

Snack chips fried in corn oil alleviate cardiovascular disease risk factors when substituted for low-fat or high-fat snacks¹⁻³

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ABSTRACT

Background: The perception that all high-fat snacks are unhealthy may be wrong.

Objective: We aimed to assess whether replacing low-fat and high-fat snacks with snacks rich in polyunsaturated fatty acids (PUFAs) and low in saturated and *trans* fatty acids would improve cardiovascular health.

Design: Thirty-three adults participated in a randomized crossover trial of 3 controlled feeding phases of 25 d each in which a different type of snack was provided: low-fat (30.8% of energy from fat, 5.2% of energy from PUFAs), high-PUFA (36.3% of energy from fat, 9.7% of energy from PUFAs), or high-fat (37.9% of energy from fat, 5.8% of energy from PUFAs) snack.

Results: Each diet reduced LDL- and total cholesterol concentrations, but reductions were greater with the low-fat and the high-PUFA diets than with the high-fat diet: LDL cholesterol (11.8% and 12.5% compared with 8.8%, respectively; $P = 0.03$ and 0.01), total cholesterol (10.5% and 10.7% compared with 7.9%, respectively; $P = 0.03$ and 0.02). The high-PUFA diet tended to reduce triacylglycerol concentrations (9.4%; $P = 0.06$), and this change was greater than that with the low-fat ($P = 0.028$) and high-fat ($P = 0.0008$) diets.

Conclusions: These data show that snack type affects cardiovascular health. Consuming snack chips rich in PUFA and low in saturated or *trans* fatty acids instead of high-saturated fatty acid and *trans* fatty acid or low-fat snacks leads to improvements in lipid profiles concordant with reductions in cardiovascular disease risk. *Am J Clin Nutr* 2007;85:1503–10.

KEY WORDS Snacks, polyunsaturated fat, *trans* fat, saturated fat, cholesterol, cardiovascular disease, corn oil

INTRODUCTION

In the past, emphasis was placed on adopting a low-fat diet because such a diet had been shown to effectively reduce total and LDL cholesterol (1, 2). However, low-fat diets may lead to reductions in HDL-cholesterol (2–4) and increases in triacylglycerol (5) concentrations. More recently, research suggests that the type of dietary fat, more than total fat, affects cardiovascular disease (CVD) risk. In fact, from the Nurses' Health Study, it was estimated that replacing 5% of calories from saturated fatty acids or 2% of calories from *trans* fats with equivalent portions of polyunsaturated fatty acids (PUFAs) would markedly reduce CVD risk (6). Similar estimated reductions in CVD risk were reported when polyunsaturated fats replaced 5% of energy from

carbohydrates in the diet (6). In addition, recent data from the Women's Health Initiative show that attempts to reduce dietary fat intake to <30% of total energy intake did not lead to significantly lower rates of coronary events, stroke, or CVD (7). Moreover, the authors suggested that an increase in intakes of PUFAs, rather than a decreased intake of total fat irrespective of the type of fat, may have incurred cardiovascular benefits.

Therefore, recommendations for ways to introduce healthy fats into the diet should be made. Some high-fat foods that have traditionally been advocated for their healthy fatty acid profile include nuts and avocados. High-fat dairy products typically contain a large quantity of saturated fats, and high-fat sweets and snack foods generally have a large amount of saturated and *trans* fats. These snack foods are often considered unhealthy, and recommendations are often made to limit their consumption, mainly as part of an effort to control body weight but also to avoid saturated and *trans* fats (8). However, frying foods in vegetable oils such as corn, canola, or sunflower oil, all of which are rich in polyunsaturated and monounsaturated fat, low in saturated fat, and free of *trans* fatty acids, can produce healthier high-fat snacks. Incorporating such snacks into the diet may provide health benefits.

Snack foods make up a large proportion of the average diet in industrialized countries: 3.7 and 3.8 snacks/d, providing 790 and 629 kcal/d in Finnish men and women, respectively (9). In comparison, US adults consume 17.7% of their daily from snacks, which equates to ≈ 350 kcal/d for a 2000-kcal diet (10). Because of the contribution of snack foods to the overall diet, it can be considered that foods chosen in snacking episodes can affect health. The objective of this study was to determine whether replacing low-fat and high-fat or high-saturated fat and high-*trans* fat snack foods with snacks high in fat (mostly PUFAs) and low in saturated and *trans* fats improves CVD risk factors. We

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² Supported by GCRC grant M01 RR-00032 from the National Center for Research Resources, CNRC grant P30 DK056336, and Frito-Lay, Inc.

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Received August 24, 2006.

Accepted for publication January 23, 2007.

employed a whole food–substitution approach rather than manipulating one nutrient, because whole foods are emphasized in *Dietary Guidelines for Americans 2005* (11) and at MyPyramid.gov to better assist Americans in maintaining a healthy diet.

SUBJECTS AND METHODS

Subjects and study design

This study was conducted at the General Clinical Research Center (GCRC) of the University of Alabama at Birmingham (UAB, Birmingham, AL). A total of 45 subjects ($n = 12$ M, 33 F) aged 19–65 y were enrolled. Inclusion criteria included fasting LDL-cholesterol concentrations of 130 to 180 mg/dL, triacylglycerol concentrations < 350 mg/dL, and glucose concentrations < 126 mg/dL, a body mass index (BMI; in kg/m²) of 20 to 35, and a stable weight for ≥ 3 mo before the study. Exclusion criteria included the use of lipid-lowering medication, a history of CVD, current smoking, type 1 or type 2 diabetes, and hypertension. If subjects were taking any medication for ≥ 3 mo before starting the study, they were required to continue taking the same medication at the same dosage throughout the entire study. Subjects recorded their physical activity, by amount and type, on a daily basis and were asked to ensure that their activity levels remained constant throughout the study.

