Apolipoprotein A-I, A-II, and VLDL-B-100 metabolism in men: comparison of a low-fat diet and a high-monounsaturated fatty acid diet

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Abstract The impact of a low-fat diet and a high-MUFA diet on apolipoprotein A-I (apoA-I), apoA-II, and VLDL-apoB-100 metabolism in conditions of unrestricted (ad libitum) energy intake was compared in 65 men randomly assigned to one of two predefined experimental diets. A subsample of 18 men participated in the kinetic study. Before and after the 6–7 week dietary intervention, kinetic subjects received a primed-constant infusion of $[5,5,5\text{-}^2H_3]$L-leucine for 12 h under feeding conditions. ApoA-I production rate (PR; -31.5%; $P < 0.001$) and fractional catabolic rate (FCR; -24.3%; $P < 0.05$) were significantly decreased after the low-fat diet. These changes in apoA-I PR and FCR with the low-fat diet were also significantly different from those observed with the high-MUFA diet ($P < 0.01$ and $P < 0.05$, respectively). ApoA-II FCR was significantly increased in the high-MUFA group only. No significant within- or between-diet difference was found in VLDL-apoB-100 PR or FCR. These results emphasize the differential impact of the low-fat diet and high-MUFA diet on HDL metabolism.—Desroches, S., M-E. Paradis, M. Pérusse, W. R. Archer, J. Bergeron, P. Couture, N. Bergeron, and B. Lamarche. **Apolipoprotein A-I, A-II, and VLDL-B-100 metabolism in men: comparison of a low-fat diet and a high-monounsaturated fatty acid diet.** *J. Lipid Res.* 2004. **45:** 2331–2338.

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Increased plasma LDL-cholesterol (LDL-C) concentrations (1), increased triglyceride (TG) concentrations, and low plasma HDL-cholesterol (HDL-C) concentrations (2) are well-established risk factors for coronary heart disease (CHD). Among the different strategies to reduce the risk of CHD in the general population, the American Heart Association has recommended the adoption of a diet low in saturated fat and high in complex carbohydrate (CHO) (3), mainly for its beneficial lowering properties on LDL-C concentrations. However, controlled nutritional interventions conducted under isocaloric conditions, in which body weight was artificially held constant for experimental purposes, have been associated with undesirable reductions in plasma HDL-C concentrations and increases in TG concentrations after a low-fat/high-CHO diet (4). It is on that basis that scientists have raised concerns regarding the appropriateness of low-fat/high-CHO diets to reduce the risk of CHD. On the other hand, it has been suggested that ad libitum feeding of low-fat/high-CHO diets may not induce deleterious changes in HDL-C and TG concentrations by being associated with weight loss attributable to a spontaneous reduction of energy intake (5).

Mediterraneans show a low prevalence of CHD, despite the consumption of a diet that can provide more than 35% of energy as fat depending upon the geographical region (6). The large amount of MUFAs consumed mainly in the form of olive oil in the traditional Mediterranean diet is thought to account for part of its protective effect against CHD (7). Data from the large Nurses Health Study have also demonstrated the cardiovascular benefits attributable to a greater intake of MUFAs (8). Therefore, the consumption of high-fat diets rich in MUFAs has been advocated as a preferable alternative to low-fat/high-CHO diets, based upon the LDL-C- and TG-lowering properties of MUFAs and their relatively neutral effects on plasma HDL-C concentrations (9).

Thus, the identification of the most appropriate diet in terms of plasma HDL-C and TG modification remains a

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Abbreviations: apoA-I, apolipoprotein A-I; CHD, coronary heart disease; CHO, carbohydrate; FCR, fractional catabolic rate; HDL-C, HDL-cholesterol; IEF, isoelectric focusing; LDL-C, LDL-cholesterol; PR, production rate; TG, triglyceride.

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matter of controversy. A better understanding of the mechanisms by which different dietary interventions affect HDL and VLDL composition and metabolism may help to reveal the most effective dietary regimen to prevent CHD risk. To date, only a few kinetic studies have been conducted in humans to help understand the effects of low-fat/high-CHO diets (10–12) and high-unsaturated fatty acid diets (13–15) on apolipoprotein kinetics.

