Exchanging Carbohydrate or Protein for Fat Improves Lipid-Related Cardiovascular Risk Profile in Overweight Men and Women When Consumed Ad Libitum

Mario Kratz, PhD,† David S. Weigle, MD,† Patricia A. Breen, BSc, RN,† Kaatje E. Meeuws, BSc,‡ Verna R. Burden, MS, RD,‡ Holly S. Callahan, MSc, RD,§ Colleen C. Matthys, BSc, RD,§ and Jonathan Q. Purnell, MD‡*

Background: The impact of low-fat diets on the plasma lipoprotein profile is incompletely understood.

Methods: We conducted two 16-week dietary studies to compare the effects of a moderate-fat (mod-FAT) baseline diet with isocaloric and ad libitum low-fat diets rich in either carbohydrates (high-CHO, n = 16) or protein (high-PRO, n = 19) on plasma lipids, post–heparin lipase activities, cholesteryl ester transfer protein, and phospholipid transfer protein.

Results: Switching from the mod-FAT to the isocaloric high-CHO diet lowered plasma high-density lipoprotein cholesterol concentrations ($P < 0.001$) and tended to increase triglyceride levels ($P = 0.087$). Cholesterol content in the larger, buoyant low-density lipoprotein (LDL) fractions decreased, whereas those of the very-low-density lipoprotein, intermediate-density lipoprotein, and smaller, denser LDL fractions tended to increase. These changes were largely reversed when subjects lost weight by consuming this high-CHO diet ad libitum. Switching from the mod-FAT diet to the isocaloric high-PRO diet did not increase cholesterol content in the small-dense LDL fraction and led to decreases in both LDL and high-density lipoprotein cholesterol in plasma ($P < 0.001$ for both).

Consumption of the high-protein ad libitum diet accompanied by weight loss did not change plasma lipids further, except for a shift of cholesterol from dense low-density lipoprotein fractions to more buoyant low-density lipoprotein fractions. Cholesteryl ester transfer protein concentrations decreased with high-cholesterol feeding, whereas cholesteryl ester transfer protein concentrations and hepatic lipase and phospholipid transfer protein activities all decreased during high-protein feeding.

Conclusions: Both high-CHO and high-PRO diets improve plasma lipid–related risk of cardiovascular disease when consumed ad libitum.

Key Words: fat, carbohydrate, protein, lipoproteins, lipids, cardiovascular disease

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Elevated plasma concentrations of low-density lipoprotein (LDL) cholesterol and triglycerides and low plasma concentrations of high-density lipoprotein (HDL) cholesterol, alone or in combination, are established risk factors for coronary heart disease (CHD).[1–3] Elevated plasma triglyceride and low HDL cholesterol concentrations are often associated with a predominance of the smaller, denser LDL particles that have more recently been shown to also affect CHD risk.[4] Among HDL particles, there are data to suggest that low concentrations of HDL_2 and elevated concentrations of HDL_3 contribute to CHD risk.[5] Elevated plasma activities of proteins that alter LDL and HDL composition including cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) have also been linked to atherosclerosis.[6,7]

Many of these lipid-related risk factors for CHD may be modified by changes in body weight and diet composition. Increased body weight and particularly abdominal obesity are associated with elevated plasma triglyceride concentrations, low HDL cholesterol, and smaller, denser LDL particles.[8] Low HDL cholesterol concentrations might be partly due to the elevated plasma activity of CETP that has been described in obese subjects.[8] After weight loss, plasma activities of both CETP and PLTP decrease, accompanied by reduced triglyceride concentrations and increases in HDL cholesterol and LDL particle size.[8–10] At a stable body weight, replacing dietary fat with carbohydrate increases plasma triglyceride concentrations, lowers plasma HDL cholesterol concentrations, and reduces LDL particle size.[11–13] Isocaloric feeding, however, does not reflect free-living conditions in which substituting carbohydrates for fat results in spontaneous reductions in food intake and modest weight loss.[14] As a result of this spontaneous weight loss, some of the unfavorable consequences of a carbohydrate-rich diet on lipid concentrations might be attenuated.

Few studies have examined the effects of replacing dietary fat with protein on plasma lipid concentrations[11] and activities of proteins involved in lipoprotein processing. Many studies

From the *Fred Hutchinson Cancer Research Center, Division of Public Health Sciences, Cancer Prevention Program; †University of Washington, Department of Medicine, Division of Metabolism, Endocrinology and Nutrition, Seattle, WA; ‡Oregon Health & Science University, Center for the Study of Weight Regulation, Division of Endocrinology, Diabetes and Clinical Nutrition, Portland, OR; and §University of Washington General Clinical Research Center, Seattle, WA.

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Reprints: Mario Kratz, PhD, Fred Hutchinson Cancer Research Center, Public Health Sciences, Cancer Prevention Program, Mail Stop M4-B402, 1100 Fairview Ave N, Seattle, WA 98109. E-mail: mkratz@fhcrc.org.

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M.K. was responsible for statistical analyses and interpretation of the data and for writing the first draft of the manuscript. D.S.W. and J.Q.P. designed the research, supervised the collection of the data, and were involved in analyzing and interpreting the data. J.Q.P. also had final responsibility for the final content. P.A.B. was the study coordinator for both studies and was involved in designing the experiments and the collection of the data. H.S.C., C.C.M., K.E.M., and V.R.B. were responsible for designing the experimental diets and for supervising the production, distribution, and weigh-backs of the diets. All authors read and approved the final manuscript.

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have failed to control for the source of dietary protein[11] or differences in fat and carbohydrate composition.[15,16] In addition, previous studies replacing dietary fat with protein have often failed to control for the enhanced satiety and weight loss that occurs during ad libitum feeding.

