

Increased plasma concentrations of lipoprotein(a) during a low-fat, high-carbohydrate diet are associated with increased plasma concentrations of apolipoprotein C-III bound to apolipoprotein B-containing lipoproteins¹⁻³

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ABSTRACT

Background: Low-fat, high-carbohydrate (LFHC) diets have been shown to increase plasma concentrations of lipoprotein(a) [Lp(a)] and of triacylglycerol-rich lipoproteins (TRLs).

Objective: We tested whether increases in plasma Lp(a) induced by an LFHC diet are related to changes in TRLs.

Design: Healthy men (study 1; $n = 140$) consumed for 4 wk each a high-fat, low-carbohydrate diet (HFLC; 40% fat, 45% carbohydrate) and an LFHC diet (20% fat, 65% carbohydrate). Plasma lipids; lipoproteins; apolipoprotein (apo) B, A-I, and C-III; and Lp(a) were measured at the end of each diet. In a second group of men following a similar dietary protocol (study 2; $n = 33$), we isolated apo(a)-containing particles by immunoaffinity chromatography and determined the concentrations of apo C-III in ultracentrifugally isolated subfractions of apo B-containing lipoproteins.

Results: In study 1, plasma concentrations of Lp(a) ($P < 0.001$), triacylglycerol ($P < 0.001$), apo B ($P < 0.005$), apo C-III ($P < 0.005$), and apo C-III in apo B-containing lipoproteins (non-HDL apo C-III) ($P < 0.001$) were significantly higher with the LFHC diet than with the HFLC diet. Stepwise multiple linear regression analysis showed that the association of changes in Lp(a) with changes in non-HDL apo C-III was independent of changes in body mass index, apo B, LDL cholesterol, and HDL cholesterol. Plasma lipid and lipoprotein changes were similar in study 2, and we found that both total apo C-III and the apo C-III content of apo(a)-containing particles were increased in a TRL fraction consisting predominantly of large VLDL particles [TRL-apo(a)].

Conclusions: The increase in plasma Lp(a) with an LFHC diet is significantly associated with an increase in non-HDL apo C-III. Enrichment of TRL-apo(a) with apo C-III may contribute to this dietary effect on Lp(a) concentrations. *Am J Clin Nutr* 2007;85:1527-32.

KEY WORDS Dietary fat, dietary carbohydrate, apolipoprotein C-III, apolipoprotein B, lipoprotein(a), Lp(a)

INTRODUCTION

Reductions in dietary fat intake result in significant increases in plasma lipoprotein(a) [Lp(a)] concentrations (1, 2). Lp(a) is an LDL-like particle whose levels are strongly genetically influenced and are associated with increased risk of cardiovascular disease (3, 4). Lp(a) consists of an apolipoprotein (a) [apo(a)]

molecule attached to an LDL particle by disulfide linkage to the C-terminal region of apo B-100 of LDL (5). However, apo(a) can also be detected in triacylglycerol-rich lipoproteins (TRLs) of lower density (6, 7), and this TRL-associated form of apo(a) has been suggested to be particularly atherogenic (8, 9). Moreover, a recent transgenic mouse study provided strong evidence that high concentrations of Lp(a) inhibit hepatic clearance of chylomicron remnants, leading to enhanced atherosclerosis (10).

Because low-fat, high-carbohydrate (LFHC) diets result in increased concentrations of TRL (11), the possibility is raised that increases in Lp(a) observed with such diets are related to the increases in TRL. We therefore tested whether the effects of an LFHC diet on Lp(a) concentrations are related to changes in plasma concentrations of triacylglycerol. Moreover, because the apo C-III content of apo B-containing lipoproteins is related to the presence of subpopulations of TRLs with reduced plasma clearance and enhanced atherogenic potential (12), we also tested whether LFHC diet-induced changes in Lp(a) are related to changes in the apo C-III content of apo B-containing lipoproteins.

