

Research Article

Pistachio Consumption at 20% of Energy Does Not Significantly Change Body Composition, Blood Pressure or Blood Lipids but Improves Diet Quality in Free-Living, Healthy College-Aged Women

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Abstract

Information regarding pistachio intake on body composition, and blood lipids in healthy women is limited. The researchers wished to investigate the effects of pistachios on blood lipids, erythrocyte membrane incorporation of fatty acids, weight, body composition, and blood pressure (BP) in healthy women (N=48, age=21±0.51). The study was a randomized, free-living crossover design at two university campuses with two 10-week treatment periods; pistachios added (20% of kcals), a no pistachio control treatment, and a 15-week washout period. Measurements were taken at beginning and end of each treatment and nine randomized diet records were collected per treatment and analyzed using a mixed effects model. Neither treatment had a significant effect on blood lipids, erythrocyte fatty acid membranes, body weight, percent body fat, or BP. In the pistachio treatment, dietary energy was higher (p=0.0012) and dietary total fat, vegetable protein, total MUFA, total PUFA, insoluble dietary fiber, gamma-tocopherol, and copper, were all significantly higher than the control (p<0.001). The glycemic index and betaine were significantly higher in the control compared to the pistachio treatment diet (p=0.0010 and 0.0002, respectively). Inclusion of 20% of kcals as pistachios does not contribute to weight gain, body fat or blood lipid changes, but may increase diet quality in healthy women. Registered. Registered with ClinicalTrials.gov #NCT02849392

Keywords: Body Composition; Diet Quality; Nut Studies; University 2 : California State Polytechnic University, Pomona
Pistachios; Young Healthy Women

Abbreviations:

BP	:	Blood Pressure	iDXA	:	Dual-Energy X-Ray Absorptiometry
University 1	:	California Polytechnic State University, San Luis Obispo	DHA	:	Docosahexaenoic Acid
			EPA	:	Eicosapentaenoic Acid
			FFM	:	Fat Free Mass
			HDL	:	High Density Lipoprotein

LDL	:	Low Density Lipoprotein
NDS-R	:	Nutrition Data System for Research
MUFA	:	Monounsaturated Fatty Acid
n-3	:	Omega-3
n-6	:	Omega-6
PUFA	:	Polyunsaturated Fatty Acid
SFA	:	Saturated Fatty Acid
SAS	:	Statistical Analysis Software
TC	:	Total Cholesterol
TBM	:	Total Body Mass
TBW	:	Total Body Water
TAG	:	Triacyl Glycerides
UDD	:	Unusual Diet Diary

Introduction

One ounce (28.4g) of pistachio contains 162 kcals, 5.97 g protein, and 13.0 g total fat. Most of the total fat is monounsaturated (MUFA) (6.96 g) and polyunsaturated (PUFA) (3.78 g) fatty acids [1]. Although pistachios are rich in fat, which provides 9 kcal/g, diets high in MUFAs [2,3] or diets high in other tree nuts have not been associated with significant weight gain [3-6].

Diets rich in MUFAs have been shown to decrease the risk of heart disease by reducing low density lipoprotein (LDL) cholesterol, thereby improving the LDL Cholesterol: High Density Lipoprotein (HDL) ratio [2,3]. Published Pistachio consumption studies show decreases in blood cholesterol and blood lipids in hypercholesteremic [7-11], in healthy men [12], in healthy volunteers (average age about +10 years than our study participants) [13], during weight loss in overweight participants [14], and in participants with metabolic syndrome [15,16]. Others have examined body composition changes in metabolic syndrome or have focused on glucose metabolism and lipid profiles in type 2 diabetes [17,18] and pre-diabetes [19-22]. There have been no studies in healthy young women.

Plasma fatty acid composition reflects dietary fatty acid intake [23,24], however, erythrocyte membrane incorporation of fatty acid is a better long-term assessment of fatty acid status than plasma [23,25]. Incorporation of MUFAs and PUFAs into membranes displaces saturated and trans fatty acids and may be one of the mechanisms for decreased blood cholesterol levels. There are no studies that have investigated erythrocyte membrane incorporation of fatty acids following pistachio consumption in healthy, young women. Pistachio consumption blood pressure (BP) changes demonstrate conflicting results [9,15,26,27]. One demonstrated a decreased systolic blood pressure; others observed no changes in blood pressure [9,15,27]. Research investigating the beneficial

effects of regular pistachio consumption on body composition is limited, for example, only one study examined the effect of pistachios on visceral adipose tissue [16]. Studies that investigate body composition, erythrocyte membrane incorporation of fatty acids, blood lipids, diet quality, and blood pressure in regard to pistachio consumption are needed, especially in healthy women. The purpose of the present study was to examine the effects of pistachio consumption on body composition, erythrocyte membrane incorporation of fatty acids, blood pressure, blood lipids, and diet quality using a cross-over study with two 10-week treatment periods: a pistachio added (20% of kcals) and a no-pistachio control treatment diet in healthy, young women. The null hypothesis for the study is that there will be no differences between campuses or between treatments for all of the variables, since the women participants are young and apparently healthy (absence of disease).