The objective of the present study, therefore, was to compare the effects of a low-fat/high-CHO diet and a high-MUFA diet consumed ad libitum on plasma apolipoprotein A-I (apoA-I), apoA-II, and VLDL-apoB-100 kinetics in men.

METHODS

Study design

The study participants and design have been described in detail previously (16). Briefly, 65 men, of which 18 participated in the kinetic study, were recruited in the Québec metropolitan area. Participants were recruited to cover a wide range of adiposity and had to be nonsmokers and free of any endocrine, cardiovascular, hepatic, or renal disorder. Subjects using medication likely to interfere with lipid metabolism at the time of screening were excluded. Individuals with unusual dietary habits, such as vegetarianism, food aversions, or allergies, and those who had experienced a significant weight change within the year that preceded the study onset were also excluded. Each participant signed a consent form approved by the Laval University Ethics Committee. Subjects were randomly assigned to either a low-fat/high-CHO diet or a high-fat diet rich in MUFAs, which they consumed for 6–7 weeks. Subjects were instructed to maintain their usual level of physical activity throughout the study and to refrain from intense physical exercise for the 3 days preceding the beginning and the end of the study. Participants and laboratory technicians were blinded to dietary assignments.

Experimental diets

The low-fat/high-CHO diet and the high-MUFA diet comprised food prepared daily in the metabolic kitchen and weighed in individual portions. Both diets consisted of the same meals, which differed mainly in the amount of fat and CHO provided (**Table 1**). The diets were composed of nonhydrogenated unsaturated fats, mainly olive oil, with whole grains and vegetables as the main forms of CHOs. Simple sugars were used only in the preparation of muffins and some desserts. The nutritional composition of the experimental diets was assessed with the Canadian Nutrient File database (Health Canada, Ottawa, 1997) and Nutrition Data System for Research software (Nutrition Coordinating Center, Minneapolis, MN; database version 4.03_30,1999).

Dietary intervention

On weekdays, subjects came to the metabolic unit to consume their lunch meal and were given their next dinner and breakfast meals packaged to take home. Weekend meals were given to the participants on Fridays. The breakfast represented 20% of the daily energy intake in each diet and had to be consumed completely, whereas lunch and dinner meals each provided 40% of daily energy intake. To achieve ad libitum conditions, subjects were provided with food representing 150% of their usual daily energy intake as assessed by a 3-day food record (2 weekdays and 1 weekend day) obtained at baseline. Uneaten portions had to be returned to the metabolic unit for measurement of actual energy intake. On demand, participants were provided with 200 kcal snacks prepared in the metabolic kitchen. The macronutrient content of the snacks matched that of the two experimental diets. Subjects were asked to restrict their intake of caffeine-containing beverages to less than two per day but had free access to water and to diet/caffeine-free soft drinks. As indicated previously, compliance with the diet was judged to be excellent (16).

Anthropometric measurements

Body weight and waist circumference were measured according to standardized procedures (17).

Lipid and lipoprotein analyses

The lipoprotein-lipid and HDL profiles were determined at the beginning and the end of the dietary interventions. Plasma and HDL lipid concentrations were measured by enzymatic methods on a Technicon RA-500 analyzer (Bayer Corp., Tarrytown, NY) as previously described (18). Plasma VLDL (d < 1.006 g/ml) was isolated by ultracentrifugation, and the HDL fraction was obtained after precipitation of LDL in the infranatant (d > 1.006 g/ml) with heparin and $MnCl_2$ (19). The cholesterol, TG, and phospholipid contents of the infranatant fraction were measured before and after the precipitation step. The lipid content of the HDL_2 and HDL_3 subfractions was also determined on the Technicon RA-500 analyzer after further precipitation of HDL_2 with dextran sulfate (20). HDL particle size was determined by polyacrylamide gradient gel electrophoresis as described previously (21). Plasma apoA-I, apoA-II, and VLDL-apoB-100 concentrations were measured by nephelometry in the various samples (22). Plasma HDL's that contain apoA-I but not apoA-II (LpA-I) and HDL's that contain both apoA-I and apoA-II (LpA-I:A-II) concentrations were assessed by an electroimmunodiffusion technique using commercially available agarose gels with polyclonal anti-apoA-I and anti-apoA-II antibodies incorporated into the gels (Laboratories Sebia, Norcross, GA) as described previously (23).

