

A Macadamia Nut-Rich Diet Reduces Total and LDL-Cholesterol in Mildly Hypercholesterolemic Men and Women^{1,2}

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Abstract

Epidemiologic studies and clinical trials have demonstrated that the unique fatty acid profile of nuts beneficially affects serum lipids/lipoproteins, reducing cardiovascular disease (CVD) risk. Nuts are low in SFA and high in PUFA and monounsaturated fatty acids (MUFA). Macadamia nuts are a rich source of MUFA. A randomized, crossover, controlled feeding study (5-wk diet periods) compared a Macadamia nut-rich diet [42.5g (1.5 ounces)/8.79 MJ (2100 kcal)] [MAC; 33% total fat (7% SFA, 18% MUFA, 5% PUFA)] vs. an average American diet [AAD; 33% total fat (13% SFA, 11% MUFA, 5% PUFA)] on the lipid/lipoprotein profile of mildly hypercholesterolemic ($n = 25$; 15 female, 10 male) subjects. Serum concentrations of total cholesterol (TC) and LDL cholesterol (LDL-C) following the MAC (4.94 ± 0.17 mmol/L, 3.14 ± 0.14 mmol/L) were lower than the AAD (5.45 ± 0.17 mmol/L, 3.44 ± 0.14 mmol/L; $P < 0.05$). The serum non-HDL cholesterol (HDL-C) concentration and the ratios of TC:HDL-C and LDL-C:HDL-C were reduced following consumption of the MAC diet (3.83 ± 0.17 , 4.60 ± 0.24 , and 2.91 ± 0.17 , respectively) compared with the AAD (4.26 ± 0.17 , 4.89 ± 0.24 , and 3.09 ± 0.18 , respectively; $P < 0.05$). There was no change in serum triglyceride concentration. Thus, macadamia nuts can be included in a heart-healthy dietary pattern that reduces lipid/lipoprotein CVD risk factors. Nuts as an isocaloric substitute for high SFA foods increase the proportion of unsaturated fatty acids and decrease SFA, thereby lowering CVD risk. J. Nutr. 138: 761–767, 2008.

Introduction

Epidemiologic and clinical studies have demonstrated that an elevated LDL cholesterol (LDL-C)⁵ is a major cardiovascular disease (CVD) risk factor (1–4). Numerous randomized controlled clinical trials have reported reductions in CVD morbidity and mortality in response to reduced LDL-C concentration (5–7). Diet is the foundation for modifying lipid and lipoprotein risk factors for CVD. In addition to LDL-C, elevated triglyceride (TG) concentration and a low concentration of HDL cholesterol (HDL-C) also increase CVD risk (8,9). A diet low in saturated fat, trans fat, and cholesterol is recommended to reduce LDL-C concentration (4). Current dietary guidelines recommend 20–35%, or 25–35% of energy, from total fat (4,10). Specifically, a diet that is low in SFA (<10% and <7% energy), trans fat (<1% energy),

and dietary cholesterol (<300 mg/d and <200 mg/d, for those at risk for CVD) with 5–10% of energy from PUFA and up to 20% of energy from monounsaturated fatty acids (MUFA) (4,10,11). The National Cholesterol Education Program recommends therapeutic options to enhance lowering LDL-C concentrations in a Therapeutic Lifestyle Changes diet that includes plant sterols/stanols (2 g/d) and viscous fiber (10–25 g/d) for maximal LDL-C concentration lowering (4).

Nuts are a unique food in that they are low in SFA, rich in unsaturated fatty acids, and contain numerous bioactive compounds that beneficially affect CVD risk. Several major epidemiologic studies (12–14) and numerous clinical studies [reviewed in (15)] have demonstrated beneficial effects of nut consumption on coronary disease risk. The clinical studies have assessed the effects of different tree nuts, including walnuts, almonds, macadamia nuts, pecans, pistachios, and hazelnuts, utilizing various experimental designs in diverse population groups [reviewed in (14,15)].

Macadamia nuts are a rich source of MUFA and contain a high percentage of palmitoleic acid [16:1(n-7)]. Compared with the effects of palmitic acid and oleic acid, palmitoleic acid acted more like a SFA, as measured by increased LDL-C concentrations in hypercholesterolemic men (16). Macadamia nuts typically are eaten as a snack, and used in baking recipes (i.e. cookies), and various confectionary items (17). To date, 4 clinical trials have

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⁵ Abbreviations used: AAD, average American diet; CHO, carbohydrate; CVD, cardiovascular disease; GCRC, General Clinical Research Center; HDL-C, HDL cholesterol; IQR, interquartile range; LDL-C, LDL cholesterol; MAC, macadamia nut-rich diet; MUFA, monounsaturated fatty acid; SCD, stearoyl-CoA desaturase; TC, total cholesterol; TG, triglyceride.