We have recently completed 2 diet studies comparing the effects of dietary fat restriction and increases in either dietary carbohydrate or protein on hormones involved in weight regulation and spontaneous weight loss during ad libitum feeding.[17,18] As part of these studies, we also measured lipid-related risk factors for CHD, with an emphasis on lipoprotein subfractions and plasma activities of proteins involved in lipoprotein particle distribution. These studies allowed us to quantify the effects of dietary macronutrient changes on lipid outcomes under isocaloric conditions when weight change is not a confounder as well as the potential combined effects of diet changes and weight loss under ad libitum conditions.

MATERIALS AND METHODS

Study Population

Two dietary studies were carried out jointly at the University of Washington (UW) in Seattle, WA, and Oregon Health & Science University (OHSU) in Portland, OR. For study A (full description below), 18 healthy adults (2 men, 16 women) with a mean (range) age of 45 years (28–63 years) and body mass index of 27.1 kg/m^2 (24.5–30.2 kg/m^2) were recruited by newspaper advertisement in their local area. Of these, 2 subjects were excluded from analyses because of a lack of compliance with the ad libitum dietary regimen.[17,18] For study B (see also below), a separate group of 19 healthy adults (3 men, 16 women) with a mean (range) age of 41 years (27–62 years) and body mass index of 26.2 kg/m^2 (22.5–30.1 kg/m^2) were also recruited by newspaper advertisement.[17,18] Before being enrolled, subjects provided informed written consent. All procedures were approved by the institutional review boards of UW and OHSU.

Study Design and Diets

As we had hypothesized that any potential deleterious effects of isocaloric low-fat feedings on lipids levels would be reversed as a result of spontaneous weight loss that accompanies ad libitum feeding, we chose identical study designs for both diets[17,18] that would allow us to measure the effects of diet alone compared with that of diet and weight loss in the same individual (Fig. 1). To compare the effects of diet alone, all subjects consumed an isocaloric baseline moderate-fat diet for 2 weeks that was similar to the average American diet; this was then followed by an isocaloric low-fat diet for an additional 2 weeks. Two weeks for each dietary phase was chosen as previous studies have shown that induction of hypertriglyceridemia on a low-fat diet occurs within this time frame.[19,20] After this second isocaloric period, subjects continued to consume the same low-fat diet for another 12 weeks but under ad libitum conditions, a time frame that others had shown to allow plateauing of spontaneous weight loss.[21] During the ad libitum period, subjects were provided with food in excess of what they consumed during the isocaloric diet periods. They were instructed to eat only as much as required to be comfortably satiated and to return any leftovers to the Nutrition Research Kitchens. Returned foods were then weighed to assess the amount of food eaten.

The diet composition, expressed as percentage of energy intake, for the first 2 weeks of both studies was 30% to 35% fat, 45% to 50% carbohydrate, and 15% to 20% protein, with total calories adjusted to keep subjects weight stable (mod-FAT diet). In study A, subjects consumed 20% less fat and 20% more carbohydrates (high-CHO diet) during the second isocaloric phase and the ad libitum phase as compared with the baseline period. This high-CHO diet contained more fiber (3.3 vs 2.2 g/MJ) and less cholesterol (17 vs 27 mg/MJ) than the baseline diet. In study B, subjects consumed 15% less fat and 15% more protein (high-PRO diet) during the second isocaloric phase and the ad libitum phase as compared with the baseline period. This high-PRO diet contained slightly less fiber (2.4 vs 2.8 g/MJ) and slightly more cholesterol (22 vs 18 mg/MJ) than the baseline diet.

All meals were prepared in the Nutrition Research Kitchens of the Clinical Research Center (CRC) of UW and OHSU. All diets were quantified using ProNutra and ProNessy software (version 3.0; Viocare Technologies Inc, Princeton, NJ). At the completion of each dietary phase (the baseline diet [CRC1], the isocaloric low-FAT diet [CRC2], and the ad libitum diet [CRC3]), subjects were admitted to the CRC at their respective institutions to undergo testing as described below.

Laboratory Methods

Lipids and Lipoproteins

After a 12- to 16-hour overnight fast and after a rest period of at least 15 minutes after intravenous line placement, baseline blood was collected in 0.1% EDTA for lipid studies. Then, a heparin bolus of 60 U/kg was given, and blood was collected after 10 minutes in lithium-heparin tubes for the measurement of lipase activities. Blood was immediately centrifuged at 4°C at 3000 revolutions per minute for 15 minutes. Aliquots of plasma were then snap frozen and stored at −70°C. Total cholesterol, triglyceride, HDL cholesterol, cholesterol content of lipoprotein fractions and peak LDL particle buoyancy, HDL_2 cholesterol, HDL_3 cholesterol, and apolipoprotein B were measured at the Northwest Lipid Research Laboratory, as previously described.[22,23]

Post–Heparin Lipase Activities

The total lipolytic activity was measured in plasma after heparin bolus as previously described.[24] Lipoprotein lipase (LPL) activity was calculated as the lipolytic activity removed from the plasma by the incubation with a specific monoclonal antibody against LPL, and hepatic lipase (HL) activity was determined as the activity remaining after incubation with the LPL antibody. Enzyme activity is expressed as nanomoles of free fatty acid released per minute per milliliter of plasma at 37°C. Intra-assay and interassay coefficients of variation (CVs) were 6% and 14%, respectively, for HL, and 7% and 8%, respectively, for LPL.

