question was answered incorrectly and praised the participant if a question was answered correctly. Immediately

following this, the children were asked to complete assessment A again to conclude the session. Each participant returned after four weeks and was presented with the same S.N.A.C.K game. In total, each participant completed assessment A four times, assessment B twice, and assessment C twice.

Dietary recalls

During each study visit, the participants reported all foods and beverages consumed by the participant in the previous 24 hours through ASA24 (https://asa24.nci.nih.gov/). The ASA24 has been shown to be comparatively accurate to a standard interviewer-administered recall, with 87% of the compared nutrients/food groups showing a difference of no more than 20%.[19] To minimize the effect of user difficulty, the participants completed the recalls in the presence of their parents under the guidance of the study researchers. Healthy Eating Index (HEI 2015) scores were calculated from these recalls with the National Cancer Institute's SAS code.[20] The HEI scoring system measures diet quality through thirteen components: total fruit, whole fruits, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids, refined grains, sodium, added sugars, and saturated fats.[21] The highest HEI-2015 score that can be achieved is 100, which would indicate a diet following the 2015-2020 Dietary Guidelines for Americans as directed.[21] The HEI has been validated and demonstrated reliability in sensitivity to differences in diet quality among individuals and is able to measure diet quality independently of energy intake.[22] Furthermore, high HEI scores have been associated with a reduced risk of mortality from all-cause, cardiovascular disease, and cancer.[23]

Statistical Analysis

Assessment A was analyzed with One Way Analysis of Covariance (ANCOVA), with covariate of age. Healthy Eating Index scores for all children, and BMI subgroups (normal and overweight/obese) were assessed with ANCOVA, and covariate of age. Both used Tukey's as a *post hoc* test. Assessment B and C were analyzed with paired t-test and Wilcoxon signed ranked test, respectively. Assessment A scores were presented in a boxplot with median (Interquartile Range; IQR) with standard deviations (SD) and are reported as Least Squares means ± standard error (SE). Assessment B and C were reported as mean ± SD. The HEI scores were reported with LS means ± SE. Cronbach's alpha was performed on assessment A baseline scores (week 0) to test for internal consistency reliability. Analyses were performed with JMP, Version 15 (SAS Institute Inc., Cary, NC). *P* values of < 0.05 were considered statistically significant.

Results

A total of 55 participants were enrolled in this sub-study, and 41 children (21 female and 20 male), with a mean age of 11 ± 1.3 years, completed all assessments and were included in this analysis (Table 1). No significant differences were observed between treatments or between protein or micronutrient levels (data not shown), therefore, the analyses were pooled. Of the 41 children, 24 were considered normal weight and 17 were overweight/obese.

Table 1. Characteristics of children who participated in S.N.A.C.K. (n = 41)

Sex *n* (%)	
Male	20 (48.8)
Female	21 (51.2)
Weight *n* (%)	
Normal Weight	24 (58.5)
Overweight/Obese	17 (41.5)
Age (years)	
Range	9 – 13
Mean ± SD	11 ± 1.3
Race and Ethnicity *n* (%)	
Multiple Ethnicity	18 (43.9)
White	14 (34.2)
Hispanic	5 (12.2)
Asian	3 (7.3)
American Indian	1 (2.4)

n denotes the total number of participants.

Assessment A demonstrated adequate internal consistency reliability with an overall Cronbach $\alpha = 0.72$ and individual question Cronbach α in a range of 0.65 to 0.75[24]. At both study visits, significant improvements in Nutrition Facts Label knowledge and utilization (Assessment A) were observed immediately after the children received education (week 0: TP 1: 6.08 ± 0.43 versus TP 2: 7.20 ± 0.43, $p = <0.001$; week 4: TP 3: 7.15 ± 0.43 versus TP 4: 7.89 ± 0.43, $p = 0.002$; Figure 2). This knowledge was sustained and improved 4 weeks later as demonstrated by increases in Assessment A scores between pre-education measurements (TP 1: 6.08 ± 0.43 versus TP 3: 7.15 ± 0.43, $p = <0.001$; Figure 2) and post-education measurements (TP 2: 7.20 ± 0.43 to TP 4: 7.89± 0.43, $p = 0.003$; Figure 2).