The study design was a randomized crossover trial with 3 controlled feeding phases. The total time of involvement for subjects was 7 mo; that span included the 3 diet phases of 25 d each, which were separated by either an 8- or a 4-wk washout period, depending on the number of groups studied at any one time. During washout periods, subjects were instructed to resume their normal eating habits and were urged against consuming study products.

Screening visits

The initial screening process occurred via telephone questionnaire. If subjects reported meeting the inclusion or exclusion requirements, they proceeded to the informational meeting, but, in the screening visit, any of these subjects could be excluded on the basis of lipid concentrations. During this meeting, the clinical coordinator (AB) obtained height and weight measurements and explained the study design in detail to potential participants who fit the BMI criterion. The consent form was reviewed at this time. Persons who wished to enroll in the study were given the consent form and instructed to return for the actual screening visit, when a blood sample and blood pressure measurements were obtained. At this screening visit, the consent form was reviewed on an individual basis, and questions about the study were answered. Furthermore, questionnaires, such as the Brief Symptom Inventory (12), demographics and medications forms, were completed.

All subjects gave written informed consent. The study was approved by UAB's Institutional Review Board.

Study diets

The control low-fat (LF) diet was designed to reduce total and saturated fat intakes to 30% and <10% of energy, respectively. The other 2 diets had higher fat contents, providing 36.3% and 37.9% of energy from fat [high-PUFA (HPUFA) diet and high-fat (HF) diet, respectively] but differed in their fatty acid profile.

TABLE 1
Macronutrient content of the test diets¹

Nutrient	Low-fat diet	High-	
		polyunsaturated fat diet	High-fat diet
Carbohydrate (%)	54.8	48.6	46.0
Protein (%)	14.7	15.5	16.3
Fat (%)	30.8	36.3	37.9
PUFA (%)	5.2	9.7	5.8
MUFA (%)	14.2	15.3	15.9
SFA (%)	8.5	8.5	11.4
<i>trans</i> Fatty acids (%)	1.2	0.7	2.7
Fiber (g/d)	20.1	22.2	20.9
Sodium (mg/d)	3546	3491	3622

¹ MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

The LF diet was designed to contain amounts of saturated and *trans* fats similar to those of the HPUFA diet and to be lower in fat because of a reduction in PUFAs. The HF diet was designed to have a fat content similar to that of the HPUFA diet and a PUFA content similar to that of the LF diet but greater amounts of saturated and *trans* fats than either of the other diets. The macronutrient breakdown of the diets, including snacks, is shown in **Table 1**. The base diet was identical for all 3 diets except for the types of snacks included. Therefore, the HPUFA and HF diets were achieved by isocaloric substitution of high-fat snacks for the low-fat snacks in the LF diet. Snacks provided ≈ 12 – 15% of energy requirements (≈ 300 kcal/d), depending on the caloric prescription, which is equivalent to ≈ 2 snacks of 150 kcal each. Snack foods included fat-free cookies, crackers, and low-fat cereal bars for the LF diet; chocolate bars, high-fat cookies and crackers, and buttered popcorn for the HF diet; and corn and tortilla snack chips fried in corn oil for the HPUFA diet. The average macronutrient contents of the snacks provided are shown in **Table 2**.

Subjects came to the GCRC each weekday morning for breakfast, consumed breakfast under supervision, and were provided foods for the rest of the day and, on Fridays, for the weekend. Subjects were required to consume all foods and beverages provided and were allowed to consume additional beverages if they were noncaloric. All subjects were provided food in the amounts

TABLE 2
Macronutrient content of study snacks¹

Nutrient	Low-fat diet	High-	
		polyunsaturated fat diet	High-fat diet
Carbohydrate [g (%)]	63.65 (84.9)	32.81 (43.8)	34.93 (46.6)
Protein [g (%)]	4.21 (5.6)	3.51 (4.7)	3.67 (4.9)
Fat [g (%)]	3.17 (9.5)	17.19 (51.6)	16.25 (48.8)
PUFA [g (%)]	0.50 (1.5)	9.44 (28.3)	1.62 (4.8)
MUFA [g (%)]	0.72 (2.2)	5.02 (15.1)	5.19 (15.6)
SFA [g (%)] ²	1.42 (4.3)	2.82 (8.5)	8.86 (26.6)
<i>trans</i> Fatty acids [g (%)]	0.60 (1.8)	0.11 (0.32)	0.85 (2.6)

¹ Data are derived from food-composition analysis per 300-kcal serving of each snack. The average value of the 4 snacks included in each diet is presented. MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

required to maintain a stable body weight. The weight-maintenance energy requirements were calculated by using the Harris-Benedict equation with an activity factor of 1.35 (13). Subjects were weighed daily before breakfast. After 7 d in the study, if body weight deviated from baseline by $>1\%$, caloric provisions were adjusted. At the morning visit, daily report forms were completed to record any symptoms of illness, foods not eaten, beverages consumed, and medication taken. If a subject did not consume a study food on a given day, he or she was instructed to consume the food the following day. None of the subjects reported any deviation from the protocol at any time during the study.

Clinical measurements

At baseline and on days 15 and 25 of each phase, the following samples and measurements were collected: 20 mL of venous blood, hip and waist circumferences, blood pressure (days 1 and 25 only), body fat and fat-free mass collected by using bioelectrical impedance analysis [(BIA) BC-418; Tanita Corporation, Arlington Heights, IL], and body weight. Blood pressure was measured in duplicate while the subject was sitting and with the use of a standard blood pressure cuff. The average of the 2 readings was used in the analyses. Blood samples were centrifuged at 3000 RPM for 10 min at 4 °C (Jouan CR3i Multifunction Centrifuge; Thermo Electron Corp, Gormley, Canada), and serum was separated from red blood cells and stored in a -80 °C freezer before being analyzed for glucose and insulin. Analyses of lipid profiles and high-sensitivity C-reactive protein (hs-CRP) were performed before freezer storage. Further BIA measurements were obtained on days 2, 3, and 10 to accurately track body weight in conjunction with caloric requirements.