CETP Plasma Concentration

Cholesteryl ester transfer protein plasma concentrations were measured by a commercial sandwich enzyme-linked immunosorbent assay kit (Wako Chemicals USA, Richmond, VA) using 2 monoclonal antibodies. The intra-assay and interassay CVs were 3.1% and 10.5%, respectively. As reported before, CETP mass as measured by this enzyme-linked immunosorbent assay correlates highly (r = 0.83, n = 42) with CETP activity.[25]

PLTP Activity Assay

Phospholipid transfer protein activity was determined by measuring the transfer of labeled phosphatidylcholine from vesicles to HDL_3,[26,27] without the use of plasma as a carrier as previously described.[26] This method reflects the phospholipid

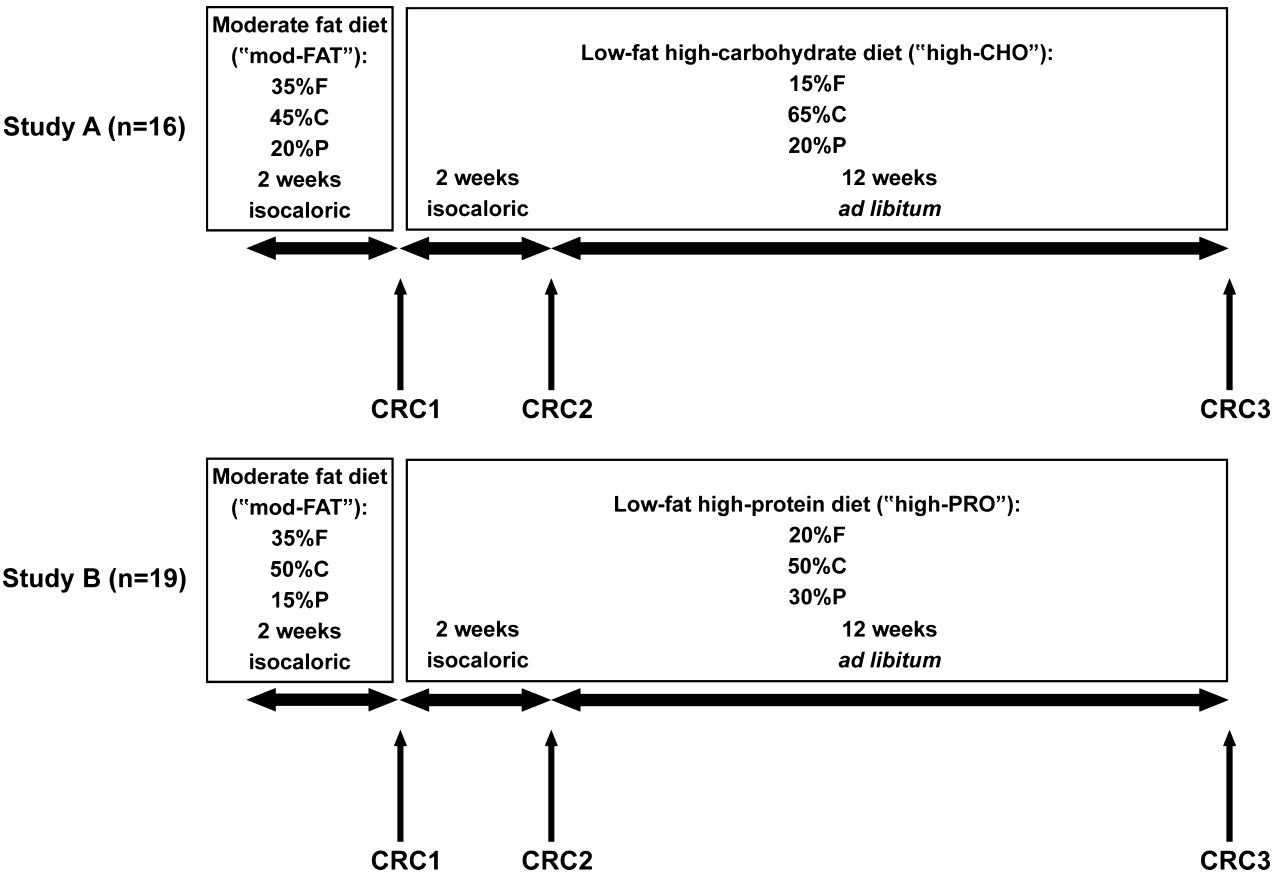

FIGURE 1. Design of the 2 studies. In both studies, subjects consumed a moderate-FAT baseline diet ("mod-FAT") for the first 2 weeks. During this time, calorie intake was adjusted to keep body weight within 1 kg of baseline. On day 14 of this period, subjects came to the CRC for blood draws. After discharge from CRC1, subjects switched to a diet rich in carbohydrates (high-CHO) in study A and to a diet rich in protein (high-PRO) in study B. For the first 2 weeks on these diets, calorie intake was kept at the level that had led to weight stability in the previous phase. This isocaloric phase was again followed by a visit to the CRC on day 28 of the study. For the last 12 weeks of the studies, subjects continued to consume these diets; now, however, they received more food and chose calorie intake freely (ad libitum). Subjects completed the study with their third visit to the CRC on day 112. F indicates fat; C, carbohydrate; P, protein.

transfer activity of PLTP but not that of CETP. Three human control plasmas were included in triplicate in each assay and used to correct for interassay variation. The intra-assay and interassay CVs were 8% and 2%, respectively.