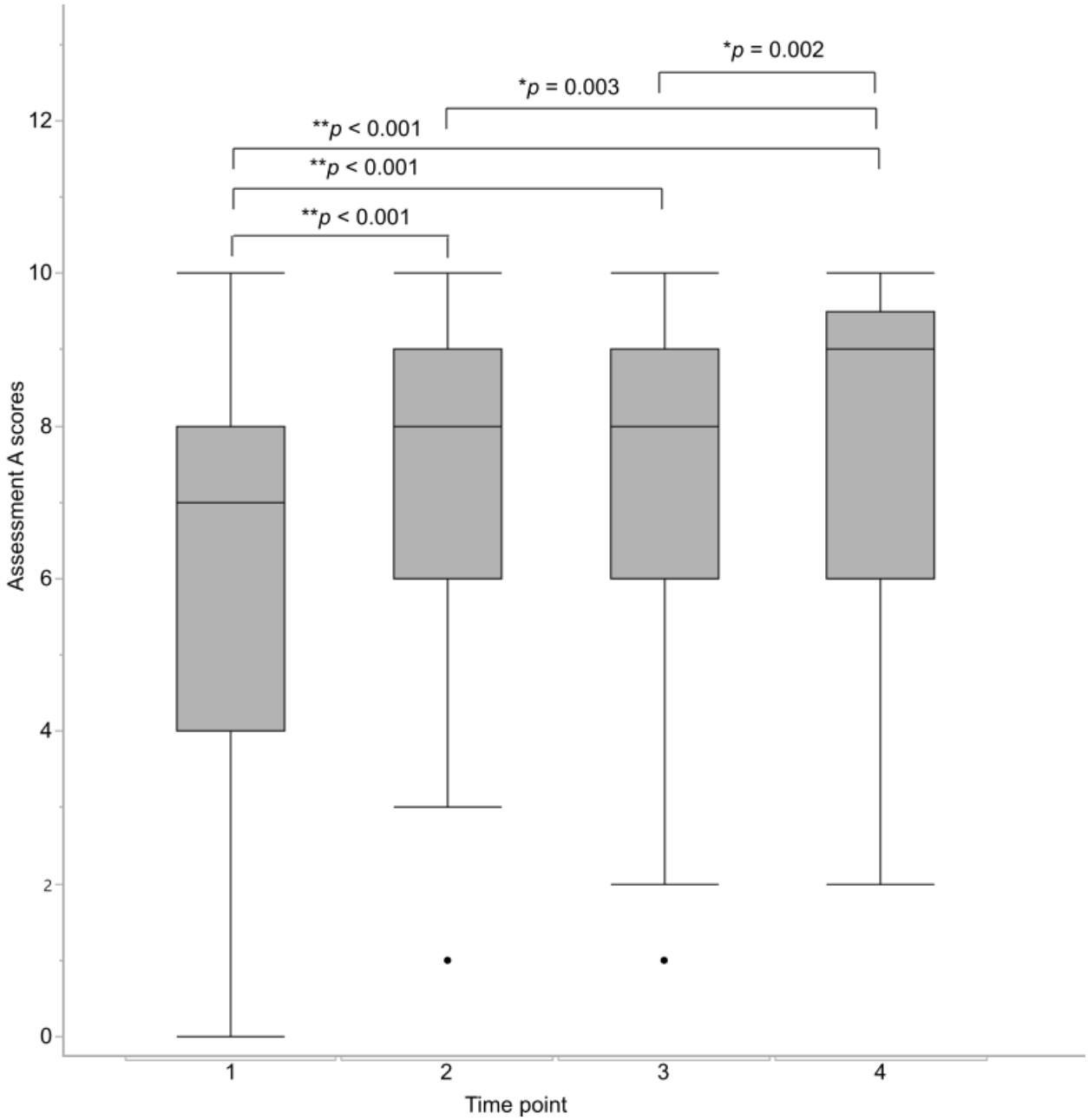

Figure 2. Comparison of mean assessment A scores at each time points. Time point 1 – pre-education at week 0, Time point 2 – post-education at week 0, Time point 3 – pre-education at week 4, and Time point 4 – post-education at week 4. Boxplots of the median and interquartile range of assessment A scores for each time point. Statistical analysis performed using One Way Analysis of Covariance (ANCOVA), with covariate of age and Tukey's *post hoc* test. $^{**}p < 0.001$ and $^{*}p < 0.05$ are statistically significant.

There were no significant differences between the mean assessment scores for test B (p = 0.32), and the children scored well for this section at both weeks, which implies that the spectrum of foods shown prior to this assessment was effective in providing a visual idea of what a “healthier” snack option looks like. The children improved their selection of the healthiest food choice, with mean assessment scores for assessment C scores significantly increasing from week 0: 4.5 ± 1.45 to week 4: 5.0 ± 1.31; p = 0.04.

Potential differences in HEI scoring components were examined between participating children with a normal BMI (< 85 percentile for age and sex) or an overweight/obese BMI (≥ 85 percentile for age and sex) After the 4 week intervention period, children in the normal weight group significantly increased their HEI component scores for “Added Sugars” (p=0.049; Figure 3), indicating decreased sugar intake A trend towards a significant increase in HEI score for dairy in all children (Week 0: 5.7 ± 0.7 versus Week 4: 6.9 ± 0.7, p = 0.06, Table 2) and was observed indicating an increase in dairy. Interestingly, total protein (p = 0.07) and total seafood and plant proteins (p = 0.07) both trended to a decrease after four weeks (Table 2), indicating a decrease in both groups of protein. The total HEI score and all other components were not significantly different (Table 2).

