Original Research

Peanut Consumption Improves Indices of Cardiovascular Disease Risk in Healthy Adults

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Key words: peanuts, monounsaturated fatty acids, cardiovascular disease, triacylglycerol, magnesium, folate, homocysteine, humans

Background: Diets containing nuts reduce cardiovascular disease (CVD) risk factors. This has primarily been attributed to their fatty acid composition, but other constituents may also contribute. Peanuts, the most widely consumed 'nut' (actually a legume), are a rich source of monounsaturated fatty acids (MUFA), magnesium and folate, but their effects on CVD risk factors are poorly characterized.

Objective: This study determined the effects of chronic peanut consumption on diet composition as well as serum lipids, magnesium and homocysteine concentrations in free-living subjects under different conditions of peanut intake.

Design: Fifteen normolipidemic adults participated in a 30-week cross-over intervention. Subjects were provided 500 (±136) kcal as peanuts during an eight-week free feeding (FF) diet. The same amount of peanuts was added during a three-week addition (ADD) diet or replaced an equal amount of other fats in the diet during an eight-week substitution (SUB) diet.

Results: Energy intake from fat was increased through greater intake of MUFA and polyunsaturated fatty acids, while saturated fatty acid intake remained relatively stable under all conditions. Triacylglycerol (TAG) was reduced by 24% during ADD ($p < 0.05$), by 17% during SUB ($p < 0.05$) and by 14% during four-weeks of FF, but then rebounded to baseline by week 8. Dietary fiber, magnesium, folate, alpha tocopherol, copper and arginine increased during all treatments ($p < 0.05$). Serum magnesium increased in 13 of 15 subjects during FF ($p < 0.05$). No changes were found in total plasma homocysteine concentration.

Conclusions: Regular peanut consumption lowers serum TAG, augments consumption of nutrients associated with reduced CVD risk and increases serum magnesium concentration.

INTRODUCTION

Epidemiological evidence supports an inverse association between nut consumption and coronary heart disease (CHD) [1–3]. A lipid lowering effect has been documented in experimental studies with almonds, walnuts, pecans, pistachios and (although actually a legume) peanuts [4–13]. Nuts contain an array of healthful nutrients, but their lipid lowering property is primarily attributed to their fatty acid composition. They contain predominantly unsaturated fatty acids and, with the exception of walnuts, have a proportionately higher monounsaturated fatty acid (MUFA) than polyunsaturated fatty acid (PUFA) content. High MUFA diets, like Mediterranean diets, are associated with reduced cardiovascular disease (CVD) mortality [14]. The predominant dietary MUFA, oleic acid, is as effective as linoleic acid in lowering LDL cholesterol (LDL-C), but does not decrease HDL cholesterol (HDL-C) [15]. High MUFA diets also do not raise triacylglycerol (TAG) or decrease HDL lipoprotein concentrations as observed with low fat, high carbohydrate diets [16,17].

In the United States, the consumption of peanuts is greater than all the other nuts combined [18]. Yet only two human feeding studies have investigated the effects of peanut consumption on CVD risk. O'Byrne *et al.* [9] reported a decrease

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of total cholesterol and LDL-C in hypercholesteremic, postmenopausal women on a low total fat, low saturated fat (SFA) and high MUFA diet, achieved through inclusion of high oleic peanuts for six months. Kris-Etherton *et al.* [10] showed beneficial effects of conventional peanuts on blood lipid concentrations with a high MUFA, low SFA diet in normocholesteremic subjects. In both studies, MUFA intake was largely substituted for SFA intake. This raises the question of whether these shifts in lipid profiles were due to the increase of MUFA or reduction of SFA. In the present study a portion of the customary dietary fat was replaced by MUFA from peanuts without manipulating SFA intake in normolipidemic subjects. In a second arm of the study, a direct effect of dietary MUFA was explored by supplementing the participant's estimated customary diet with peanuts. Given the accumulating evidence from controlled feeding trials that nuts may hold health promoting effects, it must be determined if these will also hold in free-living individuals. On a self-selected diet, the peanuts may simply be added to the diet and increase energy and nutrient intake, be substituted for nutrients resulting in little net change or alter food choice in a way that decreases the intake of selected nutrients. The effects on serum lipids would likely vary. Therefore, a third arm investigated the effects of daily peanut consumption on diet composition and blood lipid profile, without any dietary restriction.