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investigated the effect of consuming macadamia nuts on the lipid and lipoprotein profile (18–21). These studies used macadamia nuts to reduce the saturated fat in the diet by replacing it with the monounsaturated fats from macadamia nuts. The results of all 4 studies indicate that the supplementation and/or inclusion of macadamia nuts in a cholesterol-lowering diet significantly reduces LDL-C concentrations (4.0–10.7%). In addition, favorable reductions in TG concentrations (9.0–20.9%) also have been reported with diets rich in macadamia nuts vs. a habitual and low-fat diet, respectively (19,20). The few macadamia nut studies conducted to date have not evaluated the cholesterol-lowering effects of a “dose” that represents that advised in the qualified health claim for other nuts within the context of a contemporary blood cholesterol-lowering diet compared with an average American diet (AAD). Thus, the aim of this study was to evaluate the lipid and lipoprotein responses of a blood cholesterol-lowering diet that contained macadamia nuts using the serving size defined in the qualified health claim for tree nuts and peanuts [1.5 ounces (42.5 g)/8.79 MJ (2100 kcal)] vs. an AAD.

Methods and Materials

Subjects

Twenty-five moderately hypercholesterolemic males ($n = 10$) and females ($n = 15$) aged 25–65 y were recruited to participate. Subjects were reasonably healthy with no other major comorbidities. The eligibility criteria included: nonsmoker, BMI: 22–35 kg/m², LDL-C: 25–90th percentile NHANES (2.64–4.53 mmol/L), HDL-C: 10–90th percentile NHANES (0.88–1.79 mmol/L), and not on lipid-lowering medication or other medications known to affect lipid levels (subject characteristics in Table 1). Subjects were representative of the population in the U.S. that is at high risk for CVD. The Institutional Review Board at the Pennsylvania State University approved the experimental protocol and all subjects provided written informed consent before enrollment in the study.

Experimental design

A randomized, 2-period crossover design was employed. The current study was powered to detect a meaningful change in LDL-C. Data from a supplement trial by Garg et al. (18) indicated that the addition of macadamia nuts, representing 15% of the total energy intake (40–90 g/d), resulted in a significant decrease in total cholesterol (TC) (3.0%) and

LDL-C concentrations (5.3%) and a concurrent increase in HDL-C concentration (7.9%) in hypercholesterolemic men. We estimated the sample size needed to detect a meaningful change in LDL-C with our nutritional intervention to be 14 subjects, with α set to 0.05 and power set to 0.80. Based on these calculations, we sought to enroll 25 subjects. Given a 20% dropout rate, this was thought to provide an adequate sample size ($n = 20$) to detect a meaningful change in LDL-C. Subjects were recruited via advertisements in the local newspaper and fliers distributed across the campus of the Pennsylvania State University. During the initial screening, subjects were asked if they were allergic to nuts and if there were any foods that they could not eat. Subjects who reported an allergy to nuts or an aversion to consuming nuts were excluded from the study. Subjects who met the criteria during an initial phone screen reported to the General Clinical Research Center (GCRC) on the campus of the Pennsylvania State University for additional screening. At each screening, subjects completed a medical history form and an eating attitudes questionnaire and had their blood pressure and weight measured; blood was drawn for chemistry and lipid panels.

Prior to enrollment into the study, eligible subjects reported to the GCRC for baseline assessments, including weight, blood pressure, and a blood draw for outcome measurements. Subjects were randomly assigned to receive 1 of the 2 experimental diets during the first 5-wk period and the alternate diet during the next 5-wk period. Subjects consumed each diet in 2 separate 5-wk diet periods, which were separated by an approximate 2-wk compliance break, during which subjects consumed their usual diet.

Subjects consumed either breakfast or dinner at the Metabolic Diet Study Center on the campus of the Pennsylvania State University on Monday through Friday; lunches and weekend meals were prepared or packed for off-site consumption. Diet compliance, physical activity levels, and any medication changes were monitored by the staff and by the review of daily and weekly monitoring forms. Subjects' baseline body weights were maintained throughout the course of the study. Subjects were instructed to maintain their usual activities and exercise levels throughout the study.