Materials and Methods

Aims

The aims of the study are to examine the effects of a diet rich in pistachios on body composition (two different university campuses; University#1=California Polytechnic State University, San Luis Obispo, and University#2=California State Polytechnic University, Pomona); examine incorporation of healthy fats into erythrocyte membranes (University #2); determine changes in blood lipids (both campuses); assess diet quality/micronutrient quality with pistachio consumption (both campuses); assess divalent micronutrient absorption (Zn and Fe) to ensure that the fiber in the pistachios was not inhibiting uptake and absorption of these minerals (University #1).

Participants

The Institutional Review Boards at both universities approved all procedures involving human participants. Written and signed informed consent was obtained from all participants prior to beginning the study. Women at both universities were invited to participate in the study and recruitment was through mass emails and flyers posted on both campuses. The first 30 qualified women were enrolled into the study at each site (60 total). Potential participants signed Informed Consent forms and then completed a screening questionnaire. The study inclusion criteria required biological women (age 18-40 years old, with healthy body mass index), who were willing to consume pistachios, did not take nutritional supplements for 2-months before beginning of study, not allergic to nuts of any kind, and were free of chronic diseases. Participants were excluded if they did not meet the inclusion criteria or took medications that affected fat malabsorption, took laxatives, drank more than 1 ounce of ethyl alcohol per day, were planning on becoming or presently pregnant, had pacemakers or metal pins/plates in the body, or if they were considered elite athletes.

Study Design and Protocol

The study was a randomized, crossover design with two 10-week treatment periods: 20% of kcals from shelled roasted unsalted pistachios (intervention) and a no-pistachio usual diet (control) diet. Treatments were separated by a 15-week washout (rest) period. Originally, the study was based on two 12-week treatment cycles, but was changed to 10-weeks due to the time limitations of the university quarter system and concern for participant dropout and or possible compliance issues during the breaks.

Participants were randomized into either the pistachio treatment group or the control group. This study used a crossover design, therefore all subjects participated in the pistachio treatment intervention and served as their own control, which ensured that everyone could participate in both treatments. At the initial visit, half of the participants were randomized to include pistachios in their diet as snacks or in meals (20% of kcals; intervention group), and half continued their normal diets (control group) without pistachios. Since this was a free-living study, no attempt was made to alter the normal diet or to control calories, or the size and time periods between meals.

During the second treatment cycle, the control participants were given pistachios, and the participants that had consumed pistachios, were told to consume their normal diet and not consume pistachios during that treatment. All participants met with study personnel to submit diet records. During the washout, participants were asked to return to their normal diet. At baseline and end of each treatment period, height, weight, waist and hip circumference, and body composition were measured, and a 12-hour fasting blood draw was performed.

The researchers calculated the required amount of pistachios for each participant based on 20% of their usual caloric need using the Harris-Benedict Equation and the International Physical Activity Questionnaire. Every week, the researchers met with the pistachio-consuming participants to provide the weekly supply (one per day) of their individualized pre-measured packets of pistachios and to collect the diet records, the Unusual Diet Diary (UDD) [28]. Researchers met with the control subjects to collect the diet records, UDD on an “as needed” basis. Participants were asked to recall their physical activity level over the preceding week (7-day recall) using the 2002 International Physical Activity Questionnaire short questionnaire. The activity information was used to determine activity level for pistachio calorie calculation requirements. Participants were encouraged to maintain their usual levels of physical activity during the entire study.

Data Collection

Dietary Intake Analyses

Participants were trained to keep accurate food records, and

were asked to perform three randomly selected three consecutive day food records throughout the study. Food record nutrients of interest included: protein, carbohydrate, fiber, total and individual dietary fats (saturated fat, monounsaturated fat, polyunsaturated fat, as well as trans, n-3 and n-6 fatty acids), all minerals including iron and calcium and all water and fat-soluble vitamins. The Nutrition Data System for Research (NDS-R) from the University of Minnesota (University Board of Regents, 2013) was used for the diet analysis on both campuses.

Biochemical Analyses

The participants fasted for 12 hours before the blood draw and drank 1.5 cups of water in the morning before the blood draw to protect against dehydration and facilitate the blood draw. Blood was drawn and processed by trained phlebotomists at the on-campus Student Health Centers at week 1 and 10 for each treatment cycle, analyses included: Total cholesterol (TC), LDL cholesterol (calculated), HDL cholesterol and triacylglycerides (TAG), a standard blood chemistry panel and iron status. Processed samples were sent to clinical laboratories for analyses.

Erythrocyte Membrane Incorporation (Lipomics)

At University #2, blood cells were separated by centrifugation, and washed three times, frozen, then shipped overnight to Lipomics (West Sacramento, CA a division of Metabolon) to determine the fatty acid incorporation in the erythrocyte membranes according to the methods described by Burns-Whitmore, et al. [28] and Sabáte, et al. [29].