Peanuts are a rich source of magnesium (Mg), folate, fiber [19], alpha tocopherol [20,21], copper [22,23] and arginine [24,25] all of which hold CVD risk-reducing properties. Low serum Mg concentrations can increase risk of CVD [26,27] due, in part, to diminished lipoprotein lipase and lecithincholesterol acyltransferase (LCAT) activity which results in hyperlipidemia [28]. Mg infusion inhibits platelet aggregation and activity [29]. Dietary intake of Mg has declined [30] through reduced consumption of traditionally Mg-rich foods and increased losses during processing [31]. Current national average intake is only 75% of the recommended dietary allowance (RDA) [32]. Peanuts contain 170mg/100g of Mg (45% of the RDA) and lose little during processing. Thus, it was hypothesized that chronic peanut consumption would improve Mg intake and serum concentrations.

Homocysteine may impede the repair of endothelial cells, induce vascular smooth muscle cell proliferation [33] and act in a thrombogenic fashion [34]. High concentrations are considered an independent risk factor for CVD [35]. Supplementation with physiological quantities of folic acid can lower elevated plasma homocysteine [36]. Nuts are good sources of folate, and peanuts contain four times the amount of other nuts (240 μg/100 g or 60% of the RDA). It was hypothesized that regular peanut consumption would increase dietary folate intake and thereby decrease the plasma homocysteine concentration.

The present study did not assess the influence of peanut consumption on indices of fiber, alpha tocopherol, copper and arginine status, but did evaluate the impact of peanut consumption on dietary intake of these constituents.

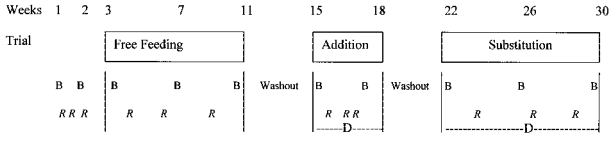
METHODS

Subjects

Seven non-pregnant or lactating female and eight male adults (33 $\pm$ 9 years of age) with no acute or chronic diseases and using no prescription medication that might have influenced study variables were recruited by public advertisement. They all were non-smokers and unrestrained eaters (score $<$ 14 on the three factor eating questionnaire [37]). Subjects were of normal weight for height (Body Mass Index (BMI) 23 $\pm$ 1.8 kg/m^2, body fat = 24.3 $\pm$ 8.5%) and had no recent history of weight gain or loss ($\pm$ 5 lbs within the prior three months). They were normolipidemic with a mean total cholesterol of 5.0 $\pm$ 0.5 mmol/L, LDL-C = 2.6 $\pm$ 0.9 mmol/L, HDL-C = 1.4 $\pm$ 0.2 mmol/L and TAG = 1.2 $\pm$ 0.6 mmol/L. They also had no family history (i.e., first degree relative) of CVD and were required to control the purchase and preparation of the majority of foods they consumed. According to the Northwest Lipid Research Clinic Fat Intake scale [38] and the Block food frequency questionnaire [39], subjects were selected with baseline dietary fat intakes similar to those of the US adult population (34% fat, 11.6% SFA, 12.6% MUFA and 7.1% PUFA of energy) [40]. Four subjects lost interest in the study and withdrew; one subject withdrew due to medical reasons unrelated to the study. The study protocol was approved by the Committee on the Use of Human Research Subjects at Purdue University and informed consent was obtained from study participants.

Experimental Design

A timeline of this 30-week cross over study is shown in Fig. 1. The three treatment arms were sequentially assigned so roughly equal numbers of participants were in each possible treatment order. In each case, dietary fat was supplied in the form of peanuts (Planter's Cocktail Peanuts, Planter's Co., Nabisco, Inc., Hanover, NJ). The free-feeding (FF) arm was an



Randomization of treatments

Treatment order	n	Baseline	Treatments		
1	3	B	FF	ADD	SUB
2	2	B	FF	SUB	ADD
3	2	B	ADD	FF	SUB
4	2	B	ADD	SUB	FF
5	3	B	SUB	FF	ADD
6	3	B	SUB	ADD	FF