Diet design

The macadamia nut-rich diet (MAC) was designed to include the amount of tree nuts, i.e. macadamia nuts (~1.5 ounces/d) that would be recommended based on the 2003 FDA Qualified Health Claim for subjects consuming 8.79 MJ (2100 kcal)/d (nutrient composition of macadamia nuts in Table 2). The macadamia nuts used in this study were roasted and one-half were salted and the other one-half were unsalted. The AAD was patterned after the typical American intake as detailed in the Continuing Survey of Food Intakes by Individuals and NHANES database and the 2 diets were matched for total fat, protein, and carbohydrate (CHO) profile in Table 3).

For the MAC diet, the macadamia nuts were incorporated into entrees and substituted for other foods and snacks. Examples of entrees that included macadamia nuts were: 1) chicken breast with vegetable rice “Mac Pao” (white rice, vegetables, unsalted, chopped macadamia nuts in an oriental sauce); 2) macadamia mango chicken salad; and 3) mixed greens salad with grilled chicken breast, apples, dried cranberries, unsalted macadamia nuts, and California salad dressing. Examples of snacks that included macadamia nuts were: 1) roasted, salted macadamia nuts; 2) cinnamon and sugar-spiced macadamia nuts; and 3) cranberry macadamia nut cookie. The menus were designed to include one-half of the portion of macadamia nuts from an entrée and one-half from a snack on a daily basis. Due to the incorporation of the macadamia nuts into the MAC diet, the AAD was comparably higher in SFA and slightly lower in dietary fiber. The higher fiber content of the MAC diet was primarily insoluble fiber. Differences in these nutrients represent the direct result of substituting macadamias for other foods in the control diet. This design allows for a direct evaluation of the contribution of macadamias nuts on the endpoints of interest, while controlling for intake of total fat, protein, energy, CHO, and cholesterol.

Menus were developed using Food Processor SQL software (ESHA Research) according to the guidelines listed above. All foods were prepared and provided to the patients following a 6-d menu cycle (sample menu in Table 4). Six different calorie levels were designed to achieve the maintenance of body weight across the range of energy needs within the

TABLE 1 Subject characteristics for all subjects and for men and women at initial screening prior to the start of the study¹

	All subjects	Men	Women
<i>n</i>	25	10	15
Age, y	50.2 ± 8.4	46.7 ± 10.3	52.5 ± 6.3
BMI, kg/m ²	26.3 ± 3.3	26.6 ± 3.6	26.1 ± 3.1
Waist circumference, ² cm	94.7 ± 8.4	95.3 ± 9.1	94.2 ± 8.1
Serum TC, mmol/L	5.40 ± 0.69	5.16 ± 0.61	5.56 ± 0.71
Serum LDL-C, mmol/L	3.46 ± 0.55	3.43 ± 0.46	3.48 ± 0.62
Serum HDL-C, mmol/L	1.32 ± 0.35	1.03 ± 0.16	1.52 ± 0.30*
Serum non-HDL-C, mmol/L	4.07 ± 0.64	4.12 ± 0.67	4.04 ± 0.65
Serum TG, mmol/L	1.33 ± 0.60	1.50 ± 0.72	1.22 ± 0.49
Serum TC:HDL-C	4.32 ± 1.10	5.11 ± 1.04	3.79 ± 0.79*
Serum LDL-C:HDL-C	2.80 ± 0.85	3.41 ± 0.76	2.39 ± 0.66*
Serum glucose, mmol/L	5.06 ± 0.53	4.98 ± 0.52	5.11 ± 0.55
Systolic blood pressure, mm Hg	120.3 ± 14.4	117.3 ± 13.5	122.3 ± 15.1
Diastolic blood pressure, mm Hg	78.5 ± 8.0	77.4 ± 8.0	79.3 ± 8.2

¹ Values are means ± SD. *Different from men, $P < 0.01$.

² Conversion factors: 1 inch = 2.54 cm, cholesterol, 1 mg/dL = 0.0259 mmol/L; TG, 1 mg/dL = 0.0113 mmol/L; glucose, 1 mg/dL = 0.0555 mmol/L.