Blood samples were analyzed for the complete lipoprotein profile by using a vertical autoprofile (VAP) technique (Atherotech Inc, Birmingham, AL) (14). Concentrations of total, HDL (including subparticles HDL₂ and HDL₃), LDL, and VLDL cholesterol and lipoprotein(a) [Lp(a)] were assessed. In addition, LDL particle density was assessed to determine LDL patterns A and B. The VAP technique is a semiautomated, modified ultracentrifugation test that separates lipoprotein fractions according to their densities in a vertical rotor; that separation by density allows for high resolution of each lipoprotein class and subclass. Each lipoprotein layer is measured with a spectrophotometer after the addition of cholesterol reagent. Cholesterol values from each layer are integrated by computer to obtain a spectrophotometric tracing of each lipoprotein subclass particle. Curves corresponding to each subclass are then deconvoluted (ie, separated) from the total profile, and cholesterol content is measured by calculating the area under the curve. The CVs for each lipid and lipoprotein subclass vary between 0.1% and 1.8% with the use of the VAP technique. Triacylglycerols were estimated by using the VAP technique. The hs-CRP analyses were conducted by Atherotech.

Fasting glucose measurements were performed on an automatic analyzer by using test kits (ILAB 600; Instrumentation Laboratories Ltd, Warrington, United Kingdom). Insulin measurements were performed by using a specific commercial enzyme-linked immunosorbent assay kit (DakoCytomation, Ely, United Kingdom). Glucose and insulin measurements were done in the Energy Metabolism and Body Composition Core Laboratory at UAB. Mean intraassay and interassay CVs, respectively,

for the measurements in our laboratory are 1.0% and 3.7% for glucose and 4.0% and 5.5% for insulin.

Statistical analysis

For all continuous response variables presented here, generalized linear models for repeated measures in SAS PROC MIXED were used in SAS software (version 9.1; SAS Institute, Cary, NC) to investigate the effect of diet on the percentage of change from baseline values. For the percentage of change in Lp(a), a few extreme outliers were observed. No valid reason was found to delete these outliers from the analysis. Therefore, we first obtained the ranks of the percentage change in Lp(a) and then analyzed those ranks as the outcome variable in a general linear model. Analyses based on ranks are statistically robust (15) and hence are not affected by outliers so long as the ordering of the observations remains the same. The correlations of the repeated measures were estimated from the data without assuming a particular covariance structure. To investigate the effect of diet on the odds that a person would have atherogenic LDL pattern B, the generalized estimating equations method applied to a logistic model was used. In this case, the covariance structure assumed was a first-order autoregressive structure (1), which assumes that the degree of correlation diminishes as 2 measurements are further separated over time.

Covariates included in the model were baseline (value of the variable before the diet was started), phase (order in which the diet was given), day (15 or 25 d after starting the diet), age, and sex. For each continuous response variable, we performed goodness-of-fit tests to compare the full model (ie, all covariates plus diet and diet \times day interaction terms) with the reduced model containing only the covariates (ie, without diet and diet \times day interaction terms) by using the likelihood ratio values of the fitted models. The effect of diet and diet \times day interaction were further investigated only when the goodness-of-fit test result was found to be significant. When the full model was applicable, the effect of diet \times day interaction was assessed and dropped if not found significant. In the end, the final models did not contain any interaction term.

A 2-tailed 5% significance level was used in all statistical testing: the overall test for diet effect, the test for interactions, and the test for comparison of diets. To control for inflation of type I error, comparisons of diets were performed only after the significance of the diet effect was established, which effectively controls the family-wise (ie, overall) error rate that is due to multiple testing when there are 3—and only 3—conditions (16), as in the present study. All analyses were done with SAS software (version 9.1). Data are reported as means \pm SEMs.

RESULTS

Of the 45 subjects enrolled in the study, 33 completed all 3 phases of the study ($n = 7$ M, 26 F; **Figure 1**). Subjects who completed the study had a mean \pm SD age of 41.8 ± 1.9 y and a BMI of 29.0 ± 0.6 . Reasons for discontinuing participation included straying from the diet ($n = 2$), food complaints ($n = 5$), did not return ($n = 2$), pregnant ($n = 1$), work conflict ($n = 1$), and death in the family ($n = 1$).

There was no significant effect of diet on the percentage change in waist circumference ($P = 0.13$), percentage body fat ($P = 0.66$), absolute fat mass ($P = 0.96$), and fat-free mass ($P = 0.12$). There was a significant effect of diet on LDL cholesterol