B= Baseline, FF = Addition without kcal lock, ADD = Addition with kcal lock
SUB = Substitution with kcal lock & replace 50% of fat energy with peanuts

Fig. 1. Schematic diagram of study design. Only one possible treatment order is shown. Diet prescription occurred during addition and substitution (D). Blood draws (B) were carried out at baseline, pre-, mid- and post treatment. Diet recalls (R) were performed unannounced on random days on three separate occasions during baseline, free feeding, addition and substitution.

eight-week trial where about 50% of dietary fat energy was provided to participants with instructions to consume the nuts daily at any time and in any manner they chose. No additional dietary advice was provided. The addition (ADD) arm was a three-week trial where about 50% of dietary fat energy was added to a prescribed diet isocaloric to each participant's estimated customary intake. The substitution (SUB) arm was an eight-week trial where participants reduced fat intake by 50%, and this was replaced with an equivalent amount of fat from peanuts. Energy requirements were determined by applying an individual activity factor to measured resting energy expenditure [41]. Wash out periods were approximately four weeks between each intervention arm of the study.

Diets

During ADD and SUB, diets were prescribed according to the American Dietetic Association Exchange list system [42]. Each participant received an individualized meal plan and an exchange booklet as a reference manual specifying portion sizes for exchanges. The ADD diet supplied 34% energy from fat, 49% from carbohydrate and 17% from protein, yielding 41% energy from fat after including the test peanuts. The SUB diet required subjects to follow a low fat diet based on 17% energy from fat, 49% from carbohydrate and 17% from protein, yielding 34% energy from fat after including the fat from peanuts. The mean ration of peanuts amounted to 89 $\pm$ 21 g/day (2113 $\pm$ 494 kJ/day). Daily peanut rations were distributed pre-weighed and numbered every three to four weeks.

Dietary Assessment

Dietary intake was assessed by random unannounced telephone interviews eliciting intake information over the previous 24 hours. Prior to the first telephone interview, participants were trained with food models to estimate portion sizes. Subjects were contacted on three separate occasions during baseline and each treatment period. Random day samples of dietary intake have been reported to offer a better estimate of customary intake than consecutive-day samples [43].

Resting Energy Expenditure

Resting energy expenditure was measured by indirect calorimetry using a metabolic cart (Sensormedics Vmax 29, SensorMedics Corporation, Yorba Linda, CA) and a ventilated respiratory canopy. After an overnight fast, subjects arrived in the laboratory, rested for ten minutes, and measurements were performed in the supine position for 45 minutes. Energy expenditure was calculated based on the Weir equation [44].

Blood Sampling and Assay

After a ten-hour overnight fast, 15 mL of blood was collected into SST vacutainers, centrifuged after clot formation and separated. This was repeated twice during baseline, before and after FF, ADD and SUB as well as during week 4 of FF and SUB. Diet induced lipid changes of the magnitude expected are measurable within two weeks [45]. Serum samples were analyzed in duplicate for total cholesterol, HDL-C, LDL-C and TAG using an automated sample analyzer (COBAS MIRA Plus, Roche Diagnostic Systems, Branchburg, NJ). Total serum Mg was determined by an enzymatic-colorimetric technique (Sigma Diagnostics, St. Louis MO, USA) at baseline (week 1 and 2) and three times during FF and SUB. Another 5 mL fasting blood sample was collected into vacutainers with EDTA at baseline and twice during FF and SUB for homocysteine analysis. Total plasma homocysteine was measured by a two-step enzyme-linked immunosorbent assay (ELISA) method using a primary antibody to homocysteine (Bio-Rad Laboratories, Fullerton, CA).

