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Title

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Permalink

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Journal

Journal of Cosmetic Dermatology, 21(9)

ISSN

1473-2130

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Publication Date

2022-09-01

DOI

10.1111/jocd.14717

Peer reviewed

ORIGINAL ARTICLE

Avocado Consumption Increased Skin Elasticity and Firmness in Women - A Pilot Study

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Funding information
Hass Avocado Board

Abstract

Background: Avocados are a rich dietary source of monounsaturated fatty acids, carotenoids, and phenolic compounds. Clinical studies have demonstrated that oral consumption of carotenoids improved skin aging. However, no studies have investigated whether oral intake of avocado will reduce skin aging.

Objectives: We therefore performed this pilot study to assess whether oral consumption of one avocado daily for 8 weeks can reduce skin aging in healthy overweight women assessing skin physical characteristics and resistance to UVB radiation.

Methods: Thirty-nine female participants (age 27–73 years) with Fitzpatrick skin type II-IV were randomly assigned to consume either one avocado daily or continue habitual diet for 8 weeks. Facial skin elasticity, firmness, pigmentation, sebum, and hydration were determined using a cutometer on the forehead and under eye. Minimal erythema dose (MED) was determined by standardized protocol at inner arm.

Results: Elasticity and firmness were increased at forehead comparing 8 weeks to baseline in the avocado group. Comparing avocado to control, change in firmness marker from baseline to week 8 indicated a significant increase in forehead skin firmness in the avocado group. We did not observe any change in hydration, pigmentation, sebum, and UVB resistance between the avocado and control group, although changes in melanin and erythema were observed in both groups over time.

Conclusions: Our findings suggest that daily oral avocado consumption may lead to enhanced elasticity and firmness of the facial skin in healthy women. Further studies of other skin locations are required to establish the connection between avocado consumption and skin aging.

KEYWORDS
avocado, elasticity, nutrition, obesity, randomized controlled trial, UVB

HENNING ET AL.

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1 | INTRODUCTION

Skin aging is a complex process causing a progressive loss in elasticity, firmness, hydration, and skin tone resulting from many intrinsic and extrinsic factors.[1] The commonly noted characteristics of older skin are increased wrinkles, skin laxity, and pigmented lesions as well as decreases in skin thickness and the elastin and collagen components.[2] The study by Kim et al demonstrated a decrease in skin elasticity with age in women and reported that adult skin shows a higher degree of fatigue than young skin.[3,4] Along with neck and forearm, the face shows different aging-related changes compared with other sites of the body because it undergoes photoaging due to the fact that the face is exposed to sunlight much more than other sites. Photoaging is characterized by severe wrinkling and pigmentation[5] caused by unprotected sun exposure and thus excessive ultraviolet radiation (UV).[5] The UV damaged connective tissue and elevated reactive oxygen species (ROS) within the skin tissues.[5]

The elastin fiber network in the dermis contributes to the skin's ability to stretch and contract and elastic fiber deficiency and their exposure to UV causes a progressive decrease in skin elasticity/firmness and plays an important role in the development of skin wrinkling and sagging.[6]

Obesity affects many organs of the body and leads to excess morbidity and mortality.[7] Less attention has been paid to the effect of obesity on skin characteristics. Cell culture studies with fibroblasts and human skin demonstrated that the presence of adipocytes in subcutaneous fat was associated with a decrease in skin elasticity and the size of adipocytes correlated inversely with dermal elastic fiber abundance.[8] In addition, a study comparing the skin of diet-induced obese mice to lean mice showed that the abundance of elastic fibers beneath the epidermis was remarkably reduced and fragmented in obese mice compared with lean mice.[9] We therefore selected women with increased waist circumference to determine the effect of avocado consumption on skin health.

Avocados are botanically designated as fruit but nutritionally belong more to the vegetable group. Avocados are rich in monounsaturated fatty acids such as oleic acid.[10] and a variety of other important phytochemicals, such as lutein, zeaxanthin, phytosterols, vitamin E, A, niacin, and folate, to the diet.[11,12] The natural combination of carotenoids, lutein, and zeaxanthin, along with the unsaturated fatty acids, provides a superior combination that increases the carotenoid absorbability.[13] It has been demonstrated that these carotenoids have the ability to reach and concentrate at the skin.[13] Oral and topical administration of lutein and zeaxanthin resulted in increased skin elasticity and hydration.[13] Topical application of saffron extract and avocado oil for anti-wrinkle topical cream for 12 weeks demonstrated an increase in skin elasticity and the area and volume of nasolabial folds were significantly reduced.[14] However, no clinical trials testing the effect of oral consumption of avocado on skin aging have been performed.