SUBJECTS AND METHODS

Subjects and experimental diets

The subjects in study 1 were 140 healthy, nonsmoking men aged >20 y who had been free of chronic disease during the previous 5 y and were not taking medication likely to interfere with lipid metabolism. They were required to have plasma total cholesterol concentrations <260 mg/dL, triacylglycerol <500 mg/dL, resting blood pressure $<160/105$ mm Hg, and body weight $<130\%$ of ideal. All subjects underwent a diet intervention involving 4 wk of a high-fat, low-carbohydrate (HFLC) diet followed by 4 wk of an LFHC diet. The HFLC diet was designed

¹ From the Children's Hospital Oakland Research Institute, Oakland, CA.

² Supported by a grant from the National Dairy Council and by a Korea Research Foundation grant funded by the Korean Government (MOEHRD) (KRF-2005-214-C00250).

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Received November 30, 2006.

Accepted for publication February 6, 2007.

to supply 40% of calories from fat (13.0% saturated, 11.0% monounsaturated, 13.8% polyunsaturated, and 3.4% *trans*), with 45% carbohydrate and 15% protein. The LFHC diet was designed to supply 20% of calories from fat (4.9% saturated, 9.9% monounsaturated, 5.1% polyunsaturated, and 2.4% *trans*), with 65% carbohydrate and 15% protein and no differences in dietary cholesterol. The ratio of simple:complex carbohydrate was \approx 50:50 with both diets.

In study 2, a total of 33 men were recruited for analyses of changes in levels and composition of apo(a)-containing particles isolated by immunoaffinity chromatography (IAC) and separated by density gradient ultracentrifugation as described below. The selection criteria were the same as those for study 1, except that men with Lp(a) concentrations <3 mg/dL were excluded. The experimental protocol including the diet intervention was identical to that of study group 1, except that the diets (HFLC versus LFHC) were consumed in a randomized crossover design.

Registered dietitians supplied the participants with personalized menus based on a 2-wk menu cycle and demonstrated the number and size of servings for the experimental LFHC diet. Nutrient composition was calculated using the Minnesota Nutrition Data System software, version 2.1, developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis (13, 14). Registered dietitians instructed the participants on the experimental diets at a group orientation class, provided written educational materials, and contacted the subjects weekly to encourage motivation and to ascertain compliance. The subjects abstained from alcohol throughout the study and were instructed to maintain their customary level of physical activity. Compliance with the experimental diet was assessed by a 4-d food record (Thursday through Sunday) of measured and weighed food intake (15) and by a daily recording of added and missed foods from the menus. If the daily diet deviations averaged $>5\%$ of total calories, the subject was considered noncompliant and the data were not included in the analyses (all subjects were compliant). Nutrient calculation of the 4-d food record was performed by using NUTRITION DATA SYSTEM software (13, 14). The subjects measured their own body weight daily at home, and the staff adjusted energy intake on the menus weekly if necessary for body weight stability. Body mass index (BMI; in kg/m^2) was calculated from weight and height measurements taken after each diet period. Subjects signed a consent form approved by the Institutional Review Boards at Children's Hospital Oakland and EO Lawrence Berkeley National Laboratory, University of California, Berkeley, and participated in a medical interview.

Laboratory measurements

Plasma samples were prepared within 2 h of collection from venous blood collected in tubes containing Na_2EDTA (1.4 g/L) and a preservative cocktail of protease and bacterial inhibitors. Blood and plasma were kept at 4°C throughout processing. Plasma total cholesterol and triacylglycerol concentrations were determined by enzymatic procedures on an Express 550 Plus analyzer (Ciba Corning, Oberlin, OH). These measurements were consistently in control as monitored by the standardization program of the Centers for Disease Control–National Heart, Lung and Blood Institute. HDL cholesterol was measured after dextran sulfate precipitation of plasma (16), and LDL cholesterol was calculated from the formula of Friedewald et al (17). Apo A-I