Anthropometric Measurements

Body composition analysis

Body composition was measured at baseline and end of treatment using the Tanita Bio-impedance Scale (G-30) TANITA Corporation, (Tokyo, Japan). Measurements taken include: body weight, body mass index (BMI), percent body fat, impedance, fat mass, fat free mass (FFM), lean body mass, total body mass (TBM), and total body water (TBW).

Body Composition/Bone Mineral Density (iDXA- Collected at University #1 site only)

Full-body DXA (iDXA, GE Healthcare) was used to determine body fat mass, body fat percentages, lean body mass and bone mineral density of each participant at University 1. The participant fasted 4-hours prior (excluding water consumption) to being measured. Even though the radiation dose given was as low as or even insignificant in comparison with natural background radiation levels [30], a pregnancy test was given to participants before the test to ensure safety of the fetus. A positive test disqualified the participant.

Body Weight and Waist-To-Hip Measurements

Body weight was measured using a calibrated scale as well as with the Tanita scale and recorded to the nearest 0.1 kg. Height was measured on a stadiometer, and recorded to the nearest 0.1 cm. Age and height was non-changing for the purpose of this study. Waist and hip circumference was measured using a flexible measuring tape and protocol was based on NHANES III standardized procedures. Personnel were thoroughly trained using CDC protocols [31]. Blood pressure was taken two times using an automatic cuff blood pressure monitor (Omron 3 series, Omron Healthcare Inc., Lake Forest, IL). Protocols were based on Mayo Clinic standardized procedures and study personnel were trained on these protocols [32].

Statistical Analyses

A sample size of 24 participants per university provides more than adequate power (80%) to detect individual changes. The statistical analyses were run using Statistical Analysis Software (SAS version 9.2). The data were analyzed using a mixed effects model, which is a model where some of the independent variables are treated as fixed effects and some as random effects. Due to the crossover nature of the study design, the participants in the dataset have repeated measurements on variables such as body weight, which are presumably related to each other. When data are collected from related units it is important to account for the possible similarities of responses from these related units. Thus, Subject ID was included in the model as an independent variable. However, even though we would like to account for the effect of observations on a participant being related, we are not interested in estimating the average response of each individual. For example, we are not interested in how much higher Subject 1's average percent body fat is compared to Subject 2's. Thus, Subject ID's effect on a response variable of interest was treated as a random effect. In contrast, we treated our other independent variables as fixed effects because we are interested in the effect size of these factors on the response variables. For example, we want to know how much higher the average percent body fat is when on diet 1 compared to diet 2. Separate mixed effects models were run for each response variable of interest, with the following predictors (or independent variables) in each case: Subject ID, diet (pistachio versus habitual), location (university 1 versus 2), and the interaction of diet and location (to investigate whether the effect of diet is modified by location).

Quality Control

Compliance

Binders containing forms and instructions for food records and the UDD were provided to participants. Three, 3-day randomized food records for a total of 9 days were collected during each treatment to obtain information regarding the participant's diet and determine if the participant was adhering to treatment protocols.

Completed forms were checked for accuracy and collected at the weekly study visits. Participants were asked to return their leftover daily bags which contained their pistachios.

Adherence and compliance to the assigned diets were assessed using a self-reported Unusual Diet Diary (UDD) [28], which was filled out by the participant when unusual quantities of foods or accidental pistachio consumption occurred, medical treatment was required, a suspected allergic reaction/side effect occurred or pharmaceuticals were consumed. Diet counseling was provided at each visit (if needed) and compliance to study protocols was evaluated by counting the number of empty pistachio bags and examining the participant's UDDs. Participants received email/text reminders about weekly meetings, meetings for diet records, and clinic instructions.

Results and Discussion

Participant Characteristics

Sixty participants were recruited for the study and 48 (80%) finished the study. Of those who failed to complete the study, most cited family, school or work pressure/problems, and one cited digestive problems. The participants in the 35-week randomized crossover trial were instructed to eat an allotted amount of pistachios (20% of kcals) during the pistachio treatment arm and not to eat pistachios during the control arm. Between both campuses, at baseline, the participants somewhat significantly differed in percent body fat $p=0.0405$ ($p<0.05$). There were no other differences in body composition. Twenty-five participants (83%) finished the study at University 1, and 25 (77%) finished at University 2. (Table 1) provides the comparisons of body composition, blood pressure and blood lipid values between participants at both campuses. Only data from the participants that finished the study was analyzed.