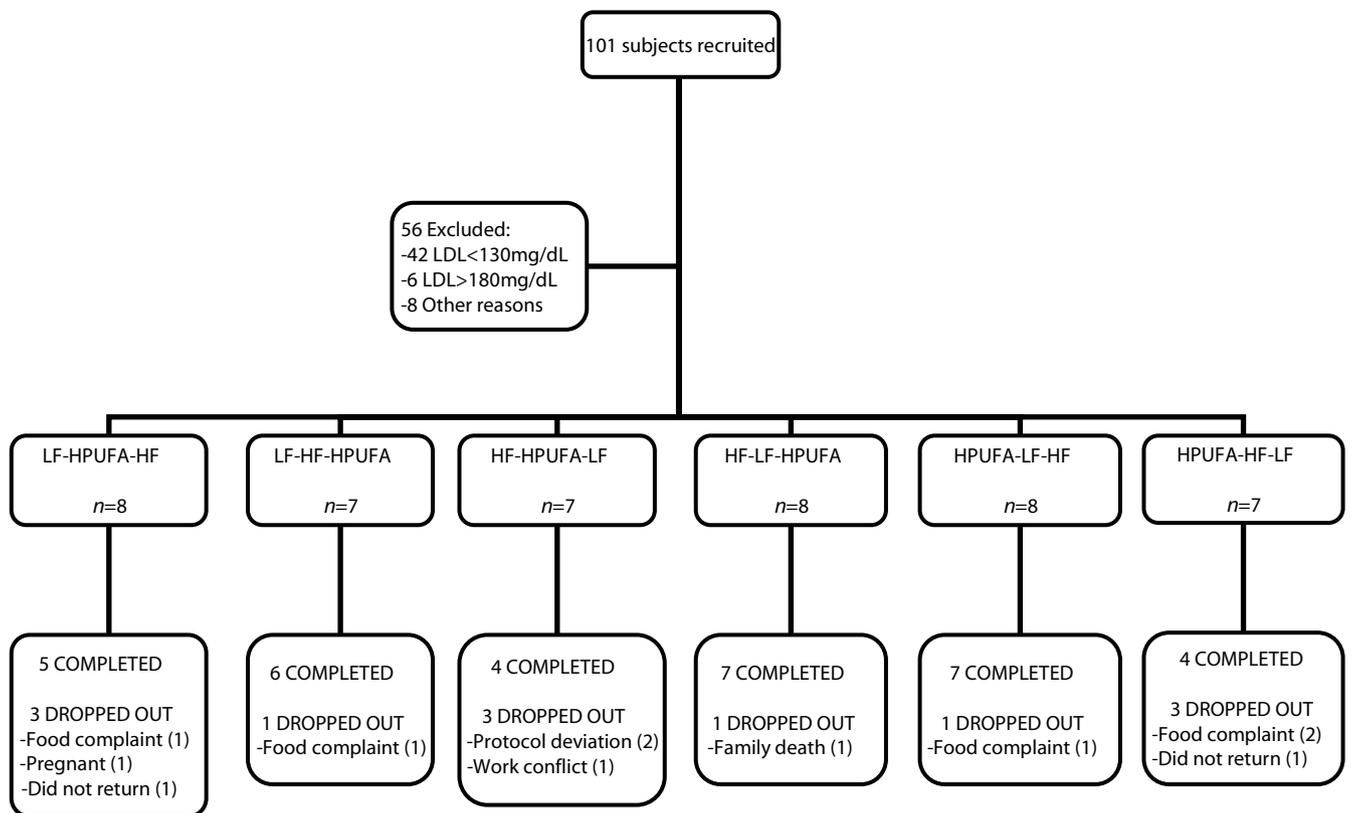


FIGURE 1. Progress of subjects in the study. HPUFA, high-polyunsaturated fatty acids; LF, low-fat; HF, high-fat.

($P = 0.02$), total cholesterol ($P = 0.03$), and triacylglycerol ($P = 0.003$) and a trend toward significance of the effect of diet on HDL cholesterol ($P = 0.09$). All 3 diets reduced LDL-cholesterol and total cholesterol concentrations (all $P < 0.0001$; **Table 3**), but HPUFA and LF diets did so to a greater extent than did the HF diet (LDL cholesterol: LF compared with HF, $P = 0.03$; HPUFA compared with HF, $P = 0.009$; total cholesterol: LF compared with HF, $P = 0.03$; HPUFA compared with HF, $P = 0.02$). The HPUFA diet tended to reduce triacylglycerol concentrations ($P = 0.06$) and the change was greater than that observed with the LF ($P = 0.0008$) and HF ($P = 0.03$) diets. We also observed a significant diet effect on non-HDL cholesterol ($P = 0.01$), and all 3 diets significantly reduced non-HDL-cholesterol concentrations from baseline (10.1%, 11.1%, and 6.9% for LF, HPUFA, and HF diets, respectively; $P < 0.0001$ for all). The reduction in non-HDL cholesterol with the HF diet was significantly less than that observed with the LF ($P = 0.03$) and HPUFA ($P = 0.004$) diets.

There was a significant diet effect on VLDL cholesterol ($P = 0.007$). The LF and HF diets did not significantly change VLDL cholesterol concentrations (4.0% and 1.8%; $P = 0.17$ and 0.53, respectively), whereas the HPUFA diet tended to reduce VLDL cholesterol concentrations (5.0%; $P = 0.08$). The change in VLDL cholesterol concentrations with HPUFA was greater than that with the LF ($P = 0.003$) and HF ($P = 0.02$) diets.

For Lp(a), because of the presence of outliers, analysis was done by first obtaining the ranks of the values of the percentage change and the ranks used as the response variable in the generalized linear model. There was a trend toward a significant effect of diet on Lp(a) ($P = 0.06$).

There tended to be a diet effect on LDL particle type ($P = 0.08$). Five subjects had an improvement in the LDL particle pattern with the LF diet, whereas 7 and 9 subjects did so with the HF and HPUFA diets, respectively. No one with LDL pattern B at baseline moved to LDL pattern A with the LF diet, whereas 2 subjects did so with the HF diet and 4 did so with the HPUFA diet. There was no significant diet effect ($P = 0.9$) on percentage change in hs-CRP ($0.7 \pm 39.3\%$, $0.5 \pm 37.9\%$, and $14.7 \pm 37.3\%$ for the LF, HF, and HPUFA diets, respectively). There was no significant effect of diet on systolic or diastolic blood pressure ($P = 0.85$ and 0.88, respectively).

DISCUSSION

Our results show that the HPUFA diet, which incorporated corn and tortilla chips fried in corn oil, resulted in the best overall CVD risk profile, although it was higher in total fat. All 3 diets lowered total and LDL cholesterol, but only the HPUFA diet tended also to reduce triacylglycerol concentrations. In addition, changes in total and LDL cholesterol were significantly greater with the HPUFA diet than with the HF diet; the change in triacylglycerol also was significantly greater with the HPUFA diet than with the LF diet. In addition to these traditional CVD risk factors, the pattern of LDL particle size was significantly more favorable with the HPUFA diet than with the LF diet. Taken together, these results indicate that substituting snack chips high in polyunsaturated fats for other high-saturated and *trans* fat or low-fat snacks leads to a significantly less atherogenic lipid profile.