and apo B were measured by immunoturbidometric assay (Bacton Assay Systems, San Marcos, CA, and Express 550 Plus analyzer). Apo C-III was measured in triplicate by sandwich-style enzyme-linked immunosorbent assay (ELISA) with purchased goat anti-human apo C-III (International Immunology Corp, Murrieta, CA). The effective range of concentration measurement on the microtiter plate was ≈ 0.25 – 5.0 ng with a CV within 10%. Validation of calibration standards and controls was made by exchange of plasma samples with the Oklahoma Medical Research Foundation/Lipid and Lipoprotein Laboratory (Director: Petar Alaupovic). Dextran sulfate precipitation (16) was used to obtain a supernatant layer depleted of apo B–containing lipoproteins and for measurement of HDL apo C-III. The concentration of apo C-III in apo B–containing lipoproteins (non-HDL apo C-III) was calculated by the difference in concentrations of HDL apo C-III and total apo C-III. Lp(a) plasma concentrations were measured in triplicate by a sandwich assay with polyclonal antibodies (International Immunology Corp). Validation of the ELISA was carried out by comparison with measurements in split plasma samples ($n = 48$) carried out by radioimmunoassay (Northwest Lipid Research Clinic, Seattle, WA) and by commercially available Lp(a) diagnostic ELISA kits (Macra Lp(a), Elkton, MD). Linear regression analysis of the results showed slopes of 1.37 ($r = 0.93$) and 0.79 ($r = 0.96$) for the 2 method comparisons, respectively. Sensitivity of the Lp(a) assay was ≤ 0.75 mg/dL; intra- and interassay variation was $\leq 10\%$.

Preparation of apo(a)-depleted plasma by immunoaffinity chromatography

Plasma was filtered by using a $0.4\text{-}\mu\text{mol}/\text{L}$ syringe filter and was adjusted to $10\ \mu\text{mol}$ Trolox/L (Aldrich, Sigma-Aldrich Corp, St Louis, MO), $1\ \text{mmol}$ diethylnitrophenyl phosphate/L, and 0.03% benzamidine. A 3-mL aliquot of the adjusted plasma was then added to an immunoaffinity column prepared from purified goat anti-human Lp(a) sera (International Immunology Corp) covalently linked to Affigel-10 (BioRad Laboratories, Hercules, CA) according to the manufacturer's instructions. The column was then incubated at 4°C with rocking overnight. An IAC-unbound fraction containing plasma depleted of apo(a)-containing particles was washed from the column with neutral buffer ($20\ \text{mmol}$ tris/L, pH 8) until a total volume of $30\ \text{mL}$ was collected. The unbound fraction was then concentrated to $2\ \text{mL}$ by using Centriprep ultrafiltration units ($10\ \text{K}$ molecular mass cutoff; Amicon Div, Beverly, MA).

Preparation of lipoprotein subfractions by density gradient ultracentrifugation

Aliquots of both untreated plasma and the apo(a)-depleted IAC-unbound fraction ($1.5\ \text{mL}$ each) were pipetted to the bottom of a 12-mL ultracentrifuge tube (Beckman catalog no. 344059; Beckman Coulter Inc, Fullerton, CA) and mixed with $3\ \text{mL}$ D_2O ($d = 1.11\ \text{g}/\text{mL}$) and $12\ \mu\text{L}$ of $10\ \text{mmol}$ Trolox/L (Aldrich). A continuous linear density gradient ($d\ 1.00$ – $1.075\ \text{g}/\text{mL}$) was then formed above the sample- D_2O mixture by using 2 high-performance fast protein liquid chromatography pumps programmed to deliver solution A (water) and solution B (D_2O ; density $1.11\ \text{g}/\text{mL}$) after passage through a mixing chamber. The total 7.5-mL gradient solution volume was delivered to a centrifuge tube positioned at a 55° angle at $1\ \text{mL}/\text{min}$ at 4°C . The tube

TABLE 1Plasma measurements during high-fat, low-carbohydrate (HFLC) and low-fat, high-carbohydrate (LFHC) diets¹