Compliance-Erythrocyte Membrane Fatty Acid Analysis

Peanut consumption was confirmed by contrasting erythrocyte membrane fatty acid composition at baseline and after the eight-week SUB treatment. Changes in erythrocyte membrane fatty acids are a sensitive indicator for recent diet [46]. A 5 mL fasting blood sample was collected into vacutainers with EDTA, the erythrocyte fraction was separated from plasma by centrifugation and frozen at -40°C. Erythrocyte membranes were prepared by hemolyzing the cells twice in deionized distilled water followed by centrifugation at 3,000 *g* for ten minutes at 4°C. The method of Lepage and Roy [47] was used to methylate the fatty acids. Fatty acid composition was determined by gas liquid chromatography using a 50 m capillary column with 0.25 mm inner diameter (CP-Sil 88, Varian Analytical Inst., Walnut Creek, CA). The temperature of the oven was 150°C for eight minutes and rose at the rate of 4°C/minute to reach a final temperature of 200°C until the analysis was completed. The temperature of the injection port and the flame ionization detector was 300°C. Nitrogen was used as the carrier gas. Peaks were identified relative to authentic standards obtained from Supelco (Bellfonte, PA) and Nu-Check-Prep (Elysian, MN). The areas under the peaks were measured by integration (Shimadzu, Columbia, MD). Data are expressed as percentages of total fatty acids.

Statistics

Treatment effects were tested by one-way repeated measures analysis of variance (ANOVA). Paired *t* tests were used for *post hoc* analyses. Data were pooled due to the lack of a gender effect assessed by two factor repeated measures ANOVA. Pearson partial correlation coefficients were computed to assess the relationships between serum Mg and lipid concentrations. As a washout period of greater than four weeks is required for erythrocyte membrane fatty acids to return to baseline, only a sub-sample of six subjects who underwent SUB as their first treatment was used for statistical analysis.

The criterion for significance was set at $p < 0.05$. Statistical analyses were performed with the SPSS software package release 10.0.5 (SPSS Inc. Chicago, IL).

RESULTS

Energy and Macronutrient Intake

Mean daily nutrient intakes are shown in Table 1 [48]. Total energy intake did not differ significantly between baseline and treatment periods. Energy intake from fat increased significantly from baseline during FF ($p < 0.01$), ADD ($p < 0.001$) and SUB ($p < 0.05$). MUFA and PUFA intake increased significantly during FF, ADD and SUB (all $p < 0.01$). SFA intake decreased from 11% to 8% during SUB ($p < 0.05$). Non fatty acid lipid material, such as glycerol or sterols, were not significantly different between treatments [48]. Nutrient comparison, with and without inclusion of peanuts during FF, reveals peanut consumption increased energy intake from fat, MUFA and PUFA (all $p < 0.01$) but not SFA (Table 1). The nutrient composition of peanuts is shown in Table 2.

Serum Lipids

Serum TAG (Fig. 2) and cholesterol concentrations during FF, ADD and SUB are presented in Table 3. During FF, there was a non-significant 14% reduction from pretreatment to week 4 ($F = 2.592$, $p = 0.093$), followed by a rebound to baseline at week 8. Mean serum TAG decreased by 24% from pretreatment to week 3 during ADD ($p < 0.05$). The TAG concentration declined by 18% from pretreatment to week 4 ($p < 0.05$) and remained 17% lower at week 8 ($p < 0.05$) during SUB. There was a trend for the LDL-C/HDL-C ratio to decline from 1.9 to 1.6 after the first four weeks of FF ($F = 2.884$, $p = 0.073$), then the ratio returned to pretreatment values by week 8. No significant changes were found throughout ADD or SUB for LDL-C/HDL-C ratios. The mean total serum cholesterol concentration did not change significantly during any of the three treatments.

Magnesium

Repeated measures ANOVA revealed a significant increase of dietary Mg intake across treatments ($p < 0.001$) (Table 1). Dietary Mg increased significantly from baseline during FF ($p < 0.001$), ADD ($p < 0.001$) and SUB ($p < 0.001$). The increase was 49% for FF, 67% for ADD and 57% for SUB. Individual and mean changes in fasting serum Mg concentrations during FF are presented in Fig. 3. Increases were observed in 13 of 15 participants. Mean fasting values increased significantly from 0.88 mmol/L at week 0 to 0.96 mmol/L at week 8 ($p < 0.05$) and from 0.90 mmol/L at week 4 to 0.96 mmol/L at week 8 ($p < 0.05$). During SUB, the mean fasting serum Mg concentration increased from 0.89 mmol/L to 0.93 mmol/L to 0.95 mmol/L at weeks 0, 4 and 8, respectively. This change was not statistically significant. Changes of serum Mg were significantly negatively correlated with reductions of total cholesterol ($r = -0.59$, $p < 0.05$), serum TAG ($r = -0.57$, $p < 0.05$), and HDL-cholesterol ($r = -0.64$, $p < 0.05$) when adjusted for unsaturated fatty acid intake (experimentally manipulated through provision of peanuts) during FF.