Cholesterol Concentrations in Lipoprotein Fractions

Lipoprotein density distribution and cholesterol content were determined by nonequilibrium density gradient ultracentrifugation using a modification of a previously described technique and a Sorvall TV-865B vertical rotor (DuPont, Wilmington, DE).[28] High-density lipoprotein is located in fractions 0 to 6, LDL in fractions 7 to 18, intermediate-density lipoprotein (IDL) in fractions 19 to 30, and very-low-density lipoprotein (VLDL) in fractions 30 to 38. Low-density lipoprotein relative flotation (Rf), a measure of LDL peak particle buoyancy, was determined by dividing the fraction number between 7 and 19 containing the peak LDL cholesterol concentration by the total number of fractions collected (equal to 38). This technique is optimized to separate subfractions of apolipoprotein B containing particles and not the denser HDL species. Low-density lipoprotein fractions were also pooled to determine the cholesterol and apolipoprotein B content.

Statistics

All analyses were performed using SPSS version 11.5 (SPSS Inc, Chicago, IL). For all variables, we performed repeated-measures analysis of variance (ANOVA) with CRC1, CRC2, and CRC3 as the 3 levels of the within-subject factor (time). Significant time effects were followed up by assessing whether the variable was consistent with a normal distribution. To this end, we checked normal plots and histograms and performed Shapiro-Wilk tests. This was the case for all tested variables. We then performed post hoc paired *t* tests, with *P* values adjusted according to Bonferroni. In case the residuals of repeated-measures ANOVA were not consistent with a normal distribution, we performed a nonparametric Friedman test to assess changes over time. These were followed up post hoc by Wilcoxon signed rank tests, again adjusted to correct for multiple testing. Correlations between LPL, HL, PLTP, and CETP on the one hand and LDL Rf, HDL_2 cholesterol, HDL_3 cholesterol, and the ratio of LDL cholesterol to apolipoprotein B on the other hand were assessed by calculating Pearson correlation coefficient for normally distributed variables and by calculating Spearman rank correlation coefficient for non–normally distributed variables. The level of significance was set to $P < 0.05$ for all tests.

RESULTS

Study A: Mod-FAT vs High-CHO Diets

Body weights were stable as planned during the isocaloric high-CHO diet ($P = 0.19$, Table 1). Whereas total-cholesterol concentrations did not change during this period compared with baseline, HDL cholesterol concentrations dropped significantly ($P < 0.001$), which was due to a reduction in both HDL_2 and HDL_3 cholesterol ($P = 0.003$ and $P = 0.04$, respectively). This was associated with a trend toward higher triglyceride concentrations ($P = 0.09$). Using nonequilibrium density gradient ultracentrifugation, cholesterol concentrations were slightly increased in all VLDL fractions (Fig. 2A). The cholesterol content of the larger, more buoyant LDL fractions significantly decreased, which was counterbalanced by a smaller (nonsignificant) increase in cholesterol in the denser LDL fractions; this was associated with a trend toward increased peak density of LDL particles (Rf, $P = 0.056$). Post–heparin lipase activities, CETP concentrations, and PLTP activity in plasma did not change during this period.

When the subjects continued to consume this low-fat, high-CHO diet during the ad libitum period, body weight decreased by 3.7 (SD, 2.3) kg ($P < 0.001$). There was a trend for a further reduction in total cholesterol, LDL cholesterol, and apolipoprotein B concentrations with this weight loss compared with the isocaloric, high-CHO diet. Total triglyceride concentrations as well as cholesterol content in VLDL and dense LDL subfractions returned to baseline; also, within the pooled LDL fractions, cholesterol and apolipoprotein B concentrations were lower, and their ratio did not change (Table 1; Fig. 2B). Cholesteryl ester transfer protein concentrations were significantly reduced with weight loss in this ad libitum, high-CHO phase compared with the baseline mod-FAT baseline phase ($P = 0.003$). No changes were found in LPL, HL, or PLTP activities compared with either previous dietary phase.

Study B: Moderate Fat vs Low-Fat, High-Protein Diets

Body weights also remained stable when subjects consumed the high-PRO diet compared with the isocaloric mod-FAT diet (Table 2). Switching to the high-PRO diet reduced total cholesterol ($P < 0.001$), reflecting reductions in LDL and HDL cholesterol ($P < 0.001$ for both). This decrease in total LDL cholesterol was predominantly due to reductions across the middle and more buoyant LDL subfractions (Fig. 3A), which was also detected as significant reductions in the cholesterol and apolipoprotein B content of the pooled LDL subfractions. Interestingly, the pooled LDL cholesterol to apolipoprotein B ratio also decreased and corresponded with stable levels of cholesterol content in the dense LDL subfractions (Fig. 3A). Although cholesterol concentrations in both HDL subfractions were lower, only the reduction in the HDL_3 subfraction was statistically significant. In contrast to exchanging carbohydrate for fat under isocaloric conditions in study A, no trend toward either an increase in triglyceride concentrations or significant increases in cholesterol content in VLDL fractions (Table 2; Fig. 3A) were found. Plasma activities of HL ($P < 0.001$) and PLTP ($P < 0.05$) were reduced as well as CETP concentrations ($P = 0.009$) during the isocaloric high-PRO diet phase.

When subjects consumed the low-fat, high-PRO diet ad libitum, they lost 4.8 (SD, 2.0) kg of weight ($P < 0.001$). In contrast to the effect of weight loss on lipids in study A, total-cholesterol

TABLE 1. Body Weight, Fasting Plasma Lipid and Apolipoprotein Concentrations, LDL Flotation Rate and Particle Composition, and Post–Heparin Lipase Activities, CETP Concentration, and Phospholipid Transfer Protein Activity During the Low-Fat, High-Carbohydrate Study (Study A)*