	HFLC diet (n = 140)	LFHC diet (n = 140)	P
Triacylglycerol (mg/dL)	113.5 ± 124.3 ²	139.5 ± 117.1	< 0.001
Total cholesterol (mg/dL)	174.7 ± 36.2	168.1 ± 34.6	< 0.001
LDL cholesterol (mg/dL)	109.5 ± 31.2	103.1 ± 27.7	< 0.001
HDL cholesterol (mg/dL)	42.9 ± 10.3	37.5 ± 8.5	< 0.001
Apo A-I (mg/dL)	115.6 ± 18.4	106.9 ± 15.9	< 0.001
Apo B (mg/dL)	89.6 ± 25.7	93.9 ± 25.7	< 0.005
Apo C-III (μg/mL)	112.7 ± 63.7	123.1 ± 66.6	< 0.005
Non-HDL apo C-III (μg/mL)	28.9 ± 35.3	47.1 ± 38.4	< 0.001
HDL apo C-III (μg/mL)	83.6 ± 35.6	75.6 ± 37.7	< 0.001
Ratio of HDL to non-HDL apo C-III	6.0 ± 8.5	3.3 ± 6.1	< 0.005
Lp(a) (mg/dL) ³	8.91 (3.41–34.6)	11.47 (3.84–38.78)	< 0.001

¹ apo, apolipoprotein.² $\bar{x} \pm SD$ (all such values).³ Median (interquartile range).

was then moved to an upright position, and 1 mL water was layered on top of the gradient. Tubes were centrifuged at 22 500 rpm, 10 °C, for 16 h in a Beckman SW 41 rotor. After centrifugation, fractions were collected from the top with volumes of 1 mL, 1 mL, 1 mL, 2 mL, 3 mL, and 4 mL for fractions 1–6, respectively. Fractions were analyzed for concentrations of triacylglycerol, total cholesterol, apo B, and apo C-III as described above. In addition, apo(a) concentrations were measured by using the Lp(a) assay procedure described above. Concentrations of apo(a)-bound apo C-III in each fraction were determined as the difference between apo C-III in fractions derived from untreated plasma and from apo(a)-depleted plasma. In addition, the size distribution of the lipoprotein particles in each density fraction was analyzed by gradient gel electrophoresis in 2–14% polyacrylamide gels with lipid staining as described previously (18). On the basis of particle diameter ranges reported elsewhere (19), particles were classified as large VLDL 1 (330–700 Å), small VLDL 2 (300–330 Å), IDL (272–300 Å), and LDL (220–272 Å). Large VLDL were found predominantly in fraction 1; small VLDL were mainly contained in fractions 2 and 3; IDL were primarily in fractions 3 and 4; and the major portion of LDL was

isolated in fractions 5 and 6, which also contained HDL and plasma proteins that are not visualized by use of this staining procedure.

Statistical analysis

SPSS 12.0 (SPSS Inc, Chicago, IL) was used for statistical analysis. Data are presented as means ± SDs. Each variable was examined for a normal distribution, and abnormally distributed variables were log-transformed. Lp(a) was expressed as medians (interquartile ranges) because of its skewed distribution. We used Pearson correlation coefficients to evaluate relations between variables. Differences in variables between the HFLC diet and the LFHC diet were evaluated by using a paired Student's *t* test. Multiple regression analysis was used to determine the factors contributing to differences in Lp(a) between the HFLC and LFHC diets. *P* values < 0.05 were considered statistically significant.

RESULTS

The subjects in study 1 (*n* = 140) were healthy men aged 37.2 ± 7.9 y (mean BMI: 24.7 ± 3.1). As shown in **Table 1**, total cholesterol (*P* < 0.001), LDL cholesterol (*P* < 0.001), HDL cholesterol (*P* < 0.001), apo A-I (*P* < 0.001), and HDL apo C-III

TABLE 2Pearson correlations between diet-induced changes (Δ ; 20% low-fat diet results minus 40% high-fat diet results) in lipoprotein(a) [Lp(a)] and those in other lipids, lipoproteins, and apolipoproteins (apos)¹

	Δ Lp(a)
Δ Triacylglycerol	0.108
Δ Total cholesterol	0.202 ²
Δ HDL cholesterol	-0.194 ²
Δ LDL cholesterol	0.208 ²
Δ Apo A-I	-0.056
Δ Apo B	0.299 ³
Δ Apo C-III	0.153
Δ non-HDL apo C-III	0.255 ⁴
Δ HDL apo C-III	-0.100