In a human clinical trial assessing the effect of diet on skin characteristics, it was found that intake of saturated and monounsaturated fat increased elasticity and green and yellow vegetables decreased wrinkling.[15] In our recent clinical trial, we assessed the effect of ellagitannins from pomegranate on skin aging. Results demonstrated improved resistance to UVB exposure after 8 weeks of consumption of pomegranate extract and juice, but no effect on skin elasticity and firmness.[16] We have found similar results with daily consumption of 1.5 oz of almonds daily.[17] It was the objective of the present study to investigate the effects of consumption of one avocado daily compared with no avocado control on skin health including elasticity, firmness, hydration, pigmentation, and UVB resistance in women with increased abdominal circumference and elevated BMI.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was a randomized, parallel group comparison of daily avocado consumption vs. habitual diet (Figure 1). Forty-one women were recruited at the UCLA Center for Human Nutrition. The clinical protocol was approved by the Institutional Review Board. The study was registered at clinicaltrials.gov (NCT05102604). All subjects gave written informed consent prior to the start of the study. Inclusion criteria were the following: ≥25 years of age, female, increased waist circumference defined as ≥35 inches non-smokers, Fitzpatrick skin type II-IV, not currently eating more than 2 avocados per month (habitual intake in the United States), and willing to maintain their normal skin care pattern for the duration of the study (avoiding excessive sun, not beginning new skin treatments). Exclusion criteria were the following: pregnant, use of skin-related prescription medication or supplements, or use of cosmetic agents with skin lightening/whitening. Unstable medical condition such as on dialysis for renal disease, cardiac, gastrointestinal, or hepatic disease, cancer (non-melanoma skin cancer >5 years ago, any cancer site >10 years without recurrence are acceptable), subjects who cannot avoid excessive exposure to either natural or artificial sunlight, used topical antibiotic or topical steroid on the face, currently taking any prescription medications that increase the risk of photosensitivity or history of taking such medications in the less than 3 months (eg, alpha-hydroxy acids in cosmetics, antibiotics ciprofloxacin, doxycycline, levofloxacin, ofloxacin, tetracycline, and trimethoprim), antifungals (flucytosine, griseofulvin, and voricanozole), antihistamines (cetirizine, diphenhydramine, loratadine, promethazine, and cyproheptadine), accutane, oral steroid use within the last 6 months longer than 7 days, elevated alcohol intake (7 + drinks/week females; 14 + drinks/week males), smoking, or participation in another clinical intervention trial within 30 days of baseline.

The study coordinator assigned participants to either consuming one avocado daily or no avocado based on randomization performed by the statistics core. The randomization algorithm involved sampling probability values from the uniform distribution without replacement, and allocating drawn probabilities to the group assignment. Permuted block design was used with sampling of 12 per
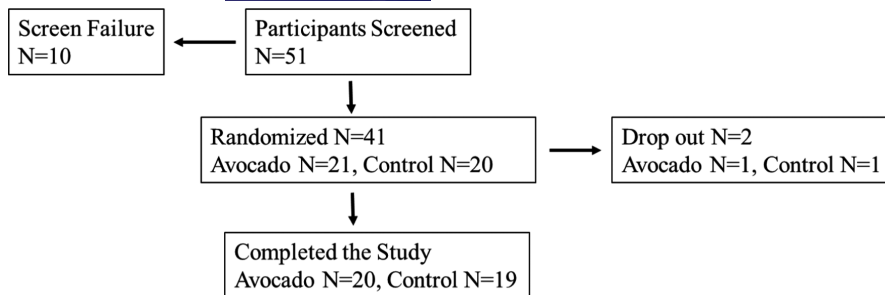
block. Throughout the study, participants were instructed to not consume any avocados outside the study.

Participants in the avocado group were provided with weekly supplies of avocado via delivery or pick up. Compliance was monitored with daily logs. All participants were instructed to maintain their habitual diet, usual activity level, and avoid sun exposure. Height, weight, Fitzpatrick skin type, and skin characteristics (melanin, hydration, sebum, erythema, and elasticity) were evaluated at baseline, and at weeks 4 and 8. Minimal erythema dose (MED) was evaluated at baseline and week 8 visits.