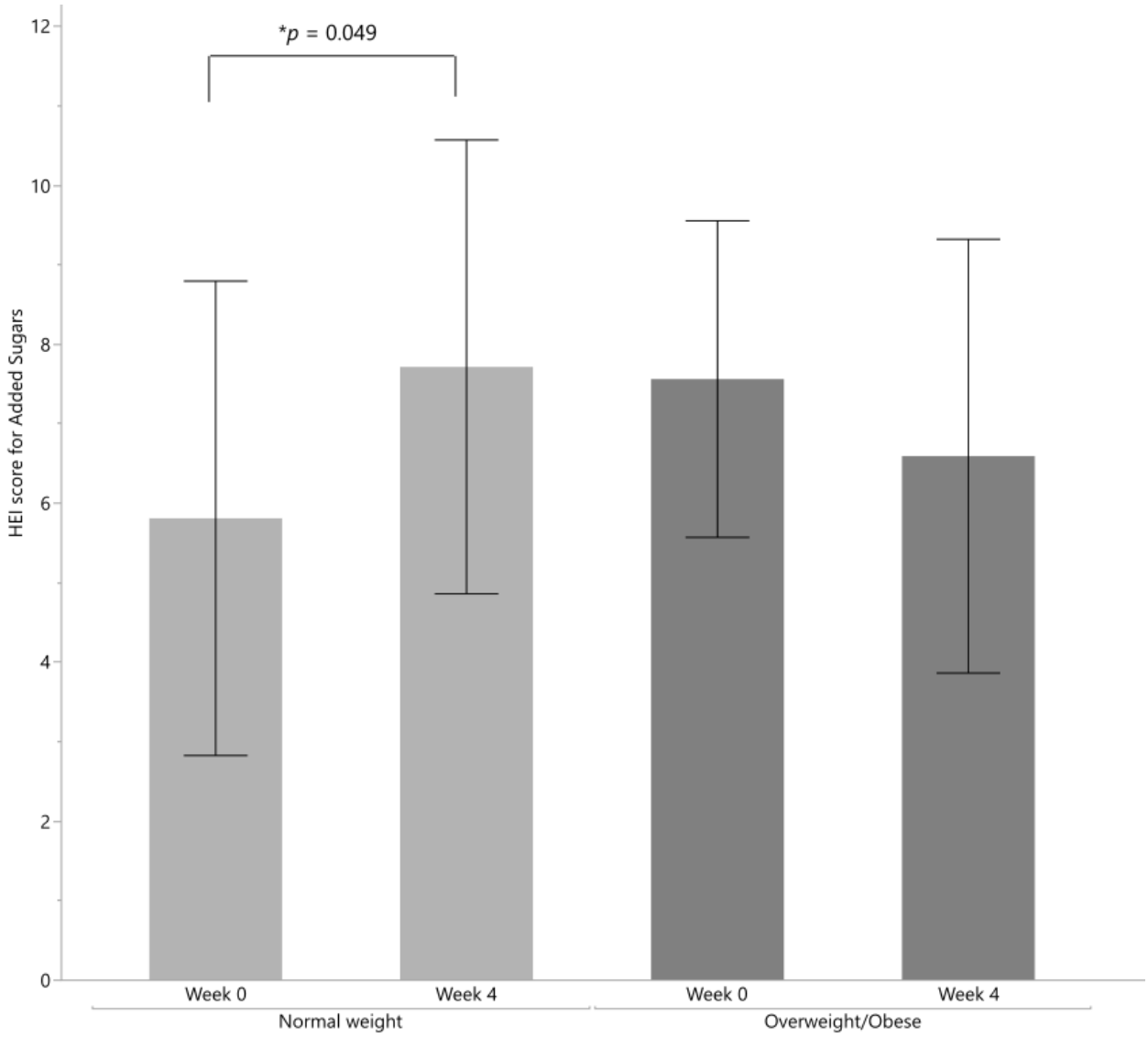

Figure 3. Change in HEI scoring component "Added Sugars" after nutrition education, between youth in the normal weight and overweight/obese category. Statistical analysis performed using One Way Analysis of Covariance (ANCOVA) for BMI subgroups (normal and overweight/obese), with covariate of age and Tukey's *post hoc* test. *$p < 0.05$ is considered statistically significant.

Table 2. Changes in HEI scores at week 0 and 4 in normal and overweight/obese youths

HEI Scoring	Normal weight			Overweight/Obese			All children		
	Week 0	Week 4		Week 0	Week 4		Week 0	Week 4	
	n = 24			n = 17			n = 41		
	LS Mean ± SE		*P*	LS Mean ± SE		*P*	LS Mean ± SE		*P*
Total Fruits	2.6 ± 0.5	2.3 ± 0.5	0.53	2.4 ± 0.4	1.8 ± 0.4	0.36	2.6 ± 0.3	2.1 ± 0.3	0.28
Whole Fruits	2.6 ± 1.3	2.4 ± 1.3	0.70	2.5 ± 0.5	2.3 ± 0.5	0.79	2.8 ± 0.3	2.5 ± 0.3	0.63
Total Vegetables	2.3 ± 0.4	2.3 ± 0.4	0.93	3.3 ± 0.4	2.8 ± 0.4	0.23	2.7 ± 0.3	2.5 ± 0.3	0.48
Greens and Beans	1.8 ± 0.6	1.5 ± 0.6	0.53	2.0 ± 0.3	1.9 ± 0.3	0.84	1.9 ± 0.4	1.7 ± 0.4	0.54
Whole Grains	3.1 ± 0.8	3.4 ± 0.8	0.74	3.7 ± 0.7	2.7 ± 0.7	0.46	3.3 ± 0.5	3.0 ± 0.5	0.72
Dairy	6.1 ± 0.7	6.8 ± 0.7	0.29	5.4 ± 1.1	7.1 ± 1.1	0.13	5.7 ± 0.7	6.9 ± 0.7	0.06
Total Protein Foods	4.3 ± 0.3	3.8 ± 0.3	0.19	3.8 ± 0.5	3.0 ± 0.5	0.22	4.1 ± 0.2	3.4 ± 0.2	0.07
Seafood and Plant Proteins	2.9 ± 0.4	1.7 ± 0.4	0.06	2.2 ± 0.5	1.8 ± 0.5	0.54	2.6 ± 0.4	1.7 ± 0.4	0.07
Fatty acids	3.9 ± 0.7	3.8 ± 0.7	0.95	4.1 ± 1.1	3.9 ± 1.1	0.87	4.1 ± 0.5	4.0 ± 0.5	0.86
Refined Grains	5.4 ± 0.6	4.6 ± 0.6	0.44	5.4 ± 0.9	5.7 ± 0.9	0.86	5.3 ± 054	5.0 ± 0.5	0.67
Sodium	4.2 ± 0.5	4.1 ± 0.5	0.95	3.5 ± 0.7	4.2 ± 0.7	0.48	3.9 ± 0.4	4.2 ± 0.4	0.67
Added Sugar	5.8 ± 0.6	7.7 ± 0.6	0.049*	7.5 ± 0.7	6.5 ± 0.7	0.24	6.5 ± 0.5	7.2 ± 0.5	0.29
Saturated Fats	4.7 ± 0.6	4.3 ± 0.6	0.56	4.4 ± 1.2	4.7 ± 1.2	0.86	4.6 ± 0.5	4.5 ± 0.5	0.87
Total HEI	49.4 ± 2.4	48.2± 2.4	0.72	49.4 ± 3.5	47.2 ± 3.5	0.63	50.3 ± 2.2	48.7 ± 2.2	0.54

*Significantly different ($p < 0.05$). Statistical analysis by Analysis of Covariance (ANCOVA), using age as covariate, for all children, and BMI subgroups (normal and overweight/obese). With Tukey's *post hoc* test.

Discussion

Childhood obesity is a critical issue and nutrition education has been one proposed action that can be employed to help alleviate this epidemic.[25] Nutrition knowledge has been correlated with food choices in seventh and eighth grade children[26]. The current study demonstrated that a single self-paced 15 to 25 minute educational session was sufficient to elicit sustained improvements in nutrition knowledge over a period of one month. Improved Nutrition Facts Label (NFL) knowledge (assessment A) post-education at week 0 (TP1 to 2) and week 4 (TP3 to 4) supports the Mastery Learning Theory, as the children were allowed to learn at their own pace. That assessment A scores remained improved at week 4 prior to the second education (TP1 to 3) also supports the Spaced Repetition theory that repeated exposure can enhance the learning of basic nutrition concepts.[6]

Assessment B and C assessed the children's ability to select the healthier snack option and required children to provide appropriate reasoning, with most participants achieving maximum points for assessment B at both visits. A significant increase in mean assessment C score ($p = 0.043$) demonstrated that more children could accurately select the healthier snack option and provide appropriate reasoning for it. Taking both results into consideration, it suggests that the children were able to accurately select or guess which snack was a healthier snack option based on its appearance and description, but prior to education, had a lack of knowledge regarding the specific nutrients that made the snack a healthier option.

Children in the normal weight category exhibited a significant decrease in reported added sugar consumption after receiving nutrition education. This is also consistent with the nutrition education provided, emphasizing the importance of reducing added sugar intake. Interestingly, this only occurred in the normal weight group; further consideration for impacting HEI scores for overweight/obese children in future implementation will be necessary. Furthermore, there was a near significant increase in dairy intake between study visits, which was a component that was encouraged as part of the nutrition education. Another intriguing finding was the trend to a decrease in protein. This could be due some replacement of participants' typical protein sources with the legume-based snacks provided as an intervention for the main study. Furthermore, the legume-based snacks were not reported in the ASA24.