Characteristic ¹	University 2	University 1	Both
Age	22.8 ± 0.93	20 ± 0.29	21 ± 0.07
Weight	60.2 ± 1.68	57.8 ± 1.46	58.9 ± 1.11
BMI	22.0 ± 0.46	21.6 ± 0.44	21.9 ± 0.33
Waist Circumference	28.9 ± 0.42	28.5 ± 0.59	28.7 ± 0.36
Triacyl Glyceride	79.0 ± 7.22	76.6 ± 7.70	7.7 ± 5.24
Total Cholesterol	151 ± 6.42	158.6 ± 4.95	155.0 ± 4.00
LDL ³	76.2 ± 4.82	79.0 ± 4.91	77.7 ± 3.41
HDL	59.1 ± 2.79	64.3 ± 2.08	61.8 ± 1.74
Cholesterol:HDL	2.64 ± 0.12	2.54 ± 0.12	2.59 ± 0.08

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Percent Body Fat²	25.8 ± 1.18	22.3 ± 1.16	24.0 ± 0.86
Systolic Blood Pressure	110 ± 2.31	105.3 ± 1.69	107.8 ± 1.45
Diastolic Blood Pressure	71.0 ± 2.06	67.3 ± 1.35	69.1 ± 1.23
Iron	113 ± 8.80	93.1 ± 9.92	102.6 ± 6.76
¹ Mean ± standard error of participants who completed the study			
² Percent body fat was significantly higher (p=0.0405) at University 2 than University 1; all other values were not significant.			
³ Calculated (Friedewald)			

Table 1: Participant Baseline Characteristics at each University, University 2 n = 23 and University 1 n = 25, Total number of participants = 48.

Dietary Compliance- Diet Composition

(Table 2) provides the data differences in selected dietary nutrients between diet treatments. To account for multiple testing, at an overall 5% alpha or significance level, we will need individual p-values to be lower than 0.001 to conclude the presence of significant relationships. Although these nutrients were not significant at an overall alpha of 5%, total protein (p=0.0055), soluble dietary fiber (p=0.0060), phosphorus (p=0.0023), and the PUFA to saturated fat (SFA) ratio were higher in the pistachio treatment while starch (p=0.0019), sodium (p=0.0191) and the glycemic load (p=0.0132) were higher in the control treatment. Energy was higher (p=0.0012) in the pistachio treatment compared to the control. Fat, vegetable protein, total MUFA, total PUFA, insoluble dietary fiber, gamma-tocopherol, and copper were also higher in the pistachio treatment (p<0.001). The glycemic index and betaine were significantly higher in the control compared to the pistachio treatment. Fat, vegetable protein, total MUFA, total PUFA, insoluble dietary fiber, gamma-tocopherol, and copper were significantly higher in the pistachio treatment, which suggests that pistachios increased dietary quality. Energy was significantly higher in the pistachio treatment compared to the control, which may indicate that participants may have added pistachios to their diet instead of incorporating them into their existing diet.

Sheridan et al. [9] found a significant decrease in saturated fat in the pistachio treatment, but our study did not. O’Neil et al. [28] found significantly higher dietary intakes of fiber, vitamin E, calcium, magnesium and potassium and a lower intake of sodium than non-tree nut consumers. We found significantly higher intakes of insoluble fiber, gamma-tocopherol, and copper, and approached significance (p<0.001) for decreased phosphorus (p=0.0023), sodium (p=0.0191), and increased potassium (p=0.0011) in the pistachio treatment (Table 2). Sauder et al. [22] found dietary sodium was significantly lower and potassium higher compared to the control in their study. Our study was a free-living group of healthy women, while the Sauder study was an iso-caloric controlled

metabolic kitchen study in people with type 2 diabetes [27]. However, both studies indicate that potassium is increased and sodium decreased when participants consume pistachios, which is a more favorable change towards a heart-healthy diet.

The glycemic index was significantly higher in the control compared to the pistachio treatment. Since the glycemic index is of concern for people with diabetes, and the glycemic index was lower in the pistachio treatment, pistachios might be a viable way to increase MUFA and PUFA while decreasing the glycemic index in the diet. More studies are needed to confirm that pistachios decrease the glycemic index of the diet, and result in lower blood glucose, which aids in the prevention of diabetic complications.

Nutrient1	Pistachio (n=48)	Control (n=48)	P value
Energy (Kcals)	1819 (43.0)	1609 (43.0)	0.0012
Fat (g)	80.88 (2.00)	58.39 (2.00)	<0.0001
Total Protein (g)	72.92 (1.50)	66.82 (1.50)	0.0055
Vegetable Protein (g)	37.11 (1.10)	29.34 (1.10)	<0.0001
MUFA ² (g)	32.78 (0.84)	20.46 (0.84)	0.0001
PUFA ³ (g)	19.96 (0.64)	13.93 (0.64)	<0.0001
Total Saturated Fat (g)	21.20 (0.76)	18.90 (0.76)	0.0365
Starch (g)	82.35 (3.80)	99.85 (3.80)	0.0019
Total Dietary Fiber (g)	24.78 (0.82)	21.52 (0.82)	0.0069
Insoluble Dietary Fiber (g)	19.05 (0.62)	14.87 (0.62)	<0.0001
Soluble Dietary Fiber (g)	5.60 (0.22)	6.48 (0.22)	0.0060
Gamma-Tocopherol (µg)	24.04 (0.67)	10.90 (0.67)	<0.0001
Vitamin B6 (mg)	2.309 (0.08)	1.879 (0.08)	0.0003
Betaine (µg)	130.0 (7.50)	172.1 (7.50)	0.0002
Total Vitamin A (IU)	8267 (582)	8720 (582)	0.5816
Lutein/Zeaxanthin (µg)	3204 (508)	3781 (508)	0.4224
Phosphorus (mg)	1254 (33.0)	1104 (33.0)	0.0023
Copper (mg)	1.890 (0.06)	1.330 (0.06)	<0.0001
Sodium (mg)	2380 (95.0)	2705 (95.0)	0.0191
Potassium (mg)	2692 (68.0)	2357 (68.0)	0.0011
Glycemic Index	53.10 (1.00)	58.13 (1.00)	0.0010
Glycemic Load	103.0 (3.70)	116.0 (3.70)	0.0132
¹ Mixed Effects Model; Least Square Mean (Standard Error of the Mean); Significance is p<0.001			
² Monounsaturated Fatty Acids (MUFA)			
³ Polyunsaturated Fatty Acids (PUFA)			