Table 1. Mean Daily Nutrient Intakes from Three Random-Day 24 Hour Recalls

	Baseline	FF with Peanuts	FF without Peanuts	ADD	SUB
Energy (kJ/day)	9570 ± 650	10300 ± 680[a]	8200 ± 590[b]	10490 ± 700	9600 ± 640
(kcal/day)	2290 ± 150	2460 ± 160[a]	1960 ± 140[b]	2510 ± 170	2300 ± 150
Fat (% energy)	30.8 ± 1.9[a]	38.8 ± 1.2[b,c,a]	22.5 ± 1.2[b]	38.8 ± 1.6[b,c]	34.9 ± 1.6[b,d]
SFA	10.6 ± 0.9[a]	10.3 ± 0.6[a]	10.2 ± 0.7	9.9 ± 0.6[a]	8.13 ± 0.5[b]
MUFA	9.2 ± 0.8[a]	13.4 ± 0.7[b,a]	6.8 ± 0.9[b]	14.7 ± 1.1[b]	12.9 ± 1.6[b]
PUFA	4.5 ± 0.4[a]	7.9 ± 0.5[b,a]	3.5 ± 0.6[b]	8.4 ± 0.6[b]	8.8 ± 0.5[b]
NFA Lipid Material	6.4 ± 1.1	7.1 ± 1.0	8.0 ± 1.3	5.7 ± 1.0	5.0 ± 0.6
Cholesterol (mg/day)	196.8 ± 23.6	177.6 ± 23.3	177.6 ± 23.3	213.1 ± 23.1	173.3 ± 22.6
Protein (% energy)	13.7 ± 0.8[a]	15.1 ± 0.7[c,a]	11.3 ± 0.7[b]	17.0 ± 0.4[b,d]	16.6 ± 0.6[b,d]
CHO (% energy)	55.8 ± 2.1[a]	48.1 ± 1.4[b,a]	45.7 ± 1.4[b]	46.9 ± 1.7[b,c]	51.0 ± 1.4[b,d]
Dietary fiber, g/day	18.37 ± 2.2[a]	26.29 ± 2.6[b,a]	18.7 ± 2.3[b]	29.08 ± 2.4[b]	28.4 ± 2.7[b]
α-TE (mg/day)	5.87 ± 0.8[a]	12.59 ± 1.0[b,c,a]	4.45 ± 0.9[b]	15.58 ± 1.9[b]	15.31 ± 1.4[b,d]
Folate (mcg/day)	318.5 ± 27[a]	458.8 ± 39[b,c,a]	244.9 ± 30.6[b]	565.2 ± 50[b,d]	563.15 ± 46[b,d]
Magnesium (mg/day)	260.1 ± 29[a]	387.8 ± 34[b,a]	238.0 ± 27.3[b]	433.7 ± 34[b]	408.2 ± 36[b]
Copper (mg/day)	1.06 ± 0.13[a]	1.88 ± 0.16[b,c,a]	0.6 ± 0.1[b]	2.17 ± 0.18[b,d]	2.02 ± 0.17[b]
Lysine/Arginine	1.25 ± 0.09[a]	0.81 ± 0.08[b,a]	1.29 ± 0.06[b]	0.89 ± 0.04[b]	0.76 ± 0.04[b]

Values are mean ± SEM, n = 15.

Means with different letters are statistically significant ($p < 0.05$).

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, NFA = non fatty acid lipid material such as glycerol or sterols [48], CHO = carbohydrate, α-TE = alpha tocopherol equivalents, FF = free feeding, ADD = addition, SUB = substitution.

Table 2. Nutrient Composition of 500 kcal of Peanuts

Fat (g)	43.9 ± 2.6
SFA	6.1 ± 0.4
MUFA	21.8 ± 1.3
PUFA	13.9 ± 0.8
Protein (g)	23.0 ± 1.4
CHO (g)	14.4 ± 0.9
Dietary Fiber (g)	7.6 ± 0.5
α-TE (mg)	7.5 ± 0.4
Folate (mcg)	213.9 ± 13
Magnesium (mg)	149.8 ± 9
Copper (mg)	1.3 ± 0.1
Arginine (mg)	2752 ± 164

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, CHO = carbohydrate, α-TE = alpha tocopherol equivalents.