Incorporating gaming into nutrition education for children has been demonstrated to be effective in a few studies. One study investigated the effectiveness of a 15 to 30 minute board-game, once a week for 20 weeks, to educate children (aged 8 to 14 years) about nutrition.[27] At 6-months post-intervention, children had a significant improvement in nutrition knowledge, dietary behavior, and long-term weight loss, compared to those who did not partake in the nutrition board-game.[27] In another study that also utilized a board-game to provide nutrition education in children 10 to 14 years a significant increase in knowledge, attitudes, and practices of healthy eating were observed, compared to the control group.[28] Although the form of the game is different, similar to the previously mentioned studies S.N.A.C.K. also utilized the concept of spaced repetition and children were allowed to learn at their own pace. An additional study investigated a nutrition program presented at

four 20-minute sessions to children aged 7 to 9 years and found a significant increase in nutrition label literacy in both students and parents.[29] These studies all demonstrated the effectiveness of short, spaced-out sessions of education in improving nutrition knowledge. The current study is different in the form of education (through a tablet) and focused specifically on promotion of healthier snack intake.

Nutrition knowledge has been associated with Nutrition Facts Label use and comprehension[30]. It is clear that engaging early nutrition education can have a positive impact in the children. When fresh snacks are unavailable, and processed snacks are chosen, it would benefit children to be equipped with the skills to utilize nutrition labels in guiding their choice of snacks. Regrettably, at this time, it should be noted that there is not a specific nutrition education mandate in schools in the US. The current study focused on skill-building with respect to utilizing the Nutrition Facts Label to make healthier snack options among children ages 9 to 13 years.

Limitations

As this study was a convenience sample within a larger dietary intervention trial, a control education group was not possible. A small sample size along with unknown participants' socioeconomic information and parents' nutrition literacy, could all affect the effectiveness of the education and should be considered in future studies. However, it should also be noted that most education studies recruited students from schools and classes, randomizing

them by clusters, while this study recruited unique individuals for this study. In addition, the duration of the study was relatively short (4 weeks), which did not allow for assessing the effects of S.N.A.C.K on retention of long-term knowledge and behavior changes. According to the Transtheoretical Model, a behavior change is considered sustained if it has been maintained for more than six months[31]. Future improvements to S.N.A.C.K. may include education towards a more refined selection of beneficial micro- and macronutrients. Ultimately, future assessments of efficacy for S.N.A.C.K. as a useful nutrition education resource for children ages 9 to 13 will need to be conducted in a controlled trial with a larger group of children, with a longer duration.

Conclusion

This study demonstrated improvements in nutrition learning that could be translated to improved dietary intakes. Signifying the efficiency of S.N.A.C.K, a short (15 to 25 minutes) gamified education that assisted children 9 to 13 years to increase their nutrition knowledge towards healthier snack selection.

Translation to Health Education Practice:

The S.N.A.C.K game was designed with the aim to equip children with the ability to choose healthier packaged snacks. To achieve this aim, the game focused on imparting knowledge about the nutrients of concern, Nutrition Facts Label and its utilization. The theories of

mastery learning and spaced repetition were incorporated in the development of S.N.A.C.K. and contributed to observed improvements in nutrition knowledge that maintained over four weeks. Further enhancements in the S.N.A.C.K. game could make it feasible to be incorporated as a nutrition education resource for children aged 9 to 13 years to improve their ability to utilize the Nutrition Facts Label to make healthier snacks and food choices.

This study meets several competencies in Area IV: Evaluation and Research, defined by the 2020 Health Education Specialist Practice Analysis II (HESPA II 2020), credentialed by the National Commission for Health Education Credentialing (NCHEC). The following sub-competencies apply: 4.1.1 align the evaluation plan with the intervention goals and objectives, 4.1.5 select an evaluation design model and the types of data to be collected, 4.1.7 select quantitative and qualitative tools consistent with assumptions and data requirements, 4.1.10 implement a pilot test to refine data collection instruments and procedures, 4.2.1 determine purpose, hypotheses, and questions, 4.2.2 comply with institutional and/or IRB requirements for research, 4.3.2 implement data collection procedures, 4.3.4 monitor data collection procedures, 4.3.5 prepare data for analysis, 4.3.6 analyze data, 4.4.1 explain how finding address the questions and/or hypotheses, 4.4.2 compare findings to other evaluations or studies, 4.4.3 identify limitations and delimitations of findings, 4.4.4 draw conclusions based on findings, 4.4.5 identify implications for practice.

Acknowledgments: We would like to acknowledge TupeloLife for their contribution to the technology of S.N.A.C.K.

Declaration of Interest Statement: The authors have no conflict of interest to declare.

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Supplemental File

Table S1. Assessment A, 10 questions quiz

No.	Questions
1.	What is the serving size of Snack A?
2.	How much saturated fat is in ½ cup of Snack A?
3.	Jade ate 1 cup of Snack A, how much saturated fat did she have?
4.	How much protein is in ½ cup of Snack A?
5.	Lee needs 9g of protein, how much of Snack A should he eat?
6.	Saturated fats are considered healthy fats.
7.	Which foods below has the most protein?
8.	Which foods below has the most sodium?
9.	Is it important to eat dietary fiber?
10.	Do apples contain added sugar?

Figure S1-A. S.N.A.C.K. game showing spectrum of foods from processed to fresh.