	CRC1 Isocaloric Mod-FAT	CRC2 Isocaloric High-CHO	CRC 3 Ad Libitum High-CHO
Body weight, kg	74.8 (10.6)[a]	74.4 (10.3)[a]	70.8 (11.2)[b]
Plasma lipids			
Total cholesterol, mmol/L	4.48 (0.75)[a]	4.30 (0.85)[a,b]	4.12 (0.75)[b]
Triglycerides, mmol/L	1.03 (0.42)	1.31 (0.58)	1.18 (0.47)
VLDL cholesterol, mmol/L	0.47 (0.18)	0.60 (0.26)	0.54 (0.21)
LDL cholesterol, mmol/L	2.67 (0.54)	2.54 (0.65)	2.41 (0.65)
HDL cholesterol, mmol/L	1.35 (0.36)[a]	1.17 (0.36)[b]	1.17 (0.28)[b]
HDL_2 cholesterol, mmol/L	0.32 (0.15)[a]	0.25 (0.12)[b]	0.26 (0.12)[a,b]
HDL_3 cholesterol, mmol/L	0.98 (0.28)[a]	0.90 (0.25)[b]	0.88 (0.18)[a,b]
Apolipoprotein B, g/L	0.82 (0.16)	0.84 (0.19)	0.79 (0.16)
Peak LDL particle buoyancy	0.293 (0.021)	0.281 (0.025)	0.288 (0.018)
LDL particle composition			
Cholesterol content	82.7 (29.5)[a]	78.1 (28.1)[a,b]	75.5 (25.5)[b]
Apolipoprotein B content	52.6 (18.1)[a]	51.6 (18.1)[a,b]	48.4 (15.9)[b]
Ratio of cholesterol to apolipoprotein B	1.56 (0.13)	1.52 (0.09)	1.48 (0.19)
Post–heparin lipase activities, CETP concentration, and phospholipid transfer protein activity in plasma			
LPL, nmol/mL per min	257 (85)	230 (83)	239 (101)
HL, nmol/mL per min	201 (80)	202 (80)	198 (87)
CETP, μg/mL	0.78 (0.36)[a]	0.72 (0.32)[a,b]	0.67 (0.32)[b]
Phospholipid transfer protein activity, μM/mL per h	15.4 (2.6)	15.5 (2.4)	14.8 (2.0)

Means with different superscript letters differ significantly from each other at $P < 0.05$ (repeated-measures ANOVA or Friedman test, with the Bonferroni correction applied to pairwise post hoc comparisons).

*All values are mean (SD), n = 16.