TABLE 3

Serum lipid concentrations and their percentage changes from baseline on days 15 and 25 combined¹

	Low-fat diet	High-polyunsaturated fat diet	High-fat diet	P ²
LDL-C				
Baseline (mg/dL)	145.5 ± 3.7	145.4 ± 3.8	144.4 ± 3.8	
Day 15 (mg/dL)	132.8 ± 3.7	131.7 ± 3.7	135.6 ± 3.7	
Day 25 (mg/dL)	130.6 ± 3.7	129.0 ± 3.8	135.7 ± 3.8	
Change (%)	-11.8 ± 1.9 ^a	-12.5 ± 1.9 ^a	-8.8 ± 1.9 ^b	0.0004
TC				
Baseline (mg/dL)	221.3 ± 4.8	219.1 ± 4.8	219.8 ± 4.8	
Day 15 (mg/dL)	204.1 ± 4.8	202.4 ± 4.9	206.8 ± 4.8	
Day 25 (mg/dL)	201.7 ± 4.8	199.8 ± 4.8	206.9 ± 4.8	
Change (%)	-10.5 ± 1.4 ^a	-10.7 ± 1.4 ^a	-7.9 ± 1.4 ^b	0.0014
HDL-C				
Baseline (mg/dL)	53.9 ± 1.7	52.2 ± 1.7	53.3 ± 1.7	
Day 15 (mg/dL)	49.4 ± 1.7	50.7 ± 1.8	49.6 ± 1.7	
Day 25 (mg/dL)	48.9 ± 1.8	50.5 ± 1.8	50.1 ± 1.8	
Change (%)	-11.1 ± 2.1	-8.2 ± 2.2	-10.2 ± 2.1	0.0015
TG				
Baseline (mg/dL)	107.8 ± 8.4	110.8 ± 7.8	99.6 ± 7.9	
Day 15 (mg/dL)	113.5 ± 8.7	104.3 ± 8.6	112.9 ± 8.6	
Day 25 (mg/dL)	118.3 ± 8.4	99.7 ± 8.6	107.3 ± 8.5	
Change (%)	6.0 ± 4.8 ^a	-9.4 ± 4.8 ^b	0.2 ± 4.8 ^a	<0.0001
VLDL-C				
Baseline (mg/dL)	21.3 ± 1.0	20.6 ± 1.0	19.9 ± 1.0	
Day 15 (mg/dL)	20.9 ± 0.8	19.0 ± 0.8	20.6 ± 0.8	
Day 25 (mg/dL)	21.4 ± 0.9	19.2 ± 0.8	20.4 ± 0.8	
Change (%)	4.0 ± 2.8 ^a	-5.0 ± 2.8 ^b	1.8 ± 2.8 ^a	<0.0001
Non-HDL-C				
Baseline (mg/dL)	167.6 ± 4.4	166.8 ± 4.3	165.5 ± 4.3	
Day 15 (mg/dL)	154.4 ± 4.1	151.6 ± 4.2	156.9 ± 4.2	
Day 25 (mg/dL)	153.5 ± 4.1	149.6 ± 4.2	157.1 ± 4.2	
Change (%)	-10.1 ± 1.6 ^a	-11.1 ± 1.6 ^a	-6.9 ± 1.6 ^b	0.0003

¹ All values are $\bar{x} \pm \text{SEM}$; $n = 33$. HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; non-HDL-C, non-HDL cholesterol; TC, total cholesterol; TG, triacylglycerols; VLDL-C, VLDL cholesterol. All baseline, Day 15, and Day 25 values are based on the fitted model (unadjusted). All change (%) values are based on the fitted model (adjusted for baseline value, age, sex, day, and phase). Values in a row with different superscript letters are significantly different, $P < 0.05$.

² Goodness-of-fit tests: P values resulting from comparing the likelihood ratio value of the fitted model with all covariates plus diet and diet \times day interaction terms versus the reduced model with all covariates but without diet and diet \times day interaction terms.

The HPUFA diet caused significantly greater reductions in total and LDL cholesterol than did the HF diet and significantly greater reductions in triacylglycerol than did the LF diet. These changes are in accordance with a report estimating predicted change in coronary disease incidence on the basis of changes in the lipoprotein profile (3). The change in coronary disease incidence was predicted to decrease by 19% in men and 16% in women when 25 g or 10% of daily calories as saturated fat was replaced with unsaturated fat from vegetable and nut oils. Controlled substitution of oils to alter dietary fatty acid profile has also shown that replacing saturated fats with polyunsaturated and monounsaturated fats leads to significant reductions in total and LDL cholesterol (17). A novel finding of the present study is that whole-food substitutions, in the form of high-fat savory snacks, can result in improvements in dietary fat intake that lead to beneficial health effects.

Our LF diet reduced total and LDL cholesterol to a significantly greater extent than did the HF diet. This finding is in agreement with data from Ginsberg et al (18). However, unlike that group, we found no significant difference in HDL cholesterol or triacylglycerol between these 2 diets. This may be due to

the shorter duration of our feeding periods—25 d, instead of the 8 wk used by Ginsberg et al (18). However, previous studies found significant effects of diet on HDL cholesterol and triacylglycerol within a 3-wk period (4, 19). Although changes in total and LDL cholesterol were significantly greater with the LF and HPUFA diets than with the HF diet in the present study, it should be noted that the HF diet also resulted in significant reductions in total and LDL cholesterol. The HF diet was designed to resemble the average American diet, as described previously (20), however, it was likely an improvement over our subjects' habitual diet.