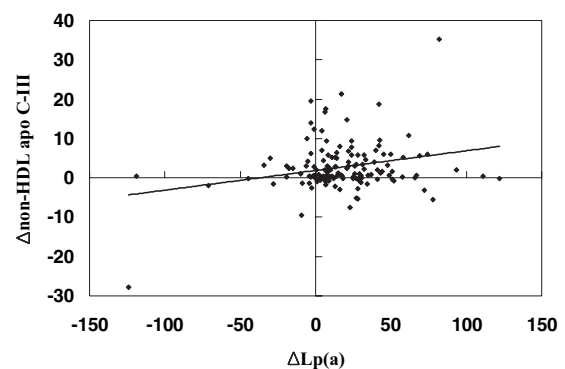
¹ Nonnormally distributed variables were log transformed before analysis.² *P* < 0.05.³ *P* < 0.001.⁴ *P* < 0.005.**FIGURE 1.** Correlation between diet-induced changes in non-HDL apolipoprotein (apo) C-III and changes in lipoprotein(a) [Lp(a)]. *r* = 0.255, *P* < 0.005.

TABLE 3Stepwise multiple regression analyses to identify factors influencing changes (Δ) in lipoprotein(a) [Lp(a)]¹

Dependent variable and model	Independent variable	Unstandardized		Adjusted		<i>R</i> ²
		β -Coefficient	Constant	β -Coefficient	<i>P</i>	
Δ Lp(a)						
1 step	Δ Apo B	0.106	2.265	0.273	0.001	0.068
2 step	Δ Apo B	0.092	1.569	0.237	0.004	0.106
	Δ non-HDL apo C-III	0.042		0.215	0.010	

¹ Regression models included age, Δ BMI, Δ apo B, Δ LDL cholesterol, Δ HDL cholesterol, and Δ non-HDL apo C-III.

($P < 0.001$) were significantly lower with the LFHC diet than with the HFLC diet, and plasma concentrations of triacylglycerol ($P < 0.001$), apo B ($P < 0.005$), apo C-III ($P < 0.005$), non-HDL apo C-III ($P < 0.001$), and Lp(a) ($P < 0.001$) were significantly higher.

Correlation analysis (Table 2) showed that the higher concentrations of Lp(a) were significantly associated with higher concentrations of total cholesterol, LDL cholesterol, and apo B and with lower concentrations of HDL cholesterol. In contrast, there was no association between diet-induced changes in Lp(a) and plasma triacylglycerol. Increases in Lp(a) with the LFHC diet were significantly related to increases in non-HDL apo C-III ($P < 0.005$; Figure 1). Stepwise multiple linear regression incorporating all the variables that were significantly associated with diet-induced Lp(a) changes showed that increases in both apo B and non-HDL apo C-III were independent predictors of increases in Lp(a) (Table 3).

On the basis of the observed associations between diet-induced changes in Lp(a) and changes in apo C-III, we further hypothesized that there might be an increase in the physical association of apo C-III with apo(a)-containing particles after the LFHC diet. To address this question, we used IAC to determine the concentrations of apo C-III bound to apo(a) in ultracentrifugally isolated lipoprotein subfractions for each of the experimental diets. These analyses were carried out in study group 2 ($n = 33$). Mean age and BMI were 48.4 ± 9.8 y and 26.6 ± 2.8 , respectively. Effects of the LFHC diet on plasma lipids and

lipoproteins in these subjects were similar to those for the subjects in study 1 (data not shown). The concentrations of apo B, apo(a), and apo C-III in each of the 6 fractions separated by density gradient ultracentrifugation are shown in Table 4. Also shown are the concentrations of apo C-III associated with apo(a)-containing particles as determined by IAC in each fraction. Compared with the HFLC diet, the LFHC diet resulted in higher concentrations of apo B and apo C-III in fraction 1, which contained mainly large VLDL. There was also a reduction in apo B in fraction 5 and an increase in fraction 6, consistent with a shift in the distribution of LDL to denser particles, and a reduction in apo C-III in fraction 6. Apo(a) was present primarily in fractions 5 and 6, but the increase induced by the LFHC diet was observed only for fraction 6. In addition, there was a small but significant increase in apo(a) in fraction 4, which was enriched in IDL. Notably, the apo C-III content of apo(a)-containing particles (Table 4) was significantly higher only in fraction 1, consistent with a dietary effect on a TRL-apo(a) subfraction enriched in apo C-III.