TABLE 2 Nutrient composition of a serving of macadamia nuts

1.5 ounces (42.5 g) macadamia nuts	
Total energy, kJ (kcal)	1335 (319)
CHO, g (% energy)	6.2 (7.8)
Protein, g (% energy)	4.1 (5.1)
Total fat, g (% energy)	30.9 (87.2)
SFA, g (% energy)	5.0 (14.1)
12:0	0.02 (<0.1)
14:0	0.21 (0.6)
16:0	2.45 (6.9)
17:0	0.01 (<0.1)
18:0	1.22 (3.4)
20:0	1.03 (2.9)
22:0	0.31 (0.9)
24:0	0.13 (0.4)
MUFA, g (% energy)	24.0 (67.7)
16:1	4.71 (13.3)
18:1	19.09 (53.9)
20:1	0.91 (2.6)
22:1	0.10 (0.3)
PUFA, g (% energy)	0.71 (2.0)
18:2	0.67 (1.9)
18:3	0.04 (0.1)
Trans fat, g (% energy)	<0.1 (<0.1)
Total fiber, g	3.8
Insoluble fiber, g	3.8
Soluble fiber, g	<1.0
Cholesterol, mg	0
Calcium, mg	18.8
Iron, mg	1.0
Vitamin E, mg	<0.3
Magnesium, mg	48.1
Phosphorus, mg	87.4
Potassium, mg	150.3
Sodium, mg	4.6

subject population. Unit foods [419 kJ (100 kcal) each] that were compositionally identical to the experimental diets were used to adjust calorie levels so that subjects maintained body weight throughout the course of the study.

TABLE 3 Predicted nutrient composition of each of the 2 experimental diets¹

	AAD	MAC
CHO, % energy	50.0	52.0
Protein, % energy	19.0	17.0
Total fat, % energy	33.0	33.0
SFA	13.0	7.0
Palmitic acid (16:0)	6.0	3.5
Stearic acid (18:0)	2.7	1.5
MUFA	12.0	18.0
Palmitoleic acid (16:1)	0.4	2.5
Oleic acid (18:1)	10.0	14.2
PUFA	5.0	5.0
Fiber, ² g/2100 kcal	21.0	23.0
Cholesterol, ² mg/2100 kcal	290.0	280.0

¹ Based on Food Processor SQL database (esha Research, Salem, OR).

² 1 kcal = 4.185 kJ.

TABLE 4 One-day sample menu for each of the 2 experimental diets

AAD	MAC
Breakfast	
Orange juice	Orange juice
Plain bagel	Plain bagel
Deli ham	Deli ham
American cheese slice	Low-fat American cheese slice
2% milk	Skim milk
Lunch	
Deli roast beef	Deli roast beef
Tomato	Tomato
Low calorie mayonnaise	Fat-free mayonnaise
White bread	White bread
Apple	Apple
M&Ms	Pretzels
Dinner	
Spaghetti with meat sauce	Spaghetti with meat sauce
Parmesan cheese	Parmesan cheese
Green beans	Green beans
Dinner roll	Dinner roll
Margarine	Margarine
JELL-O chocolate pudding snack	Low-fat strawberry yogurt
Snacks	
Pretzel twists	1.5 ounces macadamia nuts
Cheddar cheese	Granola bar

Serum samples

Twelve-hour blood samples were taken from fasting subjects by venipuncture on 2 consecutive days at the beginning of the study (baseline) and at the end of each diet period. Blood was centrifuged at 1 × g; 15 min at -4°C. Serum samples were aliquoted and stored at -80°C until the conclusion of the study when all samples were analyzed together.

Serum fatty acids. Serum fatty acids were quantified according to a standard protocol (22). Briefly, liquid/liquid solvent extraction was performed and the lower chloroform phase was removed and dried under nitrogen. The dried residue was methylated and fatty acid methyl esters were extracted in hexane and injected into a Varian gas chromatograph where the fatty acids were separated on a 60M DB-23 capillary column. The fatty acids were quantified using an internal standard method. Serum concentrations of oleic (18:1), stearic (18:0), palmitoleic (16:1), and palmitic (16:0) acids were used to calculate 2 different desaturation indices (18:1/18:0 and 16:1/16:0) as an in vivo measure of stearoyl-CoA desaturase (SCD) activity.

Serum lipids and lipoproteins. Serum TC and TG concentrations were quantified using enzymatic assays (CHOP/PAP, Boeringer, Abbott Laboratories, Diagnostic Division) conducted at the Core Laboratory of the GCRC on the Hershey Medical Center's campus of the Pennsylvania State University. HDL-C was estimated according to the modified heparin-manganese precipitate procedure of Warnick and Albers (23). LDL-C concentrations were calculated by the Friedewald equation: LDL-C = TC - (HDL-C + TG/5) (24).