Kinetic study

The kinetic study was performed at the beginning and at the end of the dietary interventions using a primed constant infusion of $[5,5,5\text{-}^2H_3]$L-leucine. After a 12 h fast, kinetic subjects were fed cookies (65% CHO, 20.2% lipids, 14.5% protein) every 30 min for 15 h. Each half-hourly portion represented 1/30th of their daily energy requirements.

Three hours after their first half-hourly meal, subjects received an intravenous bolus dose of 10 μmol/kg $[5,5,5\text{-}^2H_3]$ L-leucine, which was subsequently followed by a constant infusion at 10 μmol/kg/h. Throughout the infusion, blood samples were collected by a second intravenous line into Vacutainer tubes containing EDTA at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 h. We have recently shown that this fed-state kinetic protocol

TABLE 1. Nutritional composition of the experimental diets

Constituent	Low-Fat/High-CHO Diet	High-MUFA Diet
CHO (% energy)	58.3	44.7[a]
Fat (% energy)	25.8	40.1[a]
Saturated (% energy)	6.0	8.2[a]
Monounsaturated (% energy)	13.3	22.5[a]
Polyunsaturated (% energy)	5.1	7.6[a]
Protein (% energy)	15.9	15.2
Total fibers (g/1,000 kcal)	14.2	10.1[a]
Polyunsaturated/saturated ratio	0.87	0.93
Cholesterol (mg/1,000 kcal)	105.8	110.1

[a] Significantly different from the low-fat/high-carbohydrate (CHO) diet ($P \le 0.01$).

allowed study participants to achieve steady state rapidly, with plasma TG concentrations being maintained throughout the infusion period (24).

Isotopic enrichment measurement

ApoA-I and apoA-II were isolated from the d < 1.25 g/ml fraction obtained after centrifugation of whole plasma for 48 h at 50,000 rpm at 4°C in a Beckman 50.4 Ti rotor. Infranatant was then dialyzed overnight in a NaCl-Tris-Base-EDTA buffer, incubated with cysteamin for 4 h at 37°C, and delipidated using aceton-ethanol and diethylic ether as described previously (25). ApoA-I and apoA-II were then separated using preparative isoelectric focusing (IEF) on polyacrylamide-urea gels. VLDL-apoB-100 (d < 1.006 g/ml) was separated by SDS-PAGE according to standardized procedures (26) as described previously (27). Apolipoproteins on IEF and SDS polyacrylamide gels revealed with Coomassie blue were excised and hydrolyzed with 6 N HCl and incubated at 110°C for 24 h. Free amino acids in the hydrolysate were purified by cation-exchange chromatography and derivatized by adding propanol/acetyl chloride and heptafluorobutyric anhydride reagents. Samples were then analyzed using a gas chromatograph-mass spectrometer (GC 6890N, MS 5973N; Agilent Technologies, Palo Alto, CA). Identification of labeled and unlabeled leucine was obtained by methane-negative chemical ionization. Selective ion monitoring at $m/z = 352/349$ was used to determine the tracer/tracee ratio, which was calculated using the formula described previously (28).