LDL particle size is linked to the risk of CVD: small, dense particles (pattern B) are associated with greater risk. Diet has been shown to modify LDL particle size, with near-maximal reductions being observed within 2 wk (21). A shift toward a Mediterranean diet leads to less-dense LDL particles in women who have a high proportion of small, dense LDL particles at baseline (22) and in persons with a particular apolipoprotein E genotype (23). Mixed results have been observed, however, and another study reported that effects on particle size were the same with the high-fat, high-polyunsaturated fat, and high-saturated

fat diets (24). In those studies, dietary intake often was not strictly controlled, and the exact dietary fat profile was unknown or not reported. In controlled feeding studies, LDL particle size reduction with olive oil-, sunflower oil-, and rapeseed oil-rich diets did not differ significantly from that with a saturated fat-rich diet (25), but LDL particle density decreased with the increasing degree of hydrogenation—and hence the *trans* fatty acid content—of the diet (26). This latter study suggests that shifting dietary fat intakes from *trans* fatty acids to unsaturated fatty acids may have beneficial effects on LDL particle size. A recent study showed that dietary carbohydrate intake may also have an effect on LDL particle size (27), with the peak LDL particle diameter increasing to a greater extent with diets containing lower proportions of carbohydrates (39% and 26% of energy) than with a control diet providing 54% of energy from carbohydrates. The macronutrient distribution of the control diet in that study was similar to that of our LF diet. Our HPUFA diet had $\approx 49\%$ of energy from carbohydrates, which suggests that even modest reductions in dietary carbohydrate can have beneficial effects on the LDL pattern. Furthermore, Krauss et al (27) have observed that, in persons who start out with LDL pattern A, a low-fat, high-carbohydrate diet results in a conversion to LDL pattern B. Although the overall diet effect was not significant ($P = 0.08$) in modeling the odds of LDL pattern B, our data suggested that the odds that a person would have LDL pattern B when following an LF diet are ≈ 3.5 times the odds for a person following a HPUFA diet. This finding supports the notion that shifting dietary patterns toward a pattern with greater PUFAs, rather than carbohydrates, leads to a better LDL pattern. More research is necessary to fully understand the effect of macronutrient and fatty acid profiles on LDL density patterns.

Lp(a) is a new CVD risk factor that has been shown, in a meta-analysis of prospective studies, to have independent predictive power for coronary heart disease (28). Moreover, concomitant increases in LDL cholesterol and Lp(a) have been reported to have synergistic effects on CVD risk in hypercholesterolemic subjects (29) by enhancing arterial cholesterol deposition (30). However, this marker seems to be relatively resistant to therapies: statins are ineffective at reducing Lp(a) concentrations, and nicotinic acid produces only modest benefits (31). Our data show no effect of diet on Lp(a). This finding agrees with Brown et al (32), who also found no effect of dietary fatty acid type, whether low or high in polyunsaturated fats and low or high in cholesterol, on Lp(a). One study found significant reductions in Lp(a) when restricting carbohydrate intake (33); however, this study was of longer duration (12 wk) than the present study, and it did not have a high-carbohydrate comparison group but a carbohydrate-restricted group supplemented with 3 g dietary fiber. Furthermore, changes in Lp(a) in the present study were correlated with changes in fat mass, and, therefore, the change in body composition may have been at least partly responsible for the change in Lp(a). It is interesting that one group found that Lp(a) was increased after 5 wk of consumption of low-fat diets similar to our LF diet (34). Perhaps a longer study duration is needed to effect changes in Lp(a).

hs-CRP is an acute-phase immune reactant that has been associated with increased risk for CVD. Its concentrations have been correlated with adipose tissue, and it follows that weight loss leads to reductions in hs-CRP (35). However, studies to determine the effect of diet on hs-CRP are conflicting. A study comparing the effects of consuming a Mediterranean-style diet

and of consuming an American Heart Association Step 1 diet for 2 y found that hs-CRP was lower in the Mediterranean-diet group than in the control group (36). The Mediterranean-style diet provided a greater percentage of energy from complex carbohydrates, PUFA, and monounsaturated fat; a lower percentage of energy from saturated fat; and less overall energy than did the Step I diet. Similarly, a diet containing a combination of cholesterol-lowering foods and ingredients (eg, almonds, soy protein, viscous fiber, and phytosterols) led to reductions in CRP of 28.2%, whereas no reduction in CRP was seen with consumption of a diet very low in saturated fat (37). In contrast, others have not observed a diet effect on CRP. Desroches et al (38) found that consumption of a low-fat diet (25.8% of energy from fat), a high-fat diet (40% of energy from fat), and a high-monounsaturated fat diet (22.5% of energy from monounsaturated fat) did not affect CRP concentrations differently. Increasing the *trans* fat content of a diet also has not been found to affect CRP concentrations (39). It has been suggested that diet plays a role in modulating CRP concentrations only if a subject's body weight also changes (40, 41). Our data did not show any effect of diet on hs-CRP after a 25-d period, but we found a correlation between change in CRP and change in waist circumference. Perhaps a longer study duration or greater changes in body composition are necessary for observable changes in this biomarker. The present study did not produce any changes in body composition.

The present study had several limitations. First, subjects were mildly hyperlipidemic and normoglycemic. It is not known whether similar results would have been obtained in persons with lower or higher lipid concentrations or with type 2 diabetes. Second, the sample size was modest (ie, 33), which limited our power to examine sex and ethnicity effects. Another limitation of the present study was the length of the feeding periods. Although we found significant diet effects on some of our main endpoints, it is not implausible to postulate that 25 d may not have been enough time to initiate a change in some of our secondary endpoints, such as Lp(a) and hs-CRP. On the other hand, it is very difficult to obtain compliance with a controlled feeding protocol when the feeding period is extended beyond a few weeks. Free-living studies in which subjects are counseled to consume diets of a particular macronutrient profile may be necessary to circumvent this issue and would be a logical next step for this study. Finally, we cannot exclude the possibility that subjects were not entirely compliant with the diet requirements. However, the relatively short feeding periods and the lack of weight change are good indicators that the subjects consumed the study foods. In any case, deviations from protocol would most likely have weakened the present study and prevented our observation of changes in the variables measured.