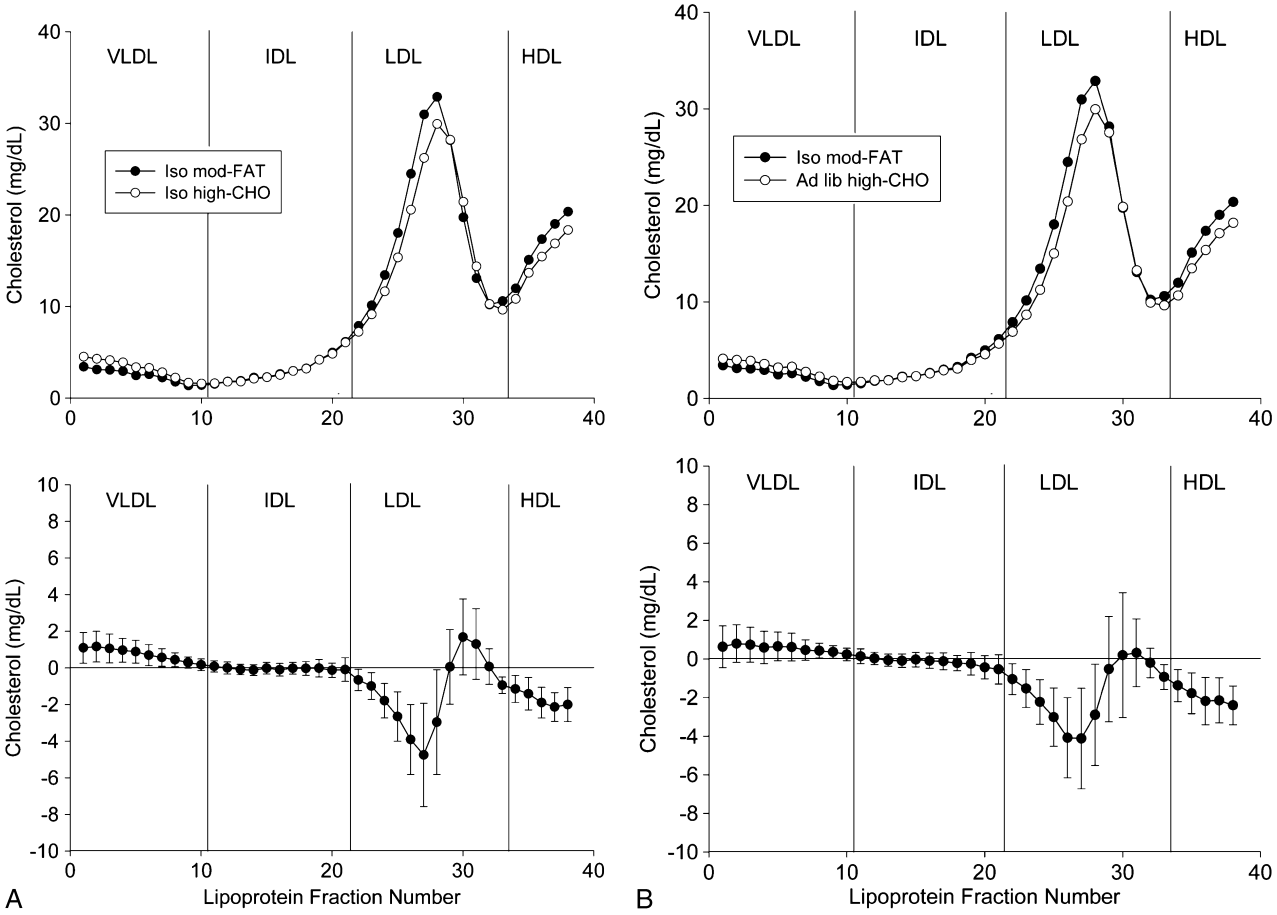

FIGURE 2. Cholesterol content in lipoprotein fractions from subjects consuming an isocaloric mod-FAT versus (A) an isocaloric low-fat, high-CHO diet and (B) an ad libitum low-fat, high-CHO diet. In each figure, the absolute cholesterol concentrations in each of the 38 individual fractions are shown on top, and the difference in cholesterol content between the 2 diet periods is displayed on the bottom. Data are means and 95% confidence intervals. Significant differences in lipoprotein fractions between groups occur when the 95% confidence intervals do not cross zero. To convert mg/dL to mmol/L, multiply by 38.6.

and LDL cholesterol concentrations tended to increase slightly, although both remained significantly lower than during the mod-FAT baseline diet. This increase in LDL cholesterol was entirely due to increases in the buoyant LDL subfractions (Fig. 3B), resulting in restoration of the baseline cholesterol to apolipoprotein B ratio in the pooled LDL subfractions. Triglyceride concentrations and total HDL cholesterol remained unchanged, although a slight increase in HDL_3 cholesterol was detected. Plasma activities of PLTP as well as the CETP concentration remained unchanged from the isocaloric low-fat, high-PRO diet period and statistically lower than the mod-FAT baseline diet; HL tended to increase and was no longer significantly lower than the baseline mod-FAT diet. Changes in LDL buoyancy (Rf) between CRC1 and CRC3 were negatively correlated with the change in HL ($r = -0.839$, $P < 0.001$) and PLTP ($r = -0.506$, $P = 0.038$), but positively with the change in LPL ($r = 0.553$, $P = 0.026$).

DISCUSSION

The design of our studies allowed us to test the effects of changes in dietary protein, carbohydrate, and fat content under both isocaloric (weight stable) and ad libitum (possibly weight loss) conditions on lipid concentrations, cholesterol content in lipoprotein subfractions, and enzyme activities of lipid transferases that are important in lipoprotein particle processing in overweight subjects.

An isocaloric reduction in total fat intake in which fatty acid composition is kept stable, with reciprocal increases in either carbohydrate or protein, results in lower plasma concentrations of total, LDL, and HDL cholesterol. We confirm previous findings that an isocaloric increase in carbohydrate is associated with a modest increase in plasma triglyceride concentrations and VLDL cholesterol concentrations, that LDL particles tend to become slightly denser,[12] and that these potentially negative metabolic effects of a low-fat, high-CHO diet were largely reversed when subjects consumed such a diet under ad libitum conditions with consequent weight loss.[29,30] We extend these findings, however, to effects on lipoprotein subparticle composition by showing that the VLDL, IDL, and dense LDL subfraction cholesterol content (and LDL peak particle density) also return to baseline with weight loss on a high-CHO diet. This latter observation of a less atherogenic LDL density with weight loss agrees with a recent report by Krauss et al.,[31] which showed that men on a fixed-calorie restriction weight-loss regimen exhibit a lower prevalence of pattern B LDL by electrophoresis on a high-CHO diet after weight loss compared with a weight-stable phase. Our analyses strongly support the hypothesis expressed in a recent meta-analysis of the effects of

TABLE 2. Body Weight, Fasting Plasma Lipid and Apolipoprotein Concentrations, LDL Flotation Rate and Particle Composition, and Post–Heparin Lipase Activities, CETP Concentration, and Phospholipid Transfer Protein Activity During the Low-Fat, High-Protein Study (Study B)*

	CRC1 Isocaloric Mod-FAT	CRC2 Isocaloric High-PROT	CRC 3 Ad Libitum High-PROT
Body weight, kg (n = 19)	72.0 (8.9)[a]	72.0 (9.1)[a]	67.2 (8.3)[b]
Plasma lipids			
Total cholesterol, mmol/L (n = 18)	4.53 (0.70)[a]	4.12 (0.67)[b]	4.27 (0.62)[b]
Triglycerides, mmol/L (n = 18)	1.00 (0.50)	1.11 (0.66)	1.03 (0.43)
VLDL cholesterol, mmol/L (n = 18)	0.47 (0.23)	0.52 (0.31)	0.47 (0.21)
LDL cholesterol, mmol/L (n = 18)	2.88 (0.67)[a]	2.56 (0.62)[b]	2.72 (0.52)[a,b]
HDL cholesterol, mmol/L (n = 18)	1.19 (0.21)[a]	1.06 (0.18)[b]	1.09 (0.18)[b]
HDL_2 cholesterol, mmol/L (n = 18)	0.32 (0.16)	0.28 (0.14)	0.28 (0.11)
HDL_3 cholesterol, mmol/L (n = 18)	0.88 (0.13)[a]	0.78 (0.10)[b]	0.82 (0.14)[a,b]
Apolipoprotein B, g/L (n = 19)	0.84 (0.17)	0.81 (0.18)	0.81 (0.16)
Peak LDL particle buoyancy (n = 19)	0.277 (0.019)	0.274 (0.026)	0.284 (0.013)
LDL particle composition			
Cholesterol content (n = 19)	95.6 (23.9)[a]	81.0 (19.1)[b]	85.7 (21.2)[b]
Apolipoprotein B content (n = 19)	62.6 (14.1)[a]	56.0 (12.5)[b]	58.0 (15.0)[a,b]
Ratio of cholesterol to apolipoprotein B (n = 19)	1.52 (0.11)[a]	1.44 (0.10)[b]	1.48 (0.10)[a]
Post–heparin lipase activities, CETP concentration, and phospholipid transfer protein activity in plasma			
LPL, nmol/mL per min (n = 16)	200 (60)	217 (57)	229 (49)
HL, nmol/mL per min (n = 16)	233 (133)[a]	170 (102)[b]	194 (117)[a,b]
CETP, μg/mL (n = 18)	1.06 (0.37)[a]	0.94 (0.29)[b]	0.94 (0.30)[b]
PLTP, μM/mL per h (n = 17)	15.3 (1.4)[a]	14.4 (1.3)[b]	14.3 (1.4)[b]