2.2 | Inner Arm Minimal Erythema Dose

We determined the lowest dose of UVB radiation capable of inducing erythema (MED) as described in detail in our previous publication.[16]

2.3 | Facial Elasticity, Melanin Index, Hydration, and Sebum Evaluation

Mechanical properties of the skin were determined with a non-invasive suction skin elasticity meter (cutometer dual MPA 580; Courage&Khazaka electronic GmbH, Koeln, Germany). We used a measuring probe with a 2-mm diameter and applied a constant suction of 500 mbar. The cutometer measures the deformation of the skin. The application of negative pressure draws the skin into the aperture of the probe. The time/strain mode was used with multiple consecutive cycles of suction application followed by a relaxation period. The resistance of the skin to negative pressure (ie, firmness) and its ability to return to its original position (ie, elasticity) are recorded. Measurements of minimum and maximum deformation are used by the software of the cutometer MPA 580 for the analysis of 9 parameters of skin elasticity and firmness (R0-R9). See Kim et al. for the description of the parameters.[3]

Melanin content and erythema were measured using a mexameter MA18 probe, hydration using the corneometer CM825 probe, and sebum using the sebumeter SM815 probe attached to the Cutometer dual MPA 580 (Courage&Khazaka electronic GmbH, Koeln, Germany) as previously described.[16] The measurements were performed in triplicates at two locations (forehead and 1 inch below the outer edge of the eye). The software of the cutometer MPA

580 allows the analysis of several parameters of skin elasticity and firmness.

2.4 | Cutometer data evaluation

R0-R9 are parameters of elasticity, firmness, and tiring from repeat suction that was evaluated from the skin deformation data as described by Kim et al.[3] R0 represents the final distension of skin sucked into the opening of the probe. The smaller the value, the firmer the skin.[3] R1 represents the difference between R0 and the skin's ability to return to the original state (smaller value = higher elasticity). R2, R5, R6, and R7 represent the overall elasticity (larger value = higher elasticity). R3, R4, and R9 represent tiring of the skin (smaller value = reduced tiring).

2.5 | Statistical Evaluation

Linear mixed-effects model was used to analyze the repeated measurements within subjects and to evaluate the change within and between avocado and habitual diet control groups. Within the intervention groups, the percentage change in each outcome between two time points (baseline to 4 and 8 weeks) was compared.

3 | RESULTS

3.1 | Characteristics of Participants

Forty-one participants were randomized. One participant dropped out from the control group without specific reasons and one from the avocado group for not willing to continue eating avocado. Thirty-nine participants completed the study (AVO $n = 20$, CTR $n = 19$). There were no statistically significant differences at baseline for age, height, weight, BMI, race, ethnicity, and skin type (Table 1). No adverse effects were reported.

There was no significant change in body weight comparing baseline to week 8 (AVO: baseline 179.4 ± 34, 8 weeks 179.8 ± 36; CTR: baseline 177.7 ± 31, 8 weeks 179.6 ± 32) or BMI (AVO: baseline 30.9 ± 6.9, 8 weeks 30.9 ± 7.3; CTR baseline 31.6 ± 6.4, 8 weeks 31.7 ± 6.7).

TABLE 1 Baseline demographics of study participants included in the final analysis

	Avocado (n = 21)	Control (n = 19)	Between-Group p-value
Age (years)	43.1 ± 13.1	48.4 ± 11.3	0.0981
Height (inches)	64.1 ± 3.3	63.1 ± 2.9	0.39
Weight (lbs)	179.4 ± 33.9	177.7 ± 31.2	0.944
BMI (kg/m²)	30.9 ± 6.9	31.6 ± 6.4	0.3464
Ethnicity[a]			0.3368
White	6 (29)	8 (40)	
Asian	5 (24)	1 (5)	
Black/AA	0 (0)	1 (5)	
Latino	7 (33)	8 (40)	
Mixed	3 (14)	1 (5)	
Skin Type[b]	3.5 ± 0.8	3.3 ± 0.7	0.4894

Note: Data are means ± SD. [a]Ethnicity, numbers in parenthesis are percentage.[b] Fitzpatrick skin type.

3.2 | Effects of Avocado Consumption on Minimal Erythema Dose

At baseline, there were no significant differences in MED between the AVO and CTR group (Table 2). MED showed a trend to increase in both groups comparing MED at 8 weeks to baseline (AVO: $p = .447$; CTR: $p = .0973$), and there was no difference between the AVO and CTR group after 8 weeks (Table 2).