The findings of the present study are in agreement with *Dietary Guidelines for Americans 2005* (11) and the American Heart Association Diet and Lifestyle Recommendations (27), which have acknowledged that fat type is more influential than total fat in reducing CVD risk and which no longer recommend "low-fat" diets (ie, $<30\%$ of energy from fat). Weight-maintaining diets containing up to $\approx 40\%$ of calories from fat may favorably affect CVD risk factors (42). It is important to note that our study does not promote the consumption of snacks above and beyond weight-maintaining energy requirements but does promote their inclusion in and contribution to a healthy diet. The role of snacks in body weight regulation is still controversial.



Some studies have found that snacking frequency is associated with increased BMI (43), whereas others have not found that (44, 45), and others have even not found snacking to prevent weight loss when incorporated in a weight-loss diet (46).

Because snacks are an integral part of most people's dietary habits, it is important to understand their nutritional contribution. The present study shows that snack foods can be incorporated into a healthy diet at a practical level and can affect metabolic risk profiles. Moreover, the study design was unique in that the only dietary manipulation was the type of snack offered—a whole-food substitution. This is a simple concept that could be very useful in educating persons about making healthy food choices. Finally, the present study shows that common beliefs about the nutritional value of foods may be quite erroneous. What constitutes a healthy snack is debatable; however, the present study shows that snack chips fried in corn oil is an effective and simple means of delivering healthy unsaturated fats into the diet while maintaining a low-saturated fat and low-*trans* fat diet. Therefore, if chosen wisely, even snack foods that are often considered to be "junk food" can contribute to a heart-healthy diet. 

We thank all of the subjects for their participation in this study. We also extend our gratitude to Betty Darnell and the GCRC kitchen and nursing staff for their help with this study, Jamy Ard for medical supervision, and W Timothy Garvey for advice on design, execution, and interpretation.

The authors' responsibilities were as follows—M-PS-O, KDH, and DBA: study concept and design; M-PS-O and DBA: obtained funding and supervised the study; IA and DBA: statistical analyses; M-PS-O, IA, BG, KDH, and DBA: analysis and interpretation of data; M-PS-O, IA, AB, BG, and DBA: drafting of the manuscript; and M-PS-O, KDH, and DBA: critical revision of the manuscript. DBA has served as a paid consultant to the sponsor and to competitor companies. KDH is employed by the sponsor. None of the other authors had any personal or financial conflict of interest.

REFERENCES

1. Sharman MJ, Gomez AL, Kraemer WJ, Volek JS. Very low-carbohydrate and low-fat diets affect fasting lipids and postprandial lipemia differently in overweight men. *J Nutr* 2004;134:880–5.
2. Vidon C, Boucher P, Cachefo A, Peroni O, Diraison F, Beylot M. Effects of isoenergetic high-carbohydrate compared with high-fat diets on human cholesterol synthesis and expression of key regulatory genes of cholesterol metabolism. *Am J Clin Nutr* 2001;73:878–84.
3. Sacks FM, Katan M. Randomized clinical trials on the effects of dietary fat and carbohydrates on plasma lipoproteins and cardiovascular disease. *Am J Med* 2002;113:13–24.
4. Meksawan K, Pendergast DR, Leddy JJ, Mason M, Horvath PJ, Awad AB. Effect of low and high fat diets on nutrient intakes and selected cardiovascular risk factors in sedentary men and women. *J Am Coll Nutr* 2004;23:131–40.
5. Mensick RP, Zock PL, Kester ADM, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003;77:1146–55.
6. Hu FB, Stampfer MJ, Manson JE, et al. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* 1997;337:1491–9.
7. Howard BV, Van Horn L, Hsia J, et al. Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative randomized controlled dietary modification trial. *JAMA* 2006;295:655–66.
8. de Graaf C. Effects of snacks on energy intake: an evolutionary perspective. *Appetite* 2006;May 2 Epub ahead of print.
9. Ovaskainen M-L, Reinivuo H, Tapanainen H, Hannila M-L, Korhonen T, Pakkala H. Snacks as an element of energy intake and food consumption. *Eur J Clin Nutr* 2006;60:494–501.
10. Nielsen SJ, Siega-Riz AM, Popkin BM. Trends in energy intake in U.S. between 1977 and 1996: similar shifts seen across age groups. *Obes Res* 2002;10:370–8.
11. US Department of Health and Human Services and US Department of Agriculture. Dietary guidelines for Americans 2005. Washington, DC: US Government Printing Office, 2005.
12. Derogatis LR. Brief symptom inventory: administrative, scoring, and procedures manual. 4th ed. Minneapolis, MN: National Computer Systems, Inc, 1993.
13. Harris J, Benedict F. A biometric study of basal metabolism in man. Washington, DC: Carnegie Institute of Washington, 1919.
14. Kulkarni KR, Garber DW, Marcovina SM, Segrest JP. Quantification of cholesterol in all lipoprotein classes by the VAP-II method. *J Lipid Res* 1994;35:159–68.
15. Jurčková J, Sen PK. Robust statistical procedures: asymptotics and interrelations. New York: J Wiley, 1996.
16. Toothaker LE. Multiple comparisons for researchers. Thousand Oaks, CA: SAGE Publications, 1991.
17. Binkoski AE, Kris-Etherton PM, Wilson TA, Mountain ML, Nicolosi RJ. Balance of unsaturated fatty acids is important to a cholesterol-lowering diet: comparison of mid-oleic sunflower oil and olive oil on CVD risk factors. *J Am Diet Assoc* 2005;105:1080–6.
18. Ginsberg HN, Kris-Etherton P, Dennis B, et al. Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: the Delta Study, protocol 1. *Arterioscler Thromb Vasc Biol* 1998;18:441–9.
19. Morgan SA, O'Dea K, Sinclair AJ. A low-fat diet supplemented with monounsaturated fat results in less HDL-C lowering than a very-low-fat diet. *J Am Diet Assoc* 1997;97:151–6.
20. National Center for Health Statistics. Health, United States, 2005: with chartbook on trends in the health of Americans, DHHS Publication No. 2005–1232. Washington, DC: US Government Printing Office, 2005.
21. Lamarche B, Desroches S, Jenkins DJ, et al. Combined effects of a dietary portfolio of plant sterols, vegetable protein, viscous fibre and almonds on LDL particle size. *Br J Nutr* 2004;92:657–63.
22. Goulet J, Lamarche B, Charest A, et al. Effect of a nutritional intervention promoting the Mediterranean food pattern on electrophoretic characteristics of low-density lipoprotein particles in healthy women from the Quebec City metropolitan area. *Br J Nutr* 2004;92:285–93.
23. Moreno JA, Perez-Jimenez F, Marin C, et al. The effect of dietary fat on LDL size is influenced by apolipoprotein E genotype in healthy subjects. *J Nutr* 2004;134:2517–22.
24. Ambring A, Friberg P, Axelsen M, et al. Effects of a Mediterranean-inspired diet on blood lipids, vascular function and oxidative stress in healthy subjects. *Clin Sci (Lond)* 2004;106:519–25.
25. Kratz M, Gulbahce E, von Eckardstein A, et al. Dietary mono- and polyunsaturated fatty acids similarly affect LDL size in health men and women. *J Nutr* 2002;132:715–8.
26. Mauger JF, Lichtenstein AH, Ausman LM, et al. Effect of different forms of dietary hydrogenated fats on LDL particle size. *Am J Clin Nutr* 2003;78:370–5.
27. Krauss RM, Blanche PJ, Rawlings RS, Fernstrom HS, Williams PT. Separate effects of reduced carbohydrate intake and weight loss on atherogenic dyslipidemia. *Am J Clin Nutr* 2006;83:1025–31.
28. Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease: meta-analysis of prospective studies. *Circulation* 2000;102:1082–5.
29. US Department of Health and Human Services. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Washington, DC: US Government Printing Office, 2002. (NIH Publication no. 02-5215.)
30. Goldstein LB, Adams R, Albert MJ, et al. Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council: cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Interdisciplinary Working Group. *Circulation* 2006;113:e873–923.
31. Kostner KM, Kostner GM. Therapy of hyper-Lp(a). *Handb Exp Pharmacol* 2005;170:519–36.
32. Brown SA, Morrisett J, Patsch JR, Reeves R, Gotto AM, Patsch W. Influence of short term dietary cholesterol and fat on human plasma Lp[a] and LDL levels. *J Lipid Res* 1991;32:1281–9.
33. Wood RJ, Volek JS, Davis SR, Dell'Ova C, Fernandez ML. Effects of a carbohydrate-restricted diet on emerging plasma markers for cardiovascular disease. *Nutr Metab* 2006;3:19 (Epub ahead of print).
34. Silaste ML, Rantala M, Alfthan G, et al. Changes in dietary fat intake