Folate and Homocysteine

Mean dietary folate increased by 44% from baseline during FF, by 78% during ADD and by 77% during SUB (all $p < 0.001$) (Table 1). Folate intakes were significantly higher during ADD ($p < 0.05$) and SUB ($p < 0.05$) when compared to FF. The mean total plasma homocysteine concentration was 6.6 μmol/L at baseline and ranged from 3–10 μmol/L. No significant changes were observed during FF or SUB (data not shown).

Micronutrient Intake and Fiber

Dietary intakes of fiber, alpha tocopherol and copper increased and the ratio of lysine to arginine decreased significantly from baseline in all treatments (all $p < 0.05$) (Table 1). During FF, these changes can be attributed to inclusion of peanuts (all $p < 0.01$) (Table 1).

Peanut consumption did not lead to significant changes of intake from the starch, fruit, other carbohydrate, milk, vegetable and meat food groups of participant's customary diet. However, participants decreased their consumption of fat exchanges ($p < 0.01$) during FF (data not shown).

Erythrocyte Membrane Fatty Acids

The mean percentage of SFA in erythrocyte membranes decreased significantly from 41.0% at baseline to 36.4% at the end of SUB ($p < 0.05$, n = 6). During the same time period, the mean percentage of PUFA increased significantly from 33.5% to 40.6% ($p < 0.05$, n = 6). Differences in MUFA were not statistically significant. The ratio of unsaturated fatty acids to SFA increased significantly from 1.45 to 1.76 ($p < 0.05$, n = 6).

DISCUSSION

Compliance

The results of the erythrocyte membrane fatty acid analysis support adherence to the dietary intervention. Among the six participants evaluated, an increased ratio of unsaturated to SFA in erythrocyte membranes was predicted and observed. The lack of an independent increase in MUFA may be explained by the high baseline erythrocyte MUFA concentrations [49] in our subjects.

Previous studies with peanuts reported lipid lowering effects when MUFA were substituted for SFA at a level of 8% [10] and 6% [9]. In this study, the intent during SUB was to increase MUFA without markedly altering SFA by replacing 50% of the customary dietary fat with the fat from peanuts. MUFA and PUFA intakes increased significantly, by about 4% each. Total fat intake increased by 4%, while estimated SFA intake decreased by about 2%. This magnitude of change in SFA has not been associated with total or LDL-C lowering effects [50]. During FF and ADD, energy from total dietary fat increased significantly from 31% to 39% by increasing MUFA and PUFA intake while SFA remained stable at baseline levels. Subjects predominantly compensated for the increased fat intake by decreasing carbohydrate intake. During ADD, subjects spontaneously commented on the high satiety value of the nuts and reported difficulties with consumption of the prescribed diets; hence, relative to baseline the addition was only 50% of predicted intake.

Lipids

The serum TAG lowering effect during SUB and ADD (17% and 24%) was slightly greater than previously reported with peanuts (13%) [10]. Replacement of carbohydrates by fat decreases serum TAG [51] and the reduction of carbohydrate was greater in our study (5% during SUB and 9% during ADD) compared to an earlier study involving peanuts (3%) [10]. During FF there was a trend for lower TAG at 4 weeks, but the concentration rebounded by week 8. The slightly smaller effect observed during FF may be partly explained by the lower serum TAG concentration prior to FF compared with ADD and SUB. According to Austin *et al.* [52] a 1 mmol/L decrease in

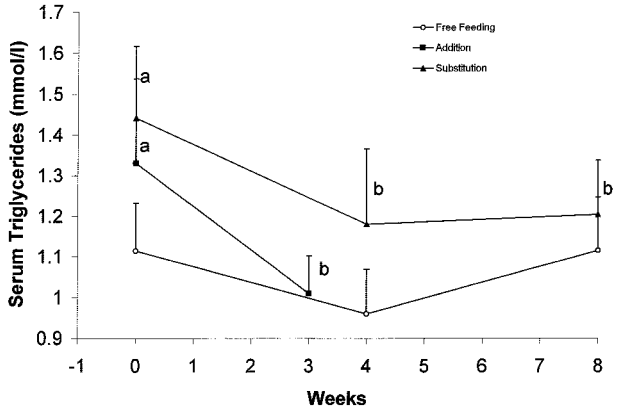


Fig. 2. Mean fasting serum triacylglycerol concentrations during free feeding, substitution and supplementation treatments (n = 15). Means with different letters are statistically significant ($p < 0.05$).