Statistical analyses

All statistical analyses were performed using SAS for Windows, release 9.1 (SAS Institute). The CV between d 1 and d 2 was calculated for each of the serum lipid and lipoprotein measurements. The interquartile range (IQR) was used to detect the presence of potential outliers based on both the levels of lipids and lipoproteins and the CV between d 1 and 2. The PROC UNIVARIATE statement in SAS was used to generate a boxplot and IQR for each of the variables at baseline. Observations that were outside of Q₁ - (1.5 × IQR) and Q₃ + (1.5 × IQR) were flagged as potential outliers; there were no outliers outside of the Q₁ - (3 × IQR) and

$Q_3 + (3 \times \text{IQR})$ range. All analyses were then completed without potential outliers to determine their impact. Final analyses represent the removal of the following number of data points for each of the lipid and lipoproteins: TC (6), LDL-C (5), HDL-C (3), and TG (3), including CVs that ranged from ~12 to 49%. The Shapiro-Wilk test of the residuals from the mixed model (PROC MIXED) was used to test for the normality of each variable. A W statistic > 0.90 indicated that the variable was normally distributed. Non-normally distributed variables were log-transformed to achieve normality. For the mixed models analysis, concentrations of serum TG were log-transformed. All analyses were performed on transformed values; all means reported represent unadjusted means.

The mixed models procedure (PROC MIXED) was used to test for effects of diet, gender, order of diet presentation, period, and their interactions on the levels of all outcome variables. Tukey-Kramer adjusted P -values < 0.05 were used to determine whether the differences in the outcome variables were significant. All of the P -values and least squares means that are presented were taken from the mixed model, including diet, gender, order, and the diet \times order interaction.

The plasma fatty acid ratios of 18:1/18:0 and 16:1/16:0 were calculated as an in vivo measure of SCD activity. Pearson correlations were performed both across all diets and within each diet to investigate possible relationships between the calculated ratios of fatty acids (18:1/18:0 and 16:1/16:0) and each of the outcome variables (i.e. TC, LDL-C, HDL-C, TG). Stepwise regression analysis was used to examine the relationship between calculated fatty acid ratios and serum TG concentrations. An increase in R^2 ($P < 0.05$) with the addition of a variable was considered significant in the regression equation. Values in the text are means \pm SE.

Results

Of the 25 individuals who started the study, 24 completed both diet periods. One subject did not complete the 2nd diet period due to time constraints; screening and diet period 1 data for this subject is included in the analyses (Table 1). The subjects represented a mildly hypercholesterolemic population with a TC concentration of 5.40 ± 0.69 mmol/L and LDL-C concentration of 3.46 ± 0.55 mmol/L. At screening, women had a higher concentration of HDL-C ($P < 0.01$) and lower ratios of TC:HDL-C and LDL-C:HDL-C ($P < 0.05$) than men (Table 1). Men and women did not differ for any of the measured endpoints at baseline.

Serum fatty acids. The changes from baseline in serum fatty acids following the 2 experimental diets reflected the predicted fatty acid compositions of the diets, indicating that participants were compliant with the study protocol (Table 5). Serum SFA were lower and MUFA were higher following consumption of the MAC diet compared with the AAD diet ($P < 0.05$). The serum PUFA concentration did not change.

Many of the individual fatty acids that are present in high concentrations in macadamia nuts (16:0, 18:0, 16:1) were present in higher concentrations in the serum following the MAC diet than the AAD diet ($P < 0.05$). The calculated ratio of 18:1/18:0 was higher following the MAC diet compared with baseline ($P < 0.001$); the ratio of 16:1/16:0 was greater following the MAC diet compared with both baseline and the AAD control diet ($P \leq 0.0001$).

Lipids and lipoproteins. The consumption of the macadamia nut-rich diet resulted in lower serum TC, LDL-C, and non-HDL-C concentrations compared with baseline and to after the AAD control diet period ($P < 0.0001$) (Table 6). The AAD also resulted in reduced LDL-C concentrations compared with baseline ($P < 0.01$). Serum TG concentrations were unchanged during the 2 experimental diets. The HDL-C concentration was lower following the MAC diet compared with both the AAD ($P < 0.001$)

TABLE 5 Fatty acid profile of serum total lipids in subjects at baseline and after consuming AAD and MAC diets for 5 wk each¹

	Baseline	AAD Diet	MAC Diet
SFA, mol %	28.29 \pm 0.41	28.94 \pm 0.42	27.20 \pm 0.41 ^a
16:0	20.37 \pm 0.33	20.73 \pm 0.34	19.43 \pm 0.33 ^{a,b}
18:0	6.14 \pm 0.10	6.15 \pm 0.10	5.87 \pm 0.10 ^a
20:0	0.24 \pm 0.05	0.35 \pm 0.05	0.40 \pm 0.05 ^b
MUFA, mol %	25.16 \pm 0.59	26.85 \pm 0.60 ^b	28.46 \pm 0.59 ^{a,b}
16:1	2.77 \pm 0.19	2.85 \pm 0.20	3.86 \pm 0.19 ^{a,b}
18:1	21.52 \pm 0.50	22.82 \pm 0.51 ^b	23.26 \pm 0.50 ^b
PUFA, mol %	46.55 \pm 0.78	44.23 \pm 0.80 ^b	44.35 \pm 0.78 ^b
(n-6)PUFA	43.26 \pm 0.77	41.16 \pm 0.79 ^b	41.12 \pm 0.77 ^b
(n-3)PUFA	3.29 \pm 0.14	3.05 \pm 0.14	3.22 \pm 0.14
18:1/18:0	3.54 \pm 0.12	3.73 \pm 0.12	4.00 \pm 0.12 ^b
16:1/16:0	0.13 \pm 0.01	0.14 \pm 0.01	0.20 \pm 0.01 ^{a,b}