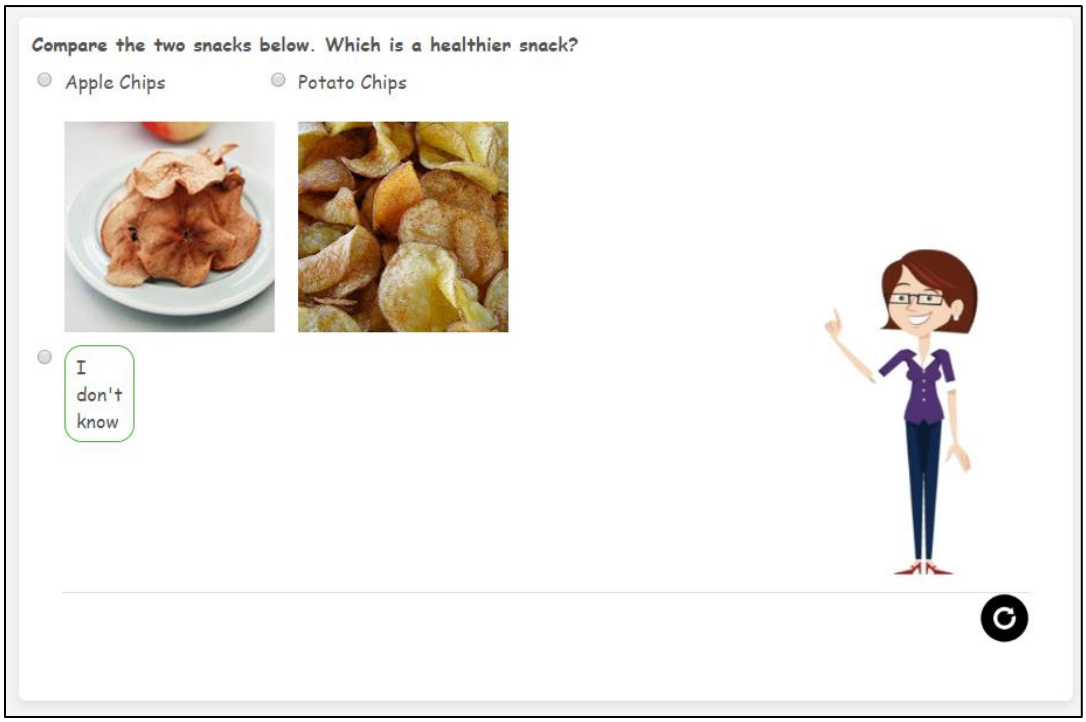

Figure S1-B. S.N.A.C.K. game to choose the healthier food option between two options.

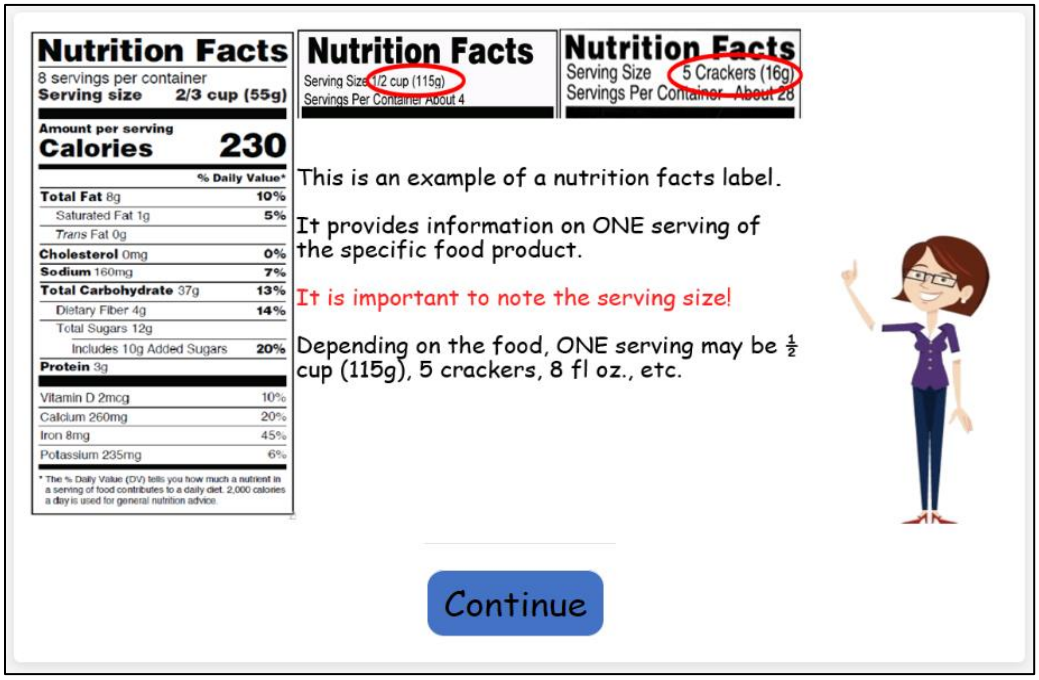

Figure S1-C. S.N.A.C.K. education about the Nutrition Facts Label.

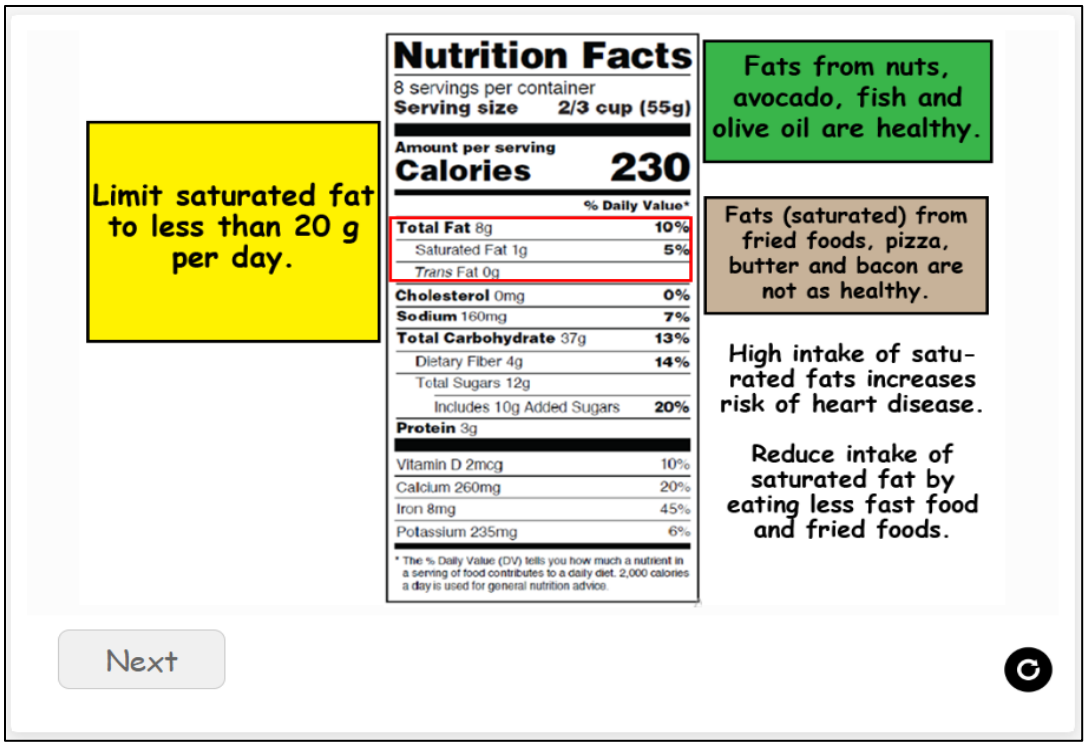

Figure S1-D. S.N.A.C.K. education about macronutrients and provided examples of food sources containing the macronutrient.