3.3 | Effects of Avocado Consumption on Elasticity and Firmness

The cutometer evaluates skin firmness and elasticity by measure of deformation of the skin when it is pulled by controlled vacuum into the circular aperture. Parameters evaluating skin firmness include R0 and R8, elasticity is evaluated by R1, R2, R5, R6, and R7, and tiring of skin by repeat suction is measured by R3, R4, and R9. Values in Tables 3-5 present means of instrument readings at each time point. Percent change data from baseline to weeks 4 and 8 are available in supplementary materials (Supplemental Tables S1-S3). After 8 weeks of AVO consumption, the forehead skin showed significant decrease in parameters R0 (increase in firmness), R1 (increase in elasticity), R3 (decrease in tiring), R4 (decrease in tiring), and R8

(increase in firmness) between week 8 and baseline. A decrease in these parameters indicates increased firmness, elasticity, and reduced tiring after repeated deformation by suction. The decrease in R0 from baseline to week 8 was significantly stronger in the AVO compared with CTR group ($p = 0.04$) (Table 3). Values for R2, R5, R6, R7, and R9 did not change from baseline to week 8 at the forehead in the AVO group; in the CTR group, only R7 was slightly increased at week 8 compared with baseline (Table 3). A decrease in R2, R6, and R7 is associated with a decrease in elasticity, and maintaining the value or showing a trend to increase demonstrates that AVO consumption maintained skin elasticity.

For the under eye location in the AVO group, R0, R1, R3, and R4 decreased significantly comparing week 8 to baseline (Table 4). At under eye, in the CTR group, R0, R1, and R4 also decreased significantly at 8 weeks compared with baseline (Table 4). In the CTR group, R0 and R6 also decreased comparing week 4 to baseline (Table 4). At under eye, R9 decreased only in the AVO group comparing week 4 to baseline and the difference was significant between the AVO and CTR group ($p = 0.02$). A decrease in R9 is associated with reduced tiring.

3.4 | Effects of Avocado Consumption on Melanin Index, Erythema, Sebum, and Hydration

There were no significant differences in sebum, and hydration determined by cutometer reading between the AVO and CTR groups (Table 5). Both groups showed similar changes at 4 and 8 weeks in melanin and erythema. At the under eye location, in both groups, the melanin index decreased and the erythema index increased significantly at 4 and 8 weeks compared with baseline (Table 5). At the forehead location, in the CTR group, the melanin index decreased significantly (weeks 4 and 8) but no change in the AVO group was observed (Table 5). In addition, the erythema index increased in the AVO group (week 4) and in the CTR group (weeks 4 and 8).

4 | DISCUSSION

We found that daily consumption of one avocado per day for 8 weeks improved firmness (R0 and R8) and elasticity (R1) and reduced tiring of repeat stretching (R3 and R4) of the forehead skin, while elasticity (R7) was also increased in the control group at 8 weeks in women. At the under eye location, improvements in firmness (R0) and elasticity

TABLE 2 Effect of avocado consumption for 8 weeks on minimal erythema dose of UVB exposure of the inner arm skin

	Avocado Baseline	Avocado Week 8	Control Baseline	Control Week 8
Minimal Erythema Dose	398.3 ± 98.5	416.0 ± 85.6	395.0 ± 107.9	415.6 ± 95.6
Exposure Time (sec)	147.5 ± 40.5	165.2 ± 47.3	152.4 ± 44.8	160.4 ± 51.0
Dermalight dose	2.83 ± 0.6	2.6 ± 0.5	2.6 ± 0.6	2.8 ± 0.5

Note: Data are means ± SD. Avocado n = 20, Control n = 19.