Table 2: Differences in Selected Dietary Nutrients Between Diet Treatments.

Serum Lipids and Lipoproteins

There were no significant differences between diet treat-

ments or campuses for blood lipids and there were no significant differences between the blood lipids at baseline and end of treatment for either the treatment or control (Table 3).

Values ¹	Treatment Diet by Visit				P value
	Pistachio Baseline ²	Pistachio End ²	Control Baseline ²	Control End ²	
Triacylglycerides (mg/dl)	80.8 (3.43)	80.8 (3.57)	82.0 (3.38)	86.0 (3.46)	0.5599
Cholesterol (mg/dl)	158 (1.90)	161 (1.88)	161 (1.88)	162 (1.90)	0.4956
LDL ³ (mg/dl)	79.4 (1.64)	82.1 (1.73)	81.5 (1.640)	81.5 (1.66)	0.4262
HDL (mg/dl)	62.8 (0.90)	63.3 (0.95)	63.6 (0.90)	63.8 (0.91)	0.8508
TC: HDL	2.59 (0.04)	2.62 (0.04)	2.61 (0.04)	2.61 (0.04)	0.6898

¹Mixed Effects Model
²Least Square Mean (Standard Error)
³Calculated (Friedewald)

Table 3: Blood Lipids at Baseline and End of Diet Treatment.

Erythrocyte Membrane Analysis

There were no significant lipid membrane differences for either treatment (Table 4).

Fatty acid $\mu\text{g}/\text{mole}\%$ ¹	Pistachio Difference ²	Control Difference ²	P-value
Total Saturated	44.1 (0.73)	44.6 (0.69)	0.6452
Total Monounsaturated	18.3 (0.42)	18.3 (0.40)	0.9640
Palmitoleic acid (16:1 n-7)	1.17 (0.22)	1.22 (0.21)	0.8681
Oleic acid (18:1 n-9)	13.5 (0.20)	13.4 (0.19)	0.6756
Vaccenic acid (18:1 n-7)	2.25 (0.16)	2.15 (0.15)	0.6724
Eicosenoic acid (20:1 n-9)	0.32 (0.01)	0.31 (0.01)	0.3386
Total Polyunsaturated	31.5 (0.95)	30.8 (0.90)	0.5854
n-6 (total)	27.0 (0.73)	26.4 (0.69)	0.6116
Linoleic (18:2 n-6)	11.4 (0.25)	11.3 (0.23)	0.9586
Gamma-linolenic acid (18:3 n-6)	0.04 (0.00)	0.05 (0.00)	0.1146
Arachidonic (20:4 n-6)	10.7 (0.47)	10.3 (0.45)	0.5416
n-3 (Total)	4.54 (0.25)	4.33 (0.24)	0.5460
Alpha-Linolenic (18:3 n-3)	0.09 (0.01)	0.09 (0.01)	0.5744
EPA (20:5 n-3) ³	0.24 (0.02)	0.22 (0.02)	0.3677
DHA (22:6 n-3) ³	2.71 (0.17)	2.57 (0.16)	0.2673

¹Fatty Acid Values Are Presented in Least Square Means with Standard Error (SE).
²Mixed Effects Model, Controlling for Diet and Visit.
³EPA = Eicosapentaenoic Acid; DHA = Docosahexaenoic Acid

Table 4: Fatty Acid Composition of Selected Erythrocyte Membrane Differences for Each Dietary Treatment (University 2 Only).

Dietary Lipids to Blood Lipids

The researchers also investigated the relationship of dietary lipids to blood lipids and membrane lipids as a change of either blood lipid (data not shown) or membrane lipids (Table 5) associated with a 1-unit change in dietary fat intake accounting for diet treatment, location, and subject-to-subject variability.