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APPENDIX B

Effects of Mango Intake on Skin Health and

Gut Microbiome Changes in Postmenopausal Women

New proposal funded by the National Mango Board, 2021 – 2023

Abstract

The role of mangos in skin health has long been advocated in traditional medicine. We have recently completed a pilot study showing a clear trend in skin wrinkle reduction when postmenopausal women consumed 85g of Ataulfo mangos, four times per week for 16 weeks. To confirm and extend these results, a larger study is proposed. Postmenopausal women with fair skin will consume either 85g (0.5 cup) or no mangos for 20 weeks. Those in the mango group will be evaluated again four weeks after the intervention is finished. The primary outcomes will be skin wrinkles at the lateral canthi (crow's feet) as assessed by a high-resolution facial modeling biophotonic system. Secondary outcomes will be skin measures of redness, hydration and carotenoid content, along with gut microbiome profiles. The information gained from this study will provide insights for consumers and health professionals, along with basic information about gut microbiome profiles after mango intake.

Objectives

Three objectives of this study exist. The first is to determine if regular mango intake (85g, four days per week for 20 continuous weeks) will result in a reduction in skin wrinkles, an increase in skin hydration and skin carotenoids, and a decrease in skin redness in postmenopausal women. The second is to characterize gut microbiome profiles during the 20-week mango intake period. The third is to determine what, if any, changes in the outcomes persist four weeks after discontinuation of mango consumption.

We hypothesize that mango intake will:

1) significantly improve skin parameters by decreasing skin wrinkles and redness, and increasing skin hydration and skin carotenoid levels.
2) increase diversity of the gut microbiome, increase the fecal levels of short chain fatty acids, and increase the presence of short chain fatty acid producing microbiota.
3) continue to show significant improvement in skin parameters and carotenoid levels four weeks after discontinuation of mango intake, while gut microbiome profiles will revert back to baseline levels.

Justification

Nutraceuticals and food-based cosmetics are a growing trend among consumers seeking to look and feel better. This trend is increasing in popularity within many fields of medicine. In a landscape of cosmetics that has many expensive options, greater understanding of the role

of dietary modulation for cosmetic indications is of high interest to the general public, to industry (1, 2) and to health professionals.

Nutritionists and health officials advocate for the consumption of diets rich in plant foods, which can reduce the risk for many chronic diseases, including select skin disorders. However, the mechanisms underlying the positive effects of high fruit and vegetable (F&V) intake are still poorly understood (3). Rather than making global recommendations (eat five to nine servings of F&V each day), more focused health messages need to be developed since the content of components such as carotenoids varies, and "all fruits and vegetables are not created equally".

Mangos are a rich source of nutrients such as carotenoids, vitamin C and fiber, as well as polyphenols and phenolic acids such as mangiferin, ellagic acid, and gallotannins (4). Mango extract has been reported to protect against photo-aging of the skin in an animal model exposed to UVB radiation, reducing the length of wrinkles and increasing collagen bundles (5). Beta-carotene and other carotenoids are known to provide skin protection from sunlight (6), but other compounds in mangos, may also be important in reducing oxidative damage in aging skin (7).

We have recently completed a pilot study assessing the effect of two amounts of mango intake (85g and 250g; each consumed four times per week for 16 continuous weeks) on skin wrinkles and carotenoid levels in postmenopausal women. Wrinkles were measured at the lateral canthi (crow's feet) on both the left and right sides. Our results show a clear trend in

wrinkle reduction among those consuming Atalufo mangos at the 85g (0.5 cup) amount. Surprisingly, those in the 250g groups showed a significant increase in wrinkles. Figure 1 shows the changes in *total* average wrinkle length (left and right sides combined). Figure 2 shows the changes in *left side* average wrinkle length.

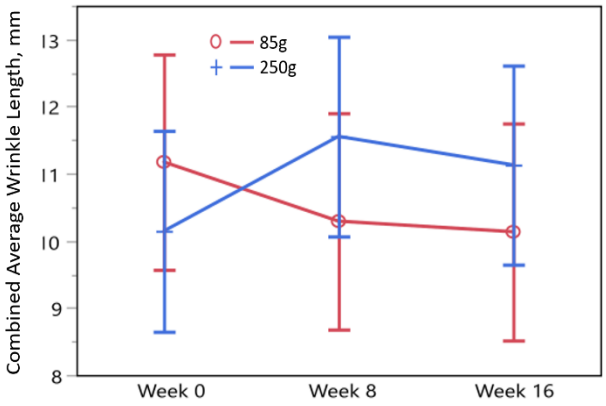


Figure 1. Changes in *total* wrinkle length

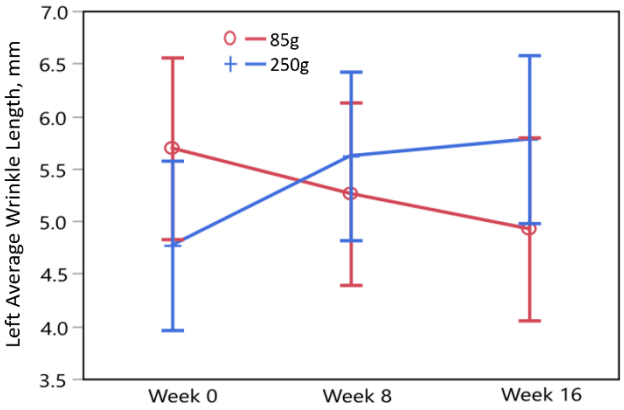


Figure 2. Changes in *left side* wrinkle length

The current proposal builds upon the results at the 85g level with a unique study design and added outcome measures of gut microbiome profiling, a topic of high interest in the scientific community (e.g., recent USDA Agriculture and Food Research Initiative grant program*).