TABLE 3 Effect of avocado consumption on skin elasticity at forehead

	Baseline	Week 4	Week 8	Baseline	Week 4	Week 8	4 wk p-value	8 wk p-value
	Avocado			Control				
R0	0.24 ± 0.05	0.21 ± 0.06	0.17 ± 0.07**	0.24 ± 0.07	0.24 ± 0.09	0.23 ± 0.08	0.4761	0.0403
R1	0.09 ± 0.03	0.08 ± 0.04	0.06 ± 0.02**	0.09 ± 0.03	0.09 ± 0.04	0.08 ± 0.03	0.599	0.1504
R2	0.62 ± 0.14	0.64 ± 0.14	0.63 ± 0.10	0.59 ± 0.11	0.62 ± 0.09	0.63 ± 0.09	0.9619	0.7092
R3	0.28 ± 0.06	0.26 ± 0.06	0.21 ± 0.08**	0.28 ± 0.07	0.28 ± 0.09	0.27 ± 0.09	0.7118	0.0594
R4	0.12 ± 0.05	0.10 ± 0.05	0.08 ± 0.04**	0.13 ± 0.04	0.11 ± 0.05	0.11 ± 0.05	0.8834	0.3963
R5	0.54 ± 0.14	0.61 ± 0.17	0.63 ± 0.25	0.49 ± 0.10	0.52 ± 0.14	0.54 ± 0.13	0.3096	0.9359
R6	0.90 ± 0.23	0.98 ± 0.28	1.09 ± 0.72	0.83 ± 0.22	0.89 ± 0.32	0.82 ± 0.27	0.9738	0.6428
R7	0.28 ± 0.06	0.30 ± 0.07	0.29 ± 0.05	0.26 ± 0.04	0.27 ± 0.04	0.29 ± 0.05*	0.5851	0.8275
R8	0.15 ± 0.05	0.14 ± 0.05	0.11 ± 0.06*	0.15 ± 0.06	0.15 ± 0.06	0.15 ± 0.07	0.6149	0.1001
R9	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.2591	0.9832

Note: Data are means ± SD. AVO $n = 20$, CRT $n = 19$. * compared with baseline (*$p \le .05$,**$p < 0.01$); p-value indicates comparison between avocado and control group.

TABLE 4 Effect of avocado consumption on skin elasticity at under eye

	Baseline	Week 4	Week 8	Baseline	Week 4	Week 8	4 wk p-value	8 wk p-value
	Avocado			Control				
R0	0.40 ± 0.12	0.33 ± 0.09	0.31 ± 0.12**	0.39 ± 0.11	0.32 ± 0.07*	0.34 ± 0.10*	0.7406	0.4485
R1	0.15 ± 0.06	0.11 ± 0.05	0.11 ± 0.05*	0.18 ± 0.06	0.14 ± 0.05	0.14 ± 0.04*	0.9291	0.8884
R2	0.66 ± 0.19	0.65 ± 0.10	0.64 ± 0.06	0.58 ± 0.16	0.57 ± 0.09	0.58 ± 0.08	0.9642	0.6116
R3	0.44 ± 0.13	0.37 ± 0.10	0.36 ± 0.13**	0.44 ± 0.13	0.37 ± 0.08	0.39 ± 0.11	0.9151	0.3904
R4	0.19 ± 0.08	0.15 ± 0.06	0.15 ± 0.07*	0.23 ± 0.08	0.19 ± 0.06	0.18 ± 0.05*	0.9182	0.8508
R5	0.56 ± 0.30	0.56 ± 0.16	0.55 ± 0.14	0.45 ± 0.19	0.54 ± 0.23	0.53 ± 0.29	0.3978	0.362
R6	0.68 ± 0.30	0.81 ± 0.28	0.78 ± 0.25	0.67 ± 0.19	0.97 ± 0.32*	0.81 ± 0.34	0.2188	0.6896
R7	0.35 ± 0.24	0.31 ± 0.07	0.31 ± 0.07	0.28 ± 0.16	0.27 ± 0.06	0.28 ± 0.05	0.5976	0.5024
R8	0.25 ± 0.09	0.22 ± 0.08	0.20 ± 0.08	0.22 ± 0.07	0.17 ± 0.03	0.20 ± 0.07	0.6765	0.358
R9	0.05 ± 0.02	0.03 ± 0.03*	0.04 ± 0.02	0.05 ± 0.02	0.05 ± 0.01	0.048 ± 0.02	0.022	0.2983

Note: Data are means ± SD. AVO $n = 20$, CRT $n = 19$. * compared with baseline (*$p \le .05$,**$p < 0.01$); p-value indicates comparison between avocado and control group.