To account for multiple testing, at an overall 5% significance level, we will need individual p-values to be lower than 0.001 to conclude the presence of significant relationships. Our analyses revealed that there were no significant relationships found (at an overall 5% significance level) between any of the dietary fat intake variables or any of the measures of fatty acids in the erythrocyte membranes, after accounting for diet (pistachio vs. control) and subject-to-subject variability (Table 5). The smallest p-value found was 0.015 for dietary PUFA and membrane 18:2 n-3 (alpha-linolenic acid-ALA), but again, it was not significant at the overall 5% significance level. Other variables that trended towards significance include total dietary fat to membrane saturated fatty acids (SFA) (p=0.034), membrane PUFAs (p=0.05), total membrane omega-3 (n-3) (p=0.035), docosahexaenoic acid (DHA) (p=0.046) and total membrane n-6 (p=0.067); dietary MUFA to membrane SFA (p=0.031), MUFA (p=0.052), PUFA (p=0.041), total n-3 (p=0.077) and total n-6 (p=0.039) and PUFA: SFA ratio to ALA (p=0.024).

We hypothesized that blood lipids, TAG, TC and LDL cholesterol, would either remain the same or decrease in the pistachio treatment. Our results confirmed a decrease in the lipids, but the findings were not significant (Table 3). It is possible that normal mean lipid values in healthy people will not show significance, since only a large drop in lipid levels would result in statistical significance in a group of 48 healthy young women. Studies in hypercholesterolemia and metabolic syndrome show a significant drop in total cholesterol and/or LDL cholesterol [7-12,15,16]. In a healthy group of men and women 10-years older than the participants of this study, TAG, LDL, and total cholesterol decreased, but the change was not significant. HDL and TC: HDL and LDL: HDL significantly increased when pistachios were substituted for 20% of energy in the diet [13]. Our study shows a similar trend but was not significant.

In the lipid correlation analysis, no statistically significant relationships were found between the dietary fat intake variables or the blood lipid measures (Table 5). Dietary MUFA and serum HDL resulted in a p=0.004, but was not significant at an overall alpha of 5% or even an overall alpha of 10% (for which the individual p-value needs to be less than 0.002). Also, non-significant, but trending to significance was dietary fat to serum HDL (p=0.015), dietary PUFA to HDL (p=0.029), dietary MUFA to TC: HDL (p=0.044), and dietary PUFA: SFA to serum TAG (p=0.010)

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measures (data not shown).

Total dietary fat affects the lipid concentration of erythrocyte membranes [25]. This is reflective in the membrane incorporation responses, which are somewhat conflicting but since dietary total fat encompasses all types of fatty acids, it is expected that many interactive effects are possible. However, dietary MUFA seemed to influence SFA incorporation (negative slope), so as MUFA increases in the diet, the lipid concentration of saturated fat decreases (Table 4). MUFA positively influences PUFA, total n-3 and total n-6 incorporation. The PUFA: SFA ratio was associated with a negative slope of total n-3, indicating as that as the ratio increases there is a decrease in n-3 incorporation. This may be related to the

inhibitory effects of SFA on the delta-6 desaturase enzymes, and possibly a competitive inhibition of other types of PUFA, including n-6, for the delta-6 desaturase enzymes and limited total n-3 incorporation into erythrocyte membranes.

No studies have investigated the effects of pistachio consumption on erythrocyte membrane incorporation; however, studies with other tree nuts, such as walnuts, clearly show an increase in membrane changes corresponding to the treatment food composition [28,33]. It is possible that uptake of essential fatty acids such as the n-3 fatty acids are incorporated at a faster rate than MUFA, or that changes in erythrocyte membrane lipids may require more than 10-weeks to show statistical significance.