Kinetic analyses

ApoA-I, apoA-II, and VLDL-apoB-100 fractional catabolic rate (FCR) was determined by fitting the tracer/tracee ratios to a monoexponential function. Using SAAM II software (University of Washington, Department of Bioengineering, Seattle, WA), data were fitted to the mathematical function $Z(t) = Z_p(1 - e^{-k(t-d)})$, where $Z(t)$ is the tracer/tracee ratio at time t, Z_p is the tracer/tracee ratio corresponding to the plateau of the curve representing the precursor amino acid pool, d is the delay time in hours, and k is the FCR in pools per hour. The VLDL-apoB-100 enrichment plateau was used as the forcing function, reflecting precursor pool enrichment for all apolipoproteins. The absolute production rate (PR) was calculated (in mg/kg/d) using the following formula:

$$PR = \frac{FCR \text{ (pools/day)} \times \text{plasma apolipoproteins pool size (mg)}}{\text{body weight (kg)}}$$

(Eq. 1)

Pool size was calculated as the plasma apoA-I, apoA-II, or VLDL-apoB-100 concentration (mg/l) multiplied by plasma volume (value fixed at 0.045 l/kg body weight) (29).

Statistical analyses

Data were analyzed using SAS (version 8.2; SAS Institute, Inc., Cary, NC). The significance of the differences within and between dietary groups was assessed with the PROC MIXED procedures for repeated measures using the least-squares mean test. Values with a skewed distribution were log-normalized. Adjustment for multiple comparisons using the Tukey procedure had no impact on the results unless otherwise specified. Correlation analyses were conducted using Spearman rank correlations.

RESULTS

Sixty-five men (mean age, 37.5 ± 11.2 years; data not shown) were randomized to either the low-fat/high-CHO diet or the high-MUFA diet. Baseline characteristics of participants assigned to each dietary protocol were comparable except for the age of participants in the kinetic subsample, who were older in the high-MUFA group than in the low-fat/high-CHO group (47.8 ± 8.2 years vs. 37.6 ± 8.3 years; $P < 0.05$). Subjects had a relatively normal lipid profile at baseline and were moderately obese as a group, with approximately two-thirds of the group with a body mass index > 27 kg/m² and the remaining one-third with a body mass index < 27 kg/m². The macronutrient intake at baseline in subjects allocated to each dietary group was comparable (data not shown).

As reported previously, the low-fat/high-CHO diet induced a clinically meaningful 20.6% reduction in LDL-C concentrations (**Table 2**) (16). The high-MUFA diet induced significant reductions in plasma LDL-C, total TG, and VLDL-apoB-100 concentrations. Changes in plasma TG and VLDL-apoB-100 concentrations were statistically different between dietary treatments ($P < 0.01$ and $P < 0.05$, respectively). Both ad libitum diets led to comparable reductions in obesity indices (16).

Ad libitum consumption of the low-fat/high-CHO diet led to a significant reduction in plasma HDL-C concentrations (−10%; $P < 0.001$; **Table 3**), which was exclusively

TABLE 2. Effects of the low-fat/high-CHO and high-MUFA diets on lipid profiles and anthropometric characteristics

Variable	Low-Fat/High-CHO Diet (n = 33)				High-MUFA Diet (n = 32)				
	Pre	Post	Percent Change	P (Post vs. Pre)[a]	Pre	Post	Percent Change	P (Post vs. Pre)[a]	P (Between Diets)[b]
Body weight (kg)	87.7 ± 14.5	85.6 ± 13.6	−2.4	<0.0001	89.4 ± 15.9	87.1 ± 15.5	−2.6	<0.0001	0.78
Body mass index (kg/m²)	28.8 ± 4.6	28.1 ± 4.5	−2.4	<0.0001	29.6 ± 5.3	28.8 ± 5.2	−2.7	<0.0001	0.68
Waist circumference (cm)	95.3 ± 13.3	92.7 ± 12.1	−2.7	<0.0001	98.5 ± 15.6	96.1 ± 15.3	−2.5	<0.0001	0.78
Total cholesterol (mmol/l)	4.43 ± 1.05	3.71 ± 0.85	−16.3	<0.0001	4.66 ± 0.97	3.95 ± 0.80	−15.2	<0.0001	0.95
LDL-C (mmol/l)	2.91 ± 0.88	2.31 ± 0.70	−20.6	<0.0001	3.13 ± 0.83	2.57 ± 0.63	−17.9	<0.0001	0.73
Triglycerides[c] (mmol/l)	1.30 ± 0.73	1.30 ± 0.81	0	0.80	1.49 ± 0.65	1.21 ± 0.61	−18.8	<0.0001	<0.01
Total cholesterol/HDL-C ratio	4.25 ± 1.37	3.95 ± 1.19	−7.1	<0.01	4.76 ± 1.19	4.19 ± 1.11	−12.0	<0.0001	0.05
Plasma apoB-100 (g/l)	0.95 ± 0.27	0.82 ± 0.24	−13.7	<0.0001	1.03 ± 0.24	0.87 ± 0.21	−15.5	<0.0001	0.35
VLDL-apoB-100[c] (g/l)	0.10 ± 0.05	0.11 ± 0.06	+10.0	0.57	0.12 ± 0.05	0.10 ± 0.05	−16.6	<0.01	<0.05