DISCUSSION

Although Lp(a) concentrations are mainly determined by genetic factors, low-fat diets have been found to induce significant increases in Lp(a) (1, 2). In the present study, we confirmed this dietary effect on Lp(a) and found that the magnitude of the increase was significantly and independently correlated with

TABLE 4Concentrations of apolipoprotein (apo) B, apo(a), total apo C-III, and apo(a)-bound apo C-III after high-fat, low-carbohydrate (HFLC) and low-fat, high-carbohydrate (LFHC) diets in lipoprotein subfractions separated by density gradient ultracentrifugation¹

Fraction	Apo B		Apo(a)		Total apo C-III		Apo(a)-bound apo C-III	
	HFLC	LFHC	HFLC	LFHC	HFLC	LFHC	HFLC	LFHC
	<i>mg/dL</i>		<i>mg/dL</i>		$\mu\text{g/mL}$		$\mu\text{g/mL}$	
1	4.3 ± 2.8^2	6.6 ± 4.5^3	$0.17 (0.08-0.48)^4$	$0.10 (0.06-0.39)$	36.3 ± 26.1	58.0 ± 46.0^5	6.6 ± 9.9	9.5 ± 11.1^6
2	1.2 ± 1.7	1.2 ± 0.7	$0.06 (0.00-0.12)$	$0.04 (0.00-0.08)$	7.60 ± 15.8	6.40 ± 5.4	3.7 ± 1.3	1.5 ± 1.6
3	1.0 ± 0.4	1.1 ± 0.5	$0.06 (0.04-0.09)$	$0.06 (0.00-0.09)$	3.60 ± 2.3	3.80 ± 2.3	0.9 ± 1.3	1.1 ± 1.0
4	6.0 ± 1.9	5.6 ± 1.7	$0.17 (0.11-0.27)$	$0.20 (0.10-0.45)^3$	6.10 ± 2.4	6.10 ± 2.6	1.6 ± 1.4	1.3 ± 1.1
5	44.6 ± 12.5	36.2 ± 11.6^6	$2.85 (0.93-7.39)$	$2.66 (0.10-7.80)$	14.6 ± 4.6	13.9 ± 4.2	3.3 ± 2.6	2.9 ± 3.2
6	23.4 ± 8.2	26.0 ± 7.4^6	$3.61 (1.07-11.9)$	$6.78 (3.35-17.85)^3$	45.0 ± 14.7	37.9 ± 9.9^7	7.2 ± 4.9	7.0 ± 4.5

¹ $n = 33$. See Subjects and Methods for characterization of the density fractions.² $\bar{x} \pm$ SD (all such values).³ $P < 0.001$.⁴ Median; interquartile range in parentheses (all such values).⁵ $P < 0.005$.⁶ $P < 0.05$.⁷ $P < 0.01$.


LFHC diet-induced increases in apo C-III in apo B-containing lipoproteins (non-HDL apo C-III), together with an increase in total plasma apo B.

Apo C-III is an 8.8-kDa protein found primarily on the surface of VLDL and HDL. In normotriacylglycerolemic subjects, apo C-III is mostly bound to apo A-containing lipoproteins; when triacylglycerol is increased, however, apo C-III becomes redistributed to apo B-containing lipoproteins and total apo C-III increases (20, 21). Clinically, concentrations of non-HDL apo C-III have been associated with progression of coronary artery disease (22, 23). Apo C-III inhibits TRL catabolism by inhibiting both lipoprotein lipase activity (24, 25) and apo E-mediated receptor clearance of TRL lipolytic remnants (26). These properties of apo C-III are consistent with evidence that patients with inherited apo C-III deficiency (24) and apo C-III gene knockout mice (27) have reduced plasma triacylglycerol concentrations. On the other hand, overexpression of the human apo C-III gene in transgenic mice (28) and certain apo C-III gene polymorphisms in humans (29, 30) result in hypertriacylglycerolemia. Along with these potentially atherogenic effects of apo C-III on lipid metabolism, recent evidence also suggests that apo C-III may directly contribute to the development of atherosclerosis by enhancing the adhesion of human monocytic cells to endothelial cells (31).