¹ Values are least-squares means \pm SE, $n = 25$. ^aDifferent from AAD, $P < 0.05$;

^bDifferent from baseline, $P < 0.05$ (post hoc Tukey comparisons from multi-factor ANOVA).

and baseline ($P < 0.0001$). Compared with the AAD control diet, the MAC diet elicited a 9.4% reduction in TC concentration and a 8.9% reduction in LDL-C concentration. The ratios of TC:HDL-C and LDL-C:HDL-C were both lower following the consumption of the MAC diet than the AAD and baseline.

Correlations and stepwise regression between SCD ratios and lipids and lipoproteins. Calculated SCD ratios were correlated with concentrations of serum TG ($r = 0.48$; $P \leq 0.0001$) and HDL-C ($r = -0.42$; $P < 0.001$) across all diets (Table 7). The calculated SCD ratio was not correlated with serum TC or LDL-C concentration. The 16:1/16:0 and 18:1/18:0 ratios were correlated with serum TG concentrations across all diets ($r = 0.41$; $P < 0.001$). Correlations also are presented for baseline values and following each of the 2 experimental diets (Table 7). Regression analysis revealed a stronger predictive value for both calculated SCD ratios following consumption of the AAD diet (16:1/16:0, $R^2 = 0.40$; $P < 0.01$ and 18:1/18:0, $R^2 = 0.37$; $P < 0.01$) compared with the MAC diet (16:1/16:0, $R^2 = 0.16$; $P < 0.05$ and 18:1/18:0, $R^2 = 0.16$; $P < 0.05$). The ratio of serum 16:1/16:0 predicted 29% of the variance in TG at baseline ($P < 0.01$); 18:1/18:0 was not a significant predictor of serum TG concentrations at baseline.

TABLE 6 Serum lipids and lipoproteins in subjects at baseline and after consuming AAD and MAC diets for 5 wk each¹

Variable ²	Baseline	AAD	MAC
TC, mmol/L	5.66 \pm 0.17	5.45 \pm 0.17 ^b	4.94 \pm 0.17 ^{a,b}
LDL-C, mmol/L	3.68 \pm 0.14	3.44 \pm 0.14 ^b	3.14 \pm 0.14 ^{a,b}
HDL-C, mmol/L	1.24 \pm 0.05	1.20 \pm 0.05 ^b	1.11 \pm 0.05 ^{a,b}
TG, mmol/L	1.51 \pm 0.15	1.59 \pm 0.15	1.55 \pm 0.15
Non-HDL-C, mmol/L	4.41 \pm 0.17	4.26 \pm 0.17	3.83 \pm 0.17 ^{a,b}
TC:HDL-C	4.79 \pm 0.24	4.89 \pm 0.24	4.60 \pm 0.24 ^a
LDL-C:HDL-C	3.15 \pm 0.17	3.09 \pm 0.18	2.91 \pm 0.17 ^{a,b}

¹ Values are least-squares means \pm SE, $n = 25$. ^aDifferent from AAD, $P < 0.05$;

^bDifferent from baseline, $P < 0.05$ (post hoc Tukey comparisons from multi-factor ANOVA).

² Conversion factors: cholesterol, 1 mg/dL = 0.0259 mmol/L; TG, 1 mg/dL = 0.0113 mmol/L.

TABLE 7 Pearson correlations between calculated SCD ratios and lipid outcomes in subjects consuming AAD and MAC diets for 5 wk

	Serum TG		Serum HDL-C	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Across all diets				
18:1/18:0	0.41	<0.001	-0.45	≤0.0001
16:1/16:0	0.41	<0.001	-0.07	NS ¹
Baseline				
18:1/18:0	0.30	NS	-0.35	NS
16:1/16:0	0.54	<0.01	-0.01	NS
AAD diet				
18:1/18:0	0.61	<0.01	-0.59	<0.01
16:1/16:0	0.63	<0.01	-0.02	NS
MAC diet				
18:1/18:0	0.40	<0.05	-0.36	NS
16:1/16:0	0.40	<0.05	0.11	NS

¹ NS, *P* ≥ 0.05.