(R1) and reduced tiring of repeat stretching (R3 and R4) were also observed in the AVO group and increase in firmness (R0) and elasticity (R1) and reduced tiring (R4) were also observed in the CTR group. Avocado consumption did not change UVB resistance compared with habitual diet control. Changes in erythema and sebum were observed in both groups and therefore might not be related to the avocado intervention. There were no significant changes in hydration and sebum formation in both groups in this study. To our knowledge, this is the first study testing the effect of oral avocado consumption on parameters of skin aging. Potential mechanism described from in vitro cell culture studies how dietary supplements might improve firmness and elasticity of the facial skin include induced elastin and collagen gene expression, resulting in enhanced fiber cross-linking in human skin explants.[6] These mechanisms have been observed in in vitro studies in human fibroblast cell culture experiments that demonstrated that a blackberry-dill extract induced elastin gene

expression, elastin promoter activity, and inhibited elastic fiber degradation by matrix metalloproteinases (MMPs) 9 and 12.[6] A clinical study using topical application of the same blackberry-dill extract for 12 weeks showed an increase in skin elasticity in middle-aged women compared with control lotion.[6] Additional studies with carotenoids also have shown that carotenoids prevent skin aging by stimulating fibroblasts to produce collagen and elastin, inhibit the activity of MMPs, and exhibit anti-inflammatory and UV-filtering effects.[18] Future cell culture or rodent studies exploring the potential mechanism how avocado consumption can improve skin firmness and elasticity will be necessary.

Improved skin elasticity is commonly associated with increased skin hydration.[19] However, a recent study investigating the topical application of a cream containing avocado oil demonstrated improved elasticity without changes in skin hydration, supporting our findings.[14]

TABLE 5 Effect of avocado consumption on hydration, melanin, erythema, and sebum at forehead and under eye

	Baseline	Week 4	Week 8	Baseline	Week 4	Week 8	4 wk p-value	8 wk p-value
	Avocado			Control				
Forehead								
Hydration	65.5 ± 13	64.0 ± 15	61.3 ± 17	61.0 ± 16	54.0 ± 11	54.8 ± 12	0.313	0.710
Melanin	420 ± 137	401 ± 118	360 ± 101	397 ± 147	350 ± 124*	313 ± 108*	0.351	0.497
Erythema	241 ± 110	281 ± 91*	288 ± 108	241 ± 98	281 ± 82*	306 ± 59*	0.632	0.272
Sebum	53.5 ± 83	67.2 ± 62	68.0 ± 68	53.4 ± 49	68.2 ± 57	57.5 ± 45	0.816	0.701
Under Eye								
Hydration	70.1 ± 16	66.5 ± 15	70.1 ± 15	71.4 ± 16	69.8 ± 19	72.4 ± 11	0.684	0.858
Melanin	406 ± 119	356 ± 58*	324 ± 84*	383 ± 171	341 ± 142*	322 ± 145*	0.673	0.707
Erythema	206 ± 109	266 ± 93*	282 ± 117*	211 ± 101	276 ± 53*	282 ± 66*	0.731	0.847
Sebum	43.8 ± 53	52.3 ± 57	45.4 ± 55	36.3 ± 34	41.9 ± 53	35.9 ± 20	0.924	0.931

Note: Data are mean ± SD. Avocado $n = 20$, Control $n = 19$. * compared with baseline ($p \leq .05$); p-value indicates comparison between avocado and control group.

Improvement in UV resistance by increased MED was previously observed following almond consumption for 12 weeks or pomegranate extract consumption for 8 weeks.[16,17] These effects were observed in women of younger age. The participants in this study were not only significantly older but also had elevated BMI and increased abdominal circumferences. Abdominal obesity is known to increase oxidant stress that may have prevented the daily avocado intake to show significant change benefit measured by MED in 8 weeks. Studies with longer consumption duration and larger sample size are needed to further evaluate effect of avocado on skin's resistance to UVB and skin characteristics.

This was a pilot study with several limitations. Potential limitations of the study include the choice of facial locations selected for the testing. Forehead skin is very different from under eye, and additional locations might provide more insight into the effect of avocado on skin elasticity and firmness. We instructed participants to avoid sun exposure and maintain their usual skincare, but did not monitor objectively. The age of women enrolled in the present study ranged from 27 to 73 years and body weight from 59 to 123 kg. For future studies to confirm the findings, a narrower range of age focusing on pre or postmenopausal women and narrower range of body weight might enhance the outcome.

CONFLICTS OF INTEREST
The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS
SMH evaluated the data and prepared manuscript, JBG recruited participant and performed skin tests, GT attained IRB approval and provided administrative assistance, JY assisted in data collection and analysis, CHT performed statistical analysis, JK contributed to study design and reviewed manuscript, DH and ZL designed study and reviewed manuscript. All authors read and approved the final manuscript.

ETHICS STATEMENT
Authors declare human ethics approval was not needed for this study.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.