Membrane Lipid Response Outcome ^{1,2}									
Diet Predictor	Saturated fatty acids	MUFA ³	PUFA ³	Total n- ³ fatty acids	Alpha-Linolenic Acid ⁴	Linoleic Acid ⁴	EPA ^{3,4}	DHA ³	Total n-6 ³ fatty acids
				(n- ³ FA ³)					
Total fat	-0.191 ± 0.082 (0.034)	-0.047 ± 0.029 (0.134)	0.216 ± 0.101 (-0.05)	0.062 ± 0.027 (-0.035)	-0.001 ± 0.001 (0.226)	-0.00004 ± 0.0002 (0.868)	0.002 ± 0.002 (-0.398)	0.038 ± 0.018 (-0.046)	0.154 ± 0.078 (-0.067)
	Cholesterol	0.017 ± 0.021	0.005 ± 0.007	-0.023 ± 0.025	-0.008 ± 0.007	-0.00002 ± 0.0002	0.000003 ± 0.0001	-0.0003 ± 0.001	-0.005 ± 0.004
SFA ³	-0.423	-0.478	(0.378)	(0.244)	(0.875)	-0.951	(0.596)	(0.252)	(0.453)
	-0.293 ± 0.340 (0.402)	-0.024 ± 0.116 (0.84)	0.342 ± 0.413 (-0.421)	0.130 ± 0.109 (-0.248)	-0.002 ± 0.003 (0.493)	-0.001 ± 0.001 (0.328)	0.014 ± 0.008 (-0.09)	0.090 ± 0.070 (-0.214)	0.212 ± 0.315 (-0.51)
PUFA ³	-0.386 ± 0.278 (0.185)	-0.113 ± 0.094 (0.248)	0.450 ± 0.338 (-0.203)	0.116 ± 0.091 (-0.224)	-0.005 ± 0.002 (0.015)	-0.0002 ± 0.001 (0.757)	0.0003 ± 0.007 (-0.965)	0.074 ± 0.059 (-0.231)	0.334 ± 0.256 (-0.213)
	MUFA ³	-0.362 ± 0.153 (0.031)	-0.111 ± 0.052 (0.052)	0.420 ± 0.188 (-0.041)	0.099 ± 0.052 (-0.077)	-0.002 ± 0.001 (0.142)	0.0002 ± 0.001 (-0.73)	0.001 ± 0.004 (0.845)	0.059 ± 0.035 (0.109)
n-3 FA ³		3.251 ± 3.334	1.210 ± 1.103	-3.383 ± 4.072	-0.527 ± 1.112	-0.007 ± 0.026	-0.013 ± 0.008	0.012 ± 0.086	-0.206 ± 0.723
	-0.345	-0.29	(0.419)	(0.643)	(0.790)	(0.129)	-0.894	(0.779)	(0.366)
PUFA ³ :SFA ³	-0.897 ± 4.843 (0.856)	-0.972 ± 1.597 (0.552)	0.878 ± 5.868 (-0.883)	-0.311 ± 1.578 (0.846)	-0.077 ± 0.031 (0.024)	0.0004 ± 0.012 (-0.977)	-0.144 ± 0.116 (0.233)	-0.213 ± 1.020 (0.837)	1.172 ± 4.434 (-0.795)
	TC ³ : SFA ³ Index	-0.027 ± 0.231 (0.908)	0.019 ± 0.077 (-0.806)	0.016 ± 0.280 (-0.955)	0.009 ± 0.075 (-0.902)	-0.001 ± 0.002 (0.683)	-0.0004 ± 0.0006 (0.538)	0.005 ± 0.006 (-0.428)	0.009 ± 0.049 (-0.848)

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¹**Parameter Estimate;** Mixed effects models was run with response being each of blood lipids, and the following predictors (explanatory variables) and expressed in parameter estimates for the “slope” (change in blood lipid measure that is associated with a 1 unit change in dietary fat intake, after accounting for type of diet, location, and subject-to-subject variability) and standard error (+) ; p-values are in parentheses and are significant at an overall alpha of 5% if the P<0.001, and at an overall alpha of 10% if P<0.002.

²Only tested at University 2.

³Abbreviations- MUFA=Monounsaturated Fatty Acids, PUFA=Polyunsaturated Fatty Acids, n-3 Fatty Acids= Omega-3, EPA=Eicosapentaenoic Acid, DHA= Docosahexaenoic Acid, n-6=omega-6, SFA=Saturated Fat, TC= Total Cholesterol.

⁴For purposes of comparison and the minute amounts measured, values for linoleic acid, alpha linolenic acid, eicosapentaenoic acid were not rounded to the nearest 10.

Table 5: Relationship of Dietary Lipids to Membrane Lipid Parameters for the “Slope” (Change in Blood Lipid Measure that is Associated with A 1-Unit Change in Dietary Fat Intake, After Accounting for Type of Diet, Location, and Subject-To-Subject Variability).

Blood Pressure and Body Composition

There were no significant differences in body composition (fat-free mass, water, fat mass) or blood pressure at baseline and end of treatment for either treatment (Table 6) and no significant differences in body composition using Idxa (Table 7).

There were no significant differences in BP at baseline and end of treatment for either treatment, or between campuses and the diet treatments (Table 6). This study did not confirm the findings of West et al. [26] in dyslipidemia, but does confirm the findings of Sheridan et al. in hypercholesterolemia, Wang, et al. in metabolic syndrome, and Sauder, et al. in type 2 diabetes [9,15,27]. Body weight and body composition values did not change over the 10-week period in both the Tanita Bioelectrical Impedence Analyses (BIA) (Table 6) and the iDXA analyses (Table 7). Gulati, et al. [16] had similar results in subjects with metabolic syndrome. An almond mastication study, found that the lower number of mastication chews, resulted in limited lipid bioavailability and higher fecal energy loss [34]. Even though our participants consumed more calories (about 210 kcals per day) during the pistachio treatment, they did not gain weight or change body composition. It is possible that the discrepancy measured energy value of pistachios in the human diet might be due to the pistachio lipids being poorly absorbed [35]. This study indicated a 5% inaccuracy in the Atwater calculations, which would have been 80.5 kcals less per day. However, during the pistachio treatment, the average increase of calories in the female participants was 210 kcals per day, which would still register about 130 extra kcals per day with the correction. Therefore, there must be other additional reasons or a combination of reasons why the consumed pistachios did not contribute to

participant weight gain. It is possible that the participants did not chew the nuts thoroughly, and this was the reason why body composition and weight did not change. It is also possible that our participants did not actually consume the nuts, but reported that they had consumed them or possibly changed their exercise patterns.