- alter plasma levels of oxidized low-density lipoprotein and lipoprotein(a). *Arterioscler Thromb Vasc Biol* 2004;24:498–503.
35. Jae SY, Fernhall B, Heffernan KS, et al. Effects of lifestyle modifications on C-reactive protein: contribution of weight loss and improved aerobic capacity. *Metabolism* 2006;55:825–31.
 36. Esposito K, Marfello R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440–6.
 37. Jenkins DJ, Kendall CW, Marchie A, et al. Effects of a dietary portfolio of cholesterol-lowering foods vs. lovastatin on serum lipids and C-reactive protein. *JAMA* 2003;290:502–10.
 38. Desroches S, Archer WR, Paradis ME, et al. Baseline plasma C-reactive protein concentrations influence lipid and lipoprotein responses to low-fat and high monounsaturated fatty acid diets in healthy men. *J Nutr* 2006;136:1005–11.
 39. Lichtenstein AH, Erkkila AT, Lamarche B, Schwab US, Jalbert SM, Ausman LM. Influence of hydrogenated fat and butter on CVD risk factors: remnant-like particles, glucose and insulin, blood pressure and C-reactive protein. *Atherosclerosis* 2003;171:97–107.
 40. Koren MS, Purnell JQ, Breen PA, Mattys CC, Callahan HS, Weigle D. Plasma C-reactive protein concentration is not affected by isocaloric dietary fat reduction. *Nutrition* 2006;22:444–8.
 41. Kasim-Karakas SE, Tsodikov A, Singh U, Jialal I. Responses of inflammatory markers to a low-fat, high-carbohydrate diet: effects of energy intake. *Am J Clin Nutr* 2006;83:774–9.
 42. Kris-Etherton PM, Binkoski AE, Zhao G, et al. Dietary fat: assessing the evidence in support of a moderate-fat diet; the benchmark based on lipoprotein metabolism. *Proc Nutr Soc* 2002;61:287–98.
 43. Berteus Forslund H, Torgerson JS, Sjostrom L, Lindroos AK. Snacking frequency in relation to energy intake and food choices in obese men and women compared to a reference population. *Int J Obes (Lond)* 2005;29:711–9.
 44. Field AE, Austin SB, Gillman MW, Rosner B, Rockett HR, Colditz GA. Snack food intake does not predict weight change among children and adolescents. *Int J Obes Relat Metab Disord* 2004;28:1210–6.
 45. Hampl JS, Heaton CL, Taylor CA. Snacking patterns influence energy and nutrient intakes but not body mass index. *J Hum Nutr Diet* 2003;16:3–11.
 46. Poston WS, Haddock CK, Pinkston MM, et al. Weight loss with meal replacement and meal replacement plus snacks: a randomized trial. *Int J Obes (Lond)* 2005;29:1107–14.