Table 3. Mean Serum Lipid Concentrations during Free-Feeding, Addition and Substitution

	PreFF	PostFF	PreADD	PostADD	PreSUB	PostSUB
Total Cholesterol (mmoles/L)	4.7 ± 0.4	5.0 ± 0.7	5.0 ± 0.5	4.9 ± 0.6	5.1 ± 0.5	4.9 ± 0.6
LDL Cholesterol (mmoles/L)	2.5 ± 0.8	2.7 ± 0.9	2.6 ± 0.7	2.6 ± 0.8	2.6 ± 0.7	2.5 ± 0.7
HDL-Cholesterol (mmoles/L)	1.3 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	1.4 ± 0.3	1.4 ± 0.3
Triacylglycerol (mmoles/L)	1.1 ± 0.5	1.1 ± 0.5	1.3 ± 0.8[a]	1.0 ± 0.4[b]	1.4 ± 0.7[a]	1.2 ± 0.5[b]

Values are mean ± SEM, n = 15.

Means with different letters are statistically significant ($p < 0.05$).

PreFF = baseline of free feeding, Post FF = after eight weeks of free feeding, PreADD = baseline of addition, PostADD = after three weeks of addition, PreSUB = baseline of substitution, PostSUB = after eight weeks of substitution.

TAG is associated with a 25% reduction in CVD risk. Assuming a linear association, the decrease of TAG during ADD (0.32 mmol/L) and SUB (0.24 mmol/L) could be expected to decrease the risk from CVD by 8% and 6%, respectively.

Previously, reductions of serum cholesterol were observed when SFA were replaced with MUFA from peanuts [9,10]. In one study, replacement of a diet containing 16% energy from SFA with a high MUFA peanut diet containing 7% of energy from SFA, total and LDL-C were lowered while HDL-C was preserved in normocholesteremic subjects [10]. In another study, a decline in total, LDL-C and HDL-C was shown in hypercholesteremic postmenopausal women who consumed a low-fat, high MUFA diet. The diet included high oleic peanuts and decreased the participant's SFA intake from 11% to 5% of energy [9]. Studies with tree nuts such as walnuts, pistachios and almonds that replaced SFA with MUFA report similar changes in blood cholesterol parameters [4,8,11,12]. In the present study no decrease in HDL was expected or observed.

Earlier studies reported changes in total and LDL-C that were not replicated here. The LDL-C lowering effect may largely be attributable to the change in SFA intake. This has been demonstrated in a study where decreases in total and LDL-C occurred when reductions of total fat (37% to 30% of energy) and SFA (16% to 9%) occurred without a change of MUFA [50]. However, when SFA intake is sustained at low (6% of energy) levels, an increase of MUFA to very high levels (25% of energy), achieved with almond consumption has been shown to decrease LDL-C in hypercholesteremic subjects [7]. A study with pecans showed decreased total and LDL-C levels with similar increases in MUFA (22.7% of energy) and comparable SFA intakes to our study. However, that same study also found increased total and LDL-C concentrations with decreased SFA intakes in their control group [13].

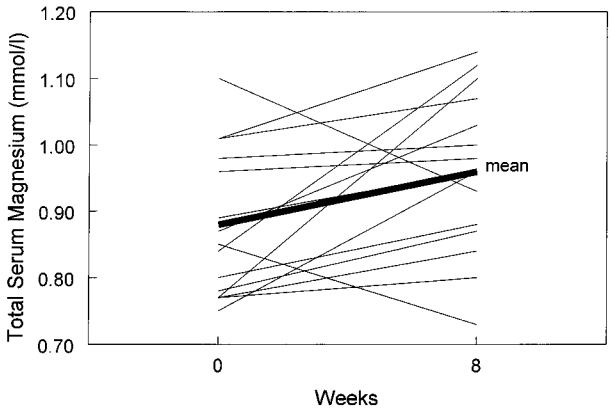


Fig. 3. Individual and mean changes in fasting serum concentration of magnesium in 15 healthy subjects who consumed 500 kcal of peanuts daily over eight weeks without restriction of the background diet.