Discussion

The results of this study demonstrate that inclusion of 1.5 ounces of macadamia nuts in a cholesterol-lowering diet significantly reduces TC and LDL-C concentrations. Although other clinical nutrition studies have consistently shown that a nut/nut oil-containing diet low in saturated fat and cholesterol beneficially affects lipids and lipoproteins vs. the control diet (usually a low-fat diet or an average American/western diet), the use of macadamia nuts as the nut source has been limited (15). The cholesterol reduction observed in clinical studies of tree nuts is typically ~25% greater than would be expected from blood cholesterol-predictive equations that are based on diet fatty acid profiles (25). Likewise, in this study we observed a 48% greater total cholesterol and a 14% greater LDL-C lowering response on the macadamia nut diet than predicted from the blood cholesterol lowering equations developed by Mensink and Katan (26). It is clear that there are other bioactive factors beyond fatty acids in nuts, including macadamia nuts, that also contribute to their cholesterol-lowering properties. The lipid-lowering effects of the nut/nut oil diet have been established as a mechanism that accounts for some of the cardioprotective effects observed with nuts (27). The present study indicates that macadamia nuts may now be added to the database of foods that serve as a rich source of unsaturated fats in the diet that can be used to replace SFA in the diet.

The decreased LDL-C concentration in this study supports the results of prior clinical nutrition studies that have shown a similar reduction in LDL-C concentration with the consumption of macadamia nuts. The results of a supplement trial by Garg et al. (18) indicated that the addition of macadamia nuts, representing 15% of the total energy intake (40–90 g/d), significantly decreased TC (3.0%) and LDL-C concentrations (5.3%) and increased HDL-C concentration (7.9%) in hypercholesterolemic men. Emerging evidence indicates that other lipid parameters, such as non-HDL-C, may be a better predictor of CVD risk compared with LDL-C concentrations in individuals with hypercholesterolemia or diabetes (28,29). In the present study, the non-HDL-C concentration was significantly lower following the MAC diet compared with after the AAD diet and baseline, indicating that multiple lipid markers of CVD risk improved. In addition, the consumption of macadamia nuts reduced the LDL:HDL ratio from 3.7 to 3.3 and the ratio of TC:HDL from 5.4 to 4.9. The results of 3 controlled feeding studies also have

demonstrated an improvement in the lipid and lipoprotein profile with the incorporation of macadamia nuts into the diet.

In a study conducted by Colquhoun et al. (20), a macadamia-enriched diet (42% total fat) reduced concentrations of TC and LDL-C and maintained concentrations of HDL-C compared with the habitual diet (37% total fat). In a later study, Curb et al. (19) compared a macadamia nut based diet (37% total fat) to a “typical American” diet (37% total fat) and a “Step 1” diet (30% total fat). Both the macadamia-based diet and the Step 1 diet reduced TC (5%, 4%; *P* < 0.01), LDL-C (4%, 5%; *P* < 0.05), and HDL-C concentrations (4%; *P* < 0.01, 6%; *P* < 0.001), respectively. Although TG concentrations were higher with the Step 1 diet (8%; *P* < 0.05) compared with the typical American diet, the macadamia nut diet reduced TG concentrations (9%; *P* < 0.05). In a recent study, inclusion of 20 g/d of macadamia nuts in bread lowered LDL-C concentrations (~7%; *P* < 0.05) compared with baseline in a population of women with normal serum cholesterol concentrations (21).

As found by Curb et al. (19), the MAC diet reduced concentrations of HDL-C compared with the AAD and baseline assessments. In addition to its traditional role of raising TC and LDL-C concentrations, SFA has been shown to increase HDL-C concentrations as well. It is estimated that for every 1% increase in SFA, HDL-C concentrations will increase by 0.011–0.013 mmol/L (29–31). The reduced HDL-C concentrations in the present study is likely due to the decreased SFA during the MAC diet (7% energy) compared with the AAD (13% energy). Results of the serum fatty acid analyses confirmed these assumptions, because the lowest concentrations of serum SFA followed the MAC diet compared with both baseline and the AAD diet (Table 6). Despite a reduction in HDL-C concentrations after the MAC diet, the ratio of TC:HDL-C was significantly lower following the MAC diet compared with the AAD diet. Epidemiologic evidence suggests that for every 1 unit decrease in the TC:HDL-C ratio, there is a 53% decrease in the risk of myocardial infarction (32). In our study, the ratio of TC:HDL-C was lower following the MAC diet (4.62 ± 0.25) compared with after the AAD (4.95 ± 0.25) (*P* < 0.01), representing an estimated ~17% reduction in risk of myocardial infarction.