Drs. Hackman and Sivamani have extensive experience in clinical studies involving fruits, nuts and human health. Dr. Hackman has been conducting clinical nutrition studies for 35 years, with his current projects focused on mangos, walnuts, strawberries and goji berries. Dr. Sivamani has written extensively about natural therapies for skin health, and is currently

conducting clinical studies on almonds and probiotics. Vivien Fam, RD and PhD graduate in March 2021, will serve as the study coordinator, having just completed that role for our current mango and skin study.

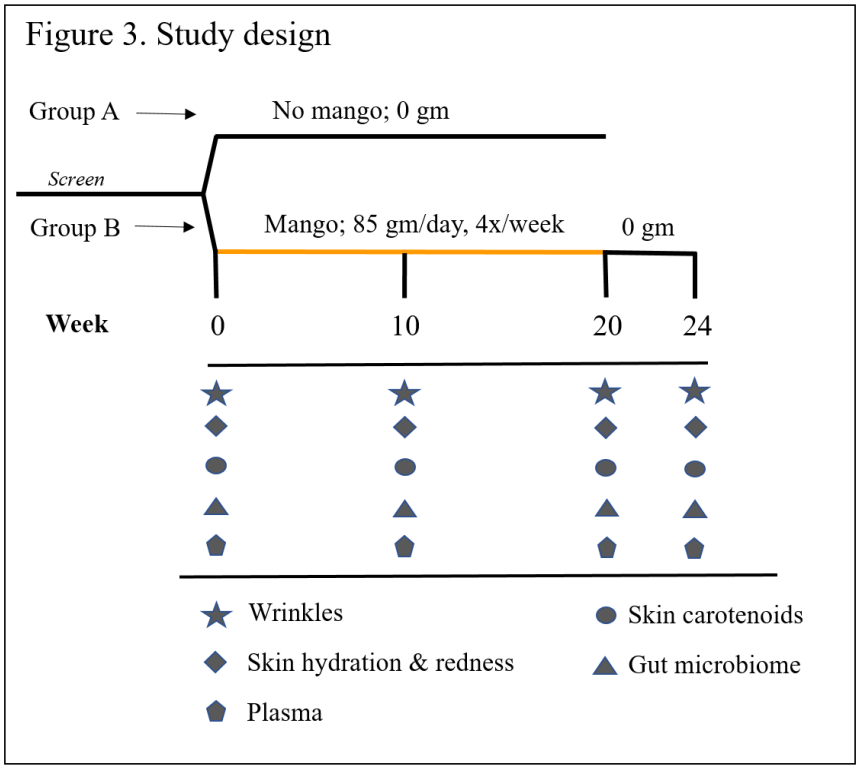
Approach

A 20-week human study is proposed to assess the effects of mango intake on skin health. Seventy postmenopausal females, 50 to 70 years of age, will be enrolled in a parallel-arm study design. Postmenopausal women are selected because they are more susceptible to facial wrinkles than younger women or men (8). Participants will have sun-sensitive skin types that are more susceptible to developing wrinkles based on previous studies with both almonds and mangos (Fitzpatrick skin type I, II or III, which is based on skin melanin content). The women will refrain from using skincare products except moisturizers and sunscreens during the intervention. They can consume dietary supplements containing no more than 100% of the Daily Value of vitamins and minerals, plus additional calcium, but no botanical extracts or probiotics. The intervention will occur at Integrative Skin Science and Research (ISSR; dba Zen Dermatology; Dr. Sivamani's clinical trials unit with a focus on skin-related nutritional studies), located in Sacramento, a large metropolitan area that will enable easy recruitment and conduct of the study. The Institutional Review Board at UC Davis will approve the research protocol. Informed consent will be obtained according to established practices.

An initial screening of volunteers will occur to assess their health condition, ensure normal clinical chemistry values, verify their skin type and skin carotenoid value, and familiarize them with the testing protocol. Once consented, baseline measures will be collected, and the women will be randomly assigned to consume either zero (Group A) or 85g (Group B) of Ataulfo mangos, four times per week for 20 weeks. The amount of mangos is based on our pilot data noted above, and we have extended the duration of the intervention to 20 weeks in order to provide more time for the mango constituents to affect skin wrinkles. Our pilot study did not include a no-mango control, which was beyond the scope of that project. For the current proposal, a no-mango control group is included, which allows adjustment of responses to accommodate what, if any, effects occur simply from going to the clinic and having measurements taken.

After 20 weeks, participants in the mango group will stop eating mangos, and will be followed for an additional four weeks to determine what, if any, effects persist. Those in the control group will be paid and given a substantial portion of frozen mangos for their participation.

Study day visits will occur as outlined in Figure 3. The primary outcomes are skin wrinkles, which will be categorized as fine or emerging wrinkles and measured for length and width. Further calculations of wrinkle severity and average scores will be calculated based on validated algorithms. Secondary outcomes are skin hydration, redness (erythema), skin carotenoid content and gut microbiome profiles.



Assessments

The primary outcome of this study is facial wrinkles at the lateral canthi ("crow's feet"; outer edge of the eye) and will be assessed with a clinically validated, high-resolution facial modeling biophotonics system (Brigh-Tex BioPhotonics, San Jose, CA). Dr. Sivamani's group has extensive experience with this system, detailing its use for the grading of facial wrinkles, facial redness, and facial scarring (9-11). The system has also been used for Dr. Sivamani's almond study (*) and our recently completed mango and skin wrinkle study.

Secondary outcome measures include erythema, measured by the same biophotonics system as above. Skin hydration will be assessed with the MoistureMeterSC (Delfin Technologies, Miami, FL). Skin carotenoid content will be measured by reflective spectroscopy (Longevity Link Corporation, Salt Lake City, UT), a non-invasive test that has been validated with plasma carotenoid content in recent studies (12, 13, 14). Gut microbiome profiles will be characterized from stool samples based on whole genome shotgun sequencing in collaboration with CosmosID (Rockville, MD). Shotgun metagenomics is an approach beyond 16S methodology and enables both taxonomic profiling at the species level, and also enables strain-level detection of particular species (*). Plasma will be collected and stored for future analysis of carotenoids or total antioxidant capacity, if warranted (Note: no funds exist in the budget for plasma analysis).

Power calculations and amount of mangos needed

Based on our recently completed mango study that found a 13% reduction in wrinkles from baseline to the 16 week time point in the 85g group, in order to have at least 80% power in a parallel group study to discern a 15% difference in wrinkle severity of the lateral canthi over a 20 week period, using two-tailed t-tests for independent means, we need 32 participants in each group. When accounting for a possible 10% dropout rate, a total of 35 participants need to be initially enrolled in each group (70 participants overall).