It is well established that high carbohydrate intake concomitant with a reduction in dietary fat results in increased concentrations of plasma triacylglycerol and TRL (11). Diet-induced changes in plasma triacylglycerol have been correlated with changes in plasma apo C-III concentrations (32). Because apo C-III is a component of TRL and their remnants, it is possible that the increase in plasma apo C-III observed in the present study was the result of increased partitioning of apo C-III from HDL to the increased plasma TRL pool. This is supported by our finding that apo C-III concentrations were higher in the fraction enriched with large VLDL after the LFHC diet. It is also conceivable that the LFHC diet results in apo C-III overproduction, but this has not been experimentally shown.

Among possible explanations for our finding that LFHC diet-induced increases in Lp(a) are correlated with increases in both plasma apo B and non-HDL apo C-III is that the metabolism of apo(a)- and apo C-III-containing TRLs is coordinately regulated in response to an LFHC diet. On the basis of our finding that the LFHC diet resulted in enrichment of apo C-III in an apo(a)-containing fraction of large VLDL, we hypothesize that the dietary effect on Lp(a) may involve increased transport of apo C-III on apo(a)-containing TRL particles [TRL-apo(a)]. This increase, however, did not account for the diet-induced increase in total plasma Lp(a). It may be that a subset of plasma Lp(a) particles is derived by catabolism of TRL-apo(a), and that the diet-induced increase in Lp(a) results, in part, from an increase in production of apo C-III-containing TRL-apo(a) precursors that undergo metabolic conversion to triacylglycerol- and apo C-III-depleted Lp(a) particles. This hypothesis is consistent with evidence that TRL-Lp(a) is secreted from the liver as a precursor of mature Lp(a) (33). It is possible that the small increase in concentration of Lp(a) that we observed in the IDL particle size range represents lipolytic remnants formed during the transformation from TRL-apo(a) to mature Lp(a). Notably, the KIV₅₋₈ peptide of apo(a) was recently reported to compete with remnant lipoproteins for hepatic uptake (10), which suggests that apo(a)-TRL may share properties with remnant particles.

A possible analytic concern is that the assay for plasma Lp(a) might not accurately measure apo(a) concentrations in TRL, and if this were the case, this could affect the apo(a) measurements in fractions 1–4 in Table 4. Another limitation is that because the 2 experimental diets differed in fatty acid composition as well as absolute content of both simple and complex carbohydrates, it is not possible to attribute the lipoprotein changes observed between the 2 diets to a specific nutrient effect.

In conclusion, we showed that an LFHC diet results in inter-related increases in non-HDL apo C-III and Lp(a). Moreover, the increased physical association of apo C-III with TRL-apo(a) particles could, at least in part, contribute to the relation between changes in Lp(a) and changes in apo C-III induced by an LFHC diet. It has been reported that normolipidemic patients with coronary artery disease have a higher proportion of apo(a) in the TRL fraction than do normolipidemic healthy subjects (9). Our findings raise the possibility that this atherogenic TRL-apo(a) fraction is enriched in particles containing apo C-III. 

We thank Laura Holl, Joseph Orr, Bahareh Sahami, and Michael Chu for technical assistance and Petar Alaupovic for helpful discussions. The author's responsibilities were as follows—RMK: principal investigator; MJS and RMK: analyzed the data; MJS: performed the biochemical analysis and wrote the article; PJB: laboratory manager; RSR: clinical coordinator; HSF: dietitian. The National Dairy Council had no role in the design or conduct of the study or in the analysis or interpretation of the study results.

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