Means with different superscript letters differ significantly from each other at $P < 0.05$ (repeated-measures ANOVA or Friedman test, with the Bonferroni correction applied to pairwise post hoc comparisons).

*All values are mean (SD).

substituting dietary carbohydrates for saturated fat that "the unfavorable effect of carbohydrates on total to HDL cholesterol might be opposed by a favorable effect of carbohydrates on body weight."[32]

We further show that when fat is substituted isocalorically by protein, plasma triglyceride concentrations and VLDL cholesterol concentrations do not increase as seen on the low-fat, high-CHO diet, and the cholesterol content in dense LDL particles does not change. Counterintuitively, the spontaneous weight loss during ad libitum consumption of a high-PRO diet did not lead to even lower lipid concentrations but instead to a slight increase in LDL cholesterol as a result of increases in the cholesterol content of more buoyant LDL particles. Despite this, total and LDL cholesterol concentrations remained below the baseline mod-FAT diet with, if anything, a slight trend toward an increase in peak LDL particle buoyancy. As observed during the high-CHO diet, HDL cholesterol concentrations remained lower despite the spontaneous weight loss during ad libitum intake. This implies that the reduction in dietary fat rather than the specific replacement with carbohydrate or protein is responsible for the reductions in HDL cholesterol and that this effect is not reversed by modest weight loss.

Both CETP and PLTP play important roles in the formation and homeostasis of HDL and LDL particles[33–35] and have been implicated as modulators of CHD risk.[7,34] Previous studies have shown that both plasma CETP concentration and PLTP activity are elevated in obesity[8,10,36] and normalize with weight loss.[8,10,36] In each of our diet studies, the plasma CETP concentration was lower or tended to be lower after subjects consumed the isocaloric low-fat diets, suggesting that lowering fat intake reduces CETP, as previously reported in animals[37] and young, normolipidemic men.[38] We observed reduced PLTP activity when subjects switched to the isocaloric high-PRO diet, but not during the high-CHO diet, and in neither diet did weight loss during the ad libitum phase result in further reductions (although there was a trend toward lower PLTP activity after subjects had consumed the high-CHO diet ad libitum, $P = 0.057$). Potential explanations for the less consistent reduction in PLTP activity with weight loss in our studies include the more modest weight loss in our subjects consuming their diets ad libitum, differences in subject selection, and a higher protein content of the very-low-calorie diets used in previous studies, which might have potentiated the reduction in PLTP plasma activity.[9,10] Finally, reductions in both CETP and PLTP may explain the greater reduction in HDL_3 cholesterol during the high-PRO diet.

Two other important enzymes involved in lipoprotein processing are HL and LPL.[39,40] In general, lower HL and higher LPL activities are felt to be beneficial and associated with less atherogenic (more buoyant) LDL and HDL particles.[39,40] Previous studies of lean men have demonstrated that switching from an isocaloric high-fat to a low-fat, high-CHO diet results in an increase in HL[41] and either an increase[41] or decrease[42] in LPL activity. We found no changes in either enzyme activity in the present study under similar dietary circumstances, which may be explained by the predominance of overweight women in our study compared with the lean men in the other studies,[41,42] and although HL is known to decrease with weight loss,[43] the likely reason that we did not find similar results here is the very modest amount of weight loss that occurs with ad libitum feeding.

A potential mechanism explaining the differences between low-fat, high-CHO diets and low-fat, high-PRO diets includes