SCD is the enzyme responsible for the biosynthesis of oleic acid (18:1) and palmitoleic acid (16:1) in vivo. Oleate and palmitoleate are the major MUFA of membrane phospholipids, TG, wax esters, and cholesterol esters. The plasma fatty acid ratios of 18:1/18:0 and 16:1/16:0, called “desaturation indices,” have been used as an in vivo measure of SCD activity in humans (33). Although the role of SCD in human lipoprotein metabolism has not been extensively evaluated, a deficiency of the SCD1 gene in animals leads to very low concentrations of VLDL, suggesting that SCD1 may be an important regulator of the rate of in vivo VLDL production (34,35). In 1 human intervention study, an increase in the ratio of 18:1/18:0 was observed in individuals with increased TG following the consumption of a low-fat, high-CHO diet (61–65% energy from CHO) compared with those with reduced TG following the same diet (33). Within the same study, the ratio of 18:1/18:0 was positively correlated with concentrations of serum TG and inversely correlated with concentrations of HDL-C, explaining 53% of the variance in TG and 17% of the variance in HDL-C (33). Our study confirms these results. There was a significant positive correlation between the ratios of 18:1/18:0 and 16:1/16:0 and the concentrations of serum TG and a significant negative correlation between the ratio of 18:1/18:0 and serum HDL-C concentration. For each test diet, the calculated ratios of 18:1/18:0 and 16:1/16:0 predicted a greater percentage of the variance in serum TG concentration following the

AAD ($R^2 = 0.37$; $P < 0.01$ and $R^2 = 0.16$; $P < 0.05$) than the MAC diet ($R^2 = 0.40$; $P < 0.01$ and $R^2 = 0.16$; $P < 0.05$). Macadamia nuts are a rich source of MUFA with 56.5% of energy from oleic acid and 13.9% of energy from palmitoleic acid. It is possible that the increased 18:1/18:0 and 16:1/16:0 following the MAC diet are due to the higher concentrations of 18:1 and 16:1 being consumed. This suggests that when individuals consume high concentrations of MUFA, the ratios of 18:1/18:0 and 16:1/16:0 become slightly less accurate as in vivo markers of SCD activity. This may indicate that when diets high in MUFA are consumed, the calculated SCD ratios are more reflective of the dietary fats and are a less reliable marker of SCD activity. Thus, directly measuring SCD is necessary to make meaningful conclusions about 18:1 and 16:1 synthesis. This is particularly important when there are no diet effects on TG concentrations, as reported herein, as would be expected because total fat was similar in the test diets. Thus, the SCD ratio was more likely affected by intake of MUFA than changes in SCD activity.

A short, informal survey to assess the acceptability of the experimental diets was sent to participants after the conclusion of the study. The response rate from the survey was 72% (18 of 25 participants returned the survey). Participants were asked to rate each question on a 5-point Likert scale with 1 representing "disagree strongly" and 5 representing "agree strongly." Participants generally enjoyed having macadamia nuts as a part of their entrée, with acceptability scores for the entrees ranging from 3.5–4.7. The acceptability of macadamia nuts as a snack was higher, with scores ranging from 4.5–4.8 for all snacks. Throughout the study, energy intake was controlled to maintain subjects' body weight. The design of our study does not address the question of whether long-term weight control can be attained in free-living situations. The available data suggest that nut consumption is not associated with increased body weight (36).

The results of the present study indicate that the inclusion of 1.5 ounces/d of macadamia nuts reduces serum TC and LDL-C concentrations in hypercholesterolemic men and women when substituted for SFA in the diet. The reduction in LDL-C concentration was similar to that observed for other tree nuts, including walnuts and almonds. The relationships reported between the ratios of 18:1/18:0 and 16:1/16:0 and concentrations of serum TG provide insight into the utility of these calculated ratios as a marker of SCD activity when diets that are high in MUFA are consumed. This study suggests that an increase in these ratios may primarily reflect the dietary fats consumed, rather than be an accurate biomarker of SCD activity when a high MUFA diet is consumed, and reinforces the importance of directly measuring SCD activity.

In summary, this study adds to the growing evidence demonstrating beneficial effects of nuts on CVD lipid risk factors. Importantly, our data demonstrate that macadamia nuts can be part of the portfolio of nuts to recommend for inclusion in a heart healthy diet.

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