Ataulfo mangos are selected for this study, as they were used in our pilot project, and are richer in beta-carotene than other varieties of mango commonly found in the United States

(15). In order to obtain 70 completed records, including fruit for people who start the study and then drop out before completion, and to provide a one-month supply of mangos to those in the no-mango control group upon completion of their involvement, a total of 710 pounds of mango pulp are needed. Assuming a yield of 40% pulp from a fresh Ataulfo mango, 1,775 pounds of fresh whole mangos are needed. Mangos will be supplied by the National Mango Board.

Anticipated shortfalls

Different Fitzpatrick skin types tend to wrinkle at different rates during the postmenopausal period (8). Participants are limited to Fitzpatrick skin types I, II and III, who have more wrinkles than those with higher skin melanin. Thus, the results may not be generalizable to all postmenopausal women, younger women, or men.

Diet and sunlight exposure can be confounding factors. Exclusion criteria will include those who consume more than five servings per day of fruits and vegetables, as well as those consuming an antioxidant supplement or regularly consume more than one serving per day of fruit juice. Participants will be asked to wear hats and/or use sunscreen when going outside. Compliance is based on self-reports, which are not always accurate.

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PERSPECTIVE AND CONCLUSION

The relationship between nutrition and skin health has come a long way since the 1700s. Initially, research was focused mainly on the effects of nutritional disorders on the skin with classic diseases such as pellagra(1), scurvy(2), and acrodermatitis enteropathica(3). As science advances and skin diseases caused by nutritional deficiencies and malabsorption become manageable, the focus has turned to identifying the role of the diet for skin health on new disorders and enhancing esthetics. As described in Chapter I, the largest organ in our body, the skin, is continuously exposed to intrinsic and extrinsic elements that can accelerate the progress of aging(4). Intrinsic factors are primarily a result of an individual's genetic constitution that could influence skin pigmentation and thickness, microvasculature structure, and sex hormones that, with aging, can impact skin dryness, thinning, and wrinkles(5). Extrinsic factors include exposure to ultraviolet radiation (UVR), air pollution, and smoking, which promote the generation of free radicals and reactive oxygen species (ROS)(6). Endogenous metabolism naturally creates ROS, and our body possesses the mechanisms to neutralize these molecules to maintain homeostasis(7). However, an increased level of ROS, beyond the skin’s ability to manage, would result in oxidative stress that could promote inflammation, damage DNA protein, and disrupt the structural integrity of the skin. This could be characterized by increased wrinkles and hyperpigmentation or decreased hydration, collagen, and elasticity of the skin. Chapter 1 also detailed the beneficial effects of mangos and their bioactive compounds, such as carotenoids, vitamins, polyphenols, on the skin and potential mechanisms. A clinical research, as described in Chapter II, was conducted to investigate the effects of mango consumption on the skin(8). The target population comprised of postmenopausal women aged 50 to 70 years old, with Fitzpatrick skin type I, II, or III, who had a higher chance of possessing

baseline wrinkles. The consumption of 85g (0.5 cup) but not 250g (1.5 cups) of mangos for 16 weeks was found to decrease wrinkles when compared to baseline measures. Furthermore, 250g of mango intake unexpectedly increased wrinkles. A similar case was also observed with another study detailed in Chapter III, that investigated unripened Jeju mandarin orange extract, which found that a lower amount was more effective in reducing wrinkles compared to a higher concentration(9). To extend our findings from Chapter II, a new study to compare the effects of 85g (0.5 cup) to a no mango intake control group on wrinkles as well as additional skin measurements and the gut microbiome, has been proposed and detailed in Appendix B.

Chapter III is essentially a list of plant-based foods and extracts that have been demonstrated to provide skin benefits, such as a reduction in wrinkles, hyperpigmentation, erythema, and roughness, and an increase in hydration, brightness, collagen, elastin, and density in humans. The goal of this chapter was to provide dietitians with the ability to provide targeted plant-based recommendations for specific skin concerns. Out of the 21 clinical studies identified, merely two examined whole foods, some used minimally processed foods, while most utilized extracts. The investigation of extracts can certainly help with the creation of new functional foods that are targeted at people with specific skin or health disorders. However, extracts are not digested, metabolized, and absorbed the same way as whole or minimally processed foods. In order to tailor dietary recommendations to different skin disorders or esthetics concerns, it is necessary to have more investigations on the effects of foods on the skin. Unlike conditions such as cardiovascular disease and diabetes, there are no official dietary recommendations for skin disorders or esthetic concerns. The effects of mango intake on cardiovascular health were also assessed through endothelial function as well as pro-inflammatory markers and described in Chapter IV. Postmenopausal women aged 50 to 70 maintained their usual diet and did not consume mangos in

the first two weeks, followed by the intake of 330 g (2 cups) of mangos for another two weeks. The study found improvements in endothelial function, assessed by peripheral artery tonometry, two hours post-consumption, but no changes were seen after two weeks of intake. No significant changes were observed with inflammatory markers after mango consumption. It is evident that dietary intake can increase the risk of obesity and diverse nutrition-related health problems, and childhood dietary habits usually persist through adulthood(10). Therefore, it is imperative to provide nutrition education to enhance good dietary habits in childhood. Appendix A details a study that described the utilization of a virtual education game aimed to improve snack choices and habits in children 9 to 13 years old. The study demonstrated that a single self-paced 15 to 25 minutes educational session was sufficient to elicit sustained improvements in nutrition knowledge over a period of one month. Furthermore, children in the normal weight category exhibited a significant decrease in reported added sugar consumption after receiving nutrition education.

Information collected from mango feeding dietary trials demonstrated the potential benefits of this fruit for skin and endothelial health. The evidence for select foods, including fruits, vegetables, nuts, and legumes, generally aligns with epidemiological studies that observed better skin outcomes and a reduced risk of skin disorders with a higher intake of a plant-based diet that include fruits and vegetables(11–13). While findings generally fall within recommendations of the 2020-2025 Dietary Guidelines for Americans(14), each food has a unique nutrient profile, and intake requirements will differ. It is with hope that this dissertation demonstrated the potential for plant-based foods for skin health and highlighted the need for more investigations on whole foods and juices to enable dietitians to provide targeted recommendations in the future.

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