Treatment Diet by Visit					
Body Composition Factor ¹	Pistachio Baseline ²	Pistachio End ²	Control Baseline ²	Control End ²	P value
BMI	21.9 (0.06)	21.9 (0.07)	21.9 (0.07)	21.9 (0.07)	0.9270
Weight (Kg)	59.6 (0.26)	59.4 (0.26)	59.1 (0.26)	59.0 (0.26)	0.8485
WC³	71.9 (0.14)	72.1 (0.14)	72.4 (0.14)	72.1 (0.14)	0.3946
% Body Fat⁴	24.5 (0.25)	25.3 (0.26)	24.2 (0.26)	25.0 (0.26)	0.9658
Systolic BP	106 (1.72)	105 (1.77)	107 (1.75)	104 (1.77)	0.6099
Diastolic BP	67.0 (0.89)	66.1 (0.91)	69.1 (0.90)	65.3 (0.91)	0.1085
¹ Mixed Effects Model					
² Least Square Mean (Standard Error of The Mean)					
³ Waist Circumference					
⁴ Tanita BIA					

Table 6: Body Composition and Blood Pressure (BP) at Baseline and End of Treatment at Both Universities.

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Treatment Diets by Visit					
Body composition factor ^{1,2}	Pistachio Baseline ²	Pistachio End ²	Control Baseline ²	Control End ²	P value
Region Fat (%)	30.2 (0.28)	30.2 (0.29)	30.0 (0.29)	30.0 (0.30)	0.9053
Fat Mass (kg)	13.7 (0.45)	13.7 (0.46)	17.1 (0.47)	17.1 (0.49)	0.9729
Lean Mass (kg)	17.2 (0.32)	17.1 (0.34)	37.3 (0.34)	37.4 (0.35)	0.7528
Fat Free Mass(kg)	37.2 (0.32)	37.1 (0.33)	39.4 (0.34)	39.6 (0.35)	0.7511
Android (% fat)	28.0 (0.44)	27.9 (0.46)	27.8 (0.49)	27.5 (0.48)	0.7663
Gynoid (% fat)	36.7 (0.31)	36.3 (0.32)	36.2 (0.33)	36.0 (0.34)	0.7436
A/G ratio	0.77 (0.01)	0.77 (0.01)	0.77 (0.01)	0.76 (0.01)	0.3920

¹Mixed Effects Model; Least Square Mean (Standard Error of The Mean)
²Only Measured at University 1 (N=25)

Table 7: Body Composition Using iDXA Analysis at Baseline and End of Diet Treatment.

Micronutrient (Fe and Zn) Status

There were no significant differences between baseline and end diet treatment for serum iron or zinc and no significant differences between campuses for serum iron, or between the pistachio and non-pistachio (control) treatments for either iron or zinc (Table 8).

Pistachios contain soluble and insoluble fiber, which might interfere with micronutrient absorption, so there was a concern that in providing 20% kcals as pistachios, micronutrient absorption may be compromised. Since pistachios contain iron and zinc and both minerals increased in the diet in both treatments and were not significantly different between treatments; this increase may demonstrate that the fiber in pistachios did not interfere with absorption of dietary iron or zinc. More research (including fecal studies) and studies of longer duration are needed to determine if pistachios enhance absorption or do not interfere with iron or zinc absorption.

Diets by Visit					
Factor1	Pista- chio Baseline	Pistachio End	Control Baseline	Control End	P value
Iron (µg/dl)	98.5 (5.95)	106 (6.30)	108 (5.95)	112 (6.02)	0.7985
Zinc (µg/ dl) ²	81.0 (1.94)	84.6 (2.11)	79.1 (2.16)	85.8 (2.00)	0.4500

¹Mixed effects model; Least square mean (standard error of the mean)
²Only tested at University 1 (n=25)

Table 8: Micronutrient Status at Baseline and End of Diet Treatment.

Conclusion

Inclusion of 20% of kcals as pistachios in the diet does not contribute to weight gain, or body fat changes, blood lipids, BP, does not interfere with absorption of dietary iron or zinc, and significantly improves dietary nutrient intake. Therefore, we fail to reject the null hypotheses for changes in body composition, blood lipids, BP, dietary iron and zinc. However, we reject the null hypothesis for dietary nutrient intake, since dietary nutrient intake significantly improved in the pistachio treatment

More research is needed to determine why the higher caloric levels in the pistachio treatment (p=0.0012) did not result in increased body fat or body composition changes.

Declarations

Ethics approval and consent to participate: The Institutional Review Boards at both universities, California Polytechnic State University, San Luis Obispo (#13-052) and California State Polytechnic University, Pomona (#12-139) approved all procedures involving human participants. Written and signed informed consent was obtained from all participants prior to beginning the study and prior to filling out the screening questionnaire. Availability of data and materials: ClinicalTrials.gov #NCT02849392. Funding: American Pistachio Growers and a grant from California State University Agricultural Research Institute.

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