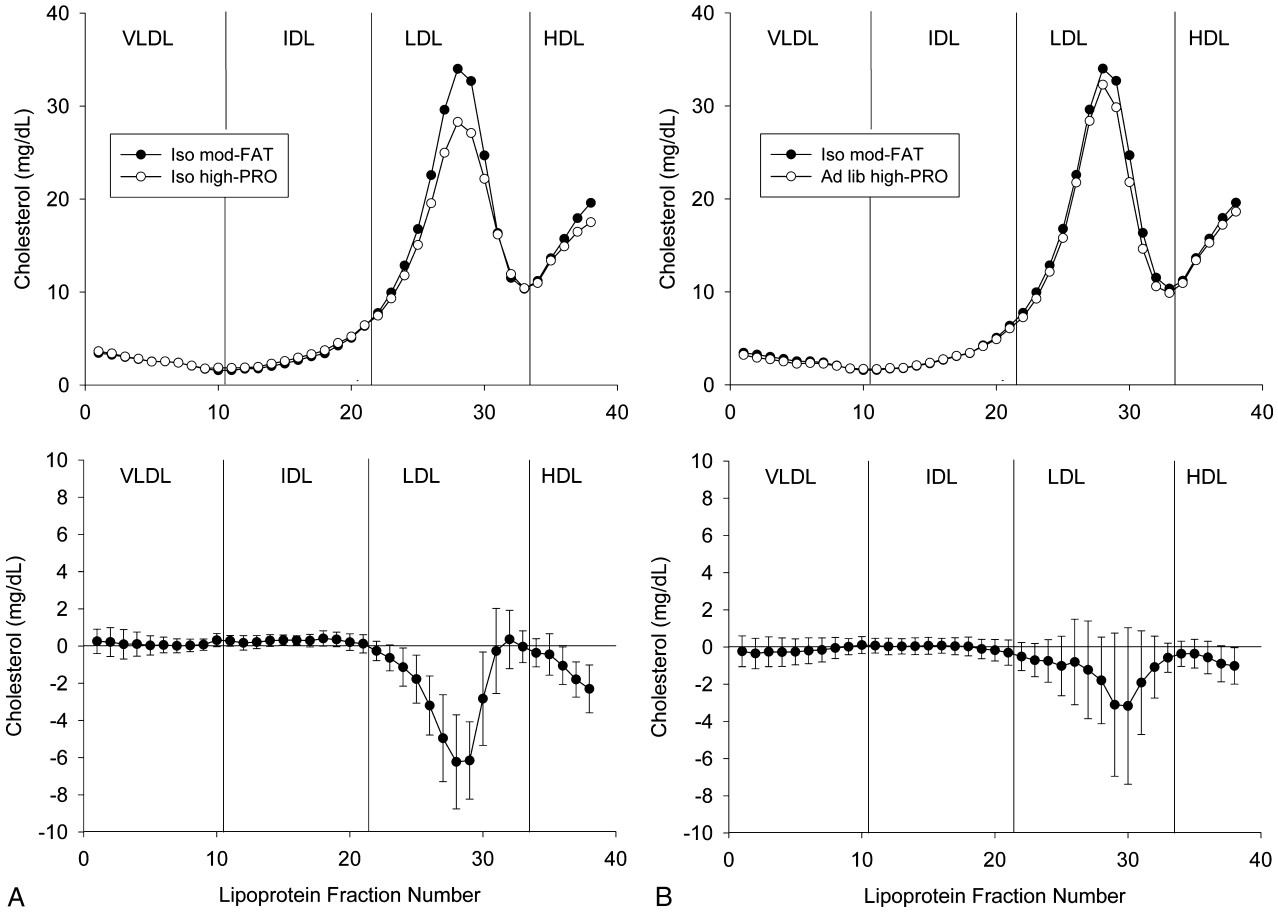

FIGURE 3. Cholesterol content in lipoprotein fractions from subjects consuming an isocaloric mod-FAT versus (A) an isocaloric low-fat, high-PROTEIN (PRO) diet and (B) an ad libitum low-fat, high-PRO diet. In each figure, the absolute cholesterol concentrations in each of the 38 individual fractions are shown on top, and the difference in cholesterol content between the 2 diet periods is displayed on the bottom. Data are means and 95% confidence intervals. Significant differences in lipoprotein fractions between groups occur when the 95% confidence intervals do not cross zero. To convert mg/dL to mmol/L, multiply by 38.6.

increased hepatic de novo lipogenesis during consumption of a high-CHO, but not high-PRO diet (reviewed in Lichtenstein[11]). This effect on lipogenesis is greater for simple sugars than for starch,[11] possibly related to the fructose content of sugars. Fructose has been shown to be removed quickly from the circulation and to enter lipogenesis in the liver.[44,45] In our study, as would be the case in most free-living individuals, the content of simple sugars increased in proportion with the overall carbohydrate content of the diet. An increased hepatic fat content has been shown to increase VLDL production as well as VLDL triglyceride content.[46] An increase in triglyceride-rich lipoproteins in plasma triggers a CETP-mediated exchange of triglycerides for cholesterol esters between triglyceride-rich lipoproteins (VLDL, IDL) and lipoproteins that are relatively richer in cholesterol esters (LDL, HDL). This exchange has 2 consequences. First, it reduces the portion of cholesterol transported in HDL. Second, it increases LDL triglycerides, which can be easily removed from LDL particles by interaction with LPL and HL, thereby lowering the overall lipid content of LDL and therewith lowering its size and increasing its density. Weight loss or even a short-term caloric deficit would be expected to counter these effects of carbohydrate-induced lipogenesis by lowering the accumulation of triglycerides in the liver, thereby reducing VLDL production. These considerations are in line with our findings in the low-fat, high-CHO study. They do not, however, provide an explanation for the lack of improvements in lipids with weight loss in the low-fat, high-PRO study.

The major strength of our study was the careful control of dietary intakes, particularly fat, carbohydrate, and protein compositions across conditions. We provided subjects with almost all of their food for a period of 16 weeks and carefully assessed ad libitum food intake by weighing back returned foods. A potential limitation to this study, however, was that subjects in both studies were still losing weight and had not yet achieved weight stability at the end of the 12-week ad libitum period.[17,18] It is possible that if a stable reduced weight had been achieved, some of the benefits of these diets on these CHD risk factors may have been less striking or even reversed.

In conclusion, we found that the reduction in body weight seen when subjects consume a low-fat, high-CHO diet ad libitum attenuates the potentially atherogenic increases in VLDL and IDL cholesterol and LDL particle density noted in previous isocaloric diet studies. A low-fat, high-PRO diet reduced LDL cholesterol without an increase in triglyceride concentrations or the cholesterol content of VLDL, IDL, or dense LDL subfractions. High-density lipoprotein cholesterol concentrations were reduced by both diets. However, the high-PRO diet caused a greater reduction in HDL_3 cholesterol in conjunction with significant reductions in HL, CETP protein concentrations, and PLTP activity. Both diets resulted in beneficial changes in lipid

concentrations in overweight subjects, especially with weight loss during ad libitum feeding. Thus, protein may be used as an alternative to carbohydrate in replacing dietary fat to treat lipid-related CHD risk factors in overweight subjects.

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