Magnesium

The baseline Mg intake of our subjects corresponded to 70% of the RDA and increased during FF, ADD and SUB to 105%, 117% and 110%, respectively. Baseline serum Mg concentrations were within the normal range (0.75 to 0.95 mmol/L) [53] but increased with peanut consumption. Even though values remained within the normal range, the increase may be desirable since the serum Mg concentration required to lower CVD risk is higher than the cut off point to prevent magnesium depletion. Risk of CVD increases with Mg concentrations below 0.81 mmol/L [27]. During FF, each of the six subjects with serum Mg concentrations below 0.81 mmol/L improved their status. During SUB, this was observed in three out of five individuals. Therefore, peanut consumption may be an effective way to increase Mg status and thereby reduce CVD risk.

Despite a significant increase in serum Mg during FF, only a transient change in TAG was observed. This may be due to the smaller magnitude of supplementation in our study (150 mg/day) compared to other studies that found significant effects on serum lipids [54,55].

Plasma Homocysteine and Dietary Folate

Results from a meta-analysis show elevated plasma homocysteine concentrations are an independent risk factor for CVD

[35]. Low folate status is frequently responsible for elevated homocysteine concentrations [36]. Peanuts are a rich source of folate (100 g contain 240 μg or 60% of the RDA) and in the present study, dietary folate intakes increased significantly from 80% of the RDA [56] to 114% and 140% during FF and SUB, respectively. However, no changes were observed for total plasma homocysteine during these treatments. Ward showed significant effects of folic acid supplementation only for the high (10.9 μmol/L) and middle tertiles (9.11 μmol/L) of baseline total plasma homocysteine, but not for the lowest (7.07 μmol/L) [36]. The mean baseline concentration of plasma homocysteine in our subjects was 6.3 μmol/L which is lower than in other studies reporting beneficial effects of folate on homocysteine concentrations [57,58]. Nevertheless, the results of the present study illustrate that dietary folate intake can be increased with incorporation of peanuts into the diet.

Peanuts are also a good source of alpha tocopherol, copper, arginine and fiber, all nutrients with CVD risk-reducing properties. Vitamin E intake has been negatively associated with CVD risk, possibly due to its antioxidant properties rendering LDL less susceptible to oxidation [20,21]. Copper supplementation can increase lipoprotein oxidation lag times [22] and factor VIII, a procoagulant, is increased during copper deficiency [59]. Arginine is a dietary precursor for nitric oxide, a tissue relaxant synthesized by vascular endothelial cells [24]. In cholesterol fed rabbits, dietary arginine leads to a comparable regression of preexisting atheromatous lesions as Lovastatin [25]. A meta-analysis shows an inverse association between dietary fiber intake and CVD [19]. According to the Continuing Survey of Food Intake by Individuals (CSFII), peanut users achieve higher micronutrient and fiber intakes compared to non-users [60]. The findings of this study further demonstrate that regular peanut consumption without other dietary advice increases dietary intake of vitamin E, copper, arginine and fiber. It is important to note that participants accommodated the nuts by decreasing the consumption of fat exchanges but did not alter intake from other food exchange groups.

Taken together, a decrease in serum TAG concentration was observed when MUFA and PUFA were increased without reducing SFA intake. This effect was most pronounced and sustained when peanuts were incorporated into an energy-balanced diet. A moderate increase in MUFA without a concomitant decrease in SFA does not appear sufficient to decrease total and LDL-C in normocholesteremic individuals. Further, this study demonstrates that peanut consumption provides beneficial effects on diet composition even when the background diet is not controlled. Dietary folate, magnesium, alpha tocopherol, copper, arginine and fiber intakes increased and serum magnesium concentration improved with incorporation of the nuts. This study provides further evidence that regular peanut consumption can lead to dietary and biochemical changes associated with reduced CVD risk.

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