Values shown are means ± SD. apoB-100, apolipoprotein B-100; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

[a] P value for the within-diet effects.

[b] P value for the between-diet effects (low-fat/high-CHO vs. high-MUFA).

[c] Values were log-transformed before statistical analyses.

TABLE 3. Effects of the low-fat/high-CHO and high-MUFA diets on HDL composition

| Variable | Low-Fat/High-CHO Diet (n = 33) | | | | High-MUFA Diet (n = 32) | | | | |
	Pre	Post	Percent Change	P (Post vs. Pre)[a]	Pre	Post	Percent Change	P (Post vs. Pre)[a]	P (Between Diets)[b]
HDL-C (mmol/L)	1.08 ± 0.19	0.97 ± 0.18	−10.2	<0.001	1.00 ± 0.17	0.98 ± 0.21	−3.0	0.35	<0.05
HDL_2-C (mmol/L)	0.36 ± 0.11	0.37 ± 0.15	+2.8	0.71	0.33 ± 0.11	0.35 ± 0.17	+6.1	0.28	0.61
HDL_3-C (mmol/L)	0.72 ± 0.13	0.60 ± 0.08	−16.7	<0.0001	0.68 ± 0.12	0.63 ± 0.10	−11.8	<0.05	<0.05
HDL_2-C/HDL_3-C	0.51 ± 0.16	0.62 ± 0.25	+21.6	<0.01	0.49 ± 0.18	0.56 ± 0.26	+14.3	0.07	0.54
HDL-TG[c] (mmol/L)	0.18 ± 0.05	0.18 ± 0.03	0	0.49	0.18 ± 0.04	0.17 ± 0.04	−0.06	0.22	0.70
HDL-PL (mmol/L)	0.99 ± 0.15	0.89 ± 0.12	−10.1	<0.0001	0.93 ± 0.15	0.87 ± 0.15	−6.5	<0.01	0.19
ApoA-I (g/L)	1.14 ± 0.13	1.04 ± 0.12	−8.8	<0.0001	1.13 ± 0.14	1.05 ± 0.11	−7.1	<0.001	0.61
ApoA-II (g/L)	0.31 ± 0.05	0.28 ± 0.04	−9.7	<0.0001	0.32 ± 0.05	0.28 ± 0.04	−12.5	<0.0001	0.43
LpA-I (g/L)	0.38 ± 0.10	0.35 ± 0.11	−7.9	0.23	0.34 ± 0.09	0.35 ± 0.11	+2.9	0.78	0.29
LpA-I:A-II (g/L)	0.77 ± 0.12	0.68 ± 0.13	−13.2	<0.01	0.78 ± 0.13	0.68 ± 0.13	−12.8	<0.001	0.57
LpA-I/LpA-I:A-II	0.51 ± 0.18	0.56 ± 0.28	+9.8	0.31	0.45 ± 0.14	0.55 ± 0.26	+18.2	<0.05	0.40
HDL particle size (Å)	82.22 ± 6.12	84.32 ± 7.23	+2.0	<0.05	80.40 ± 2.20	81.78 ± 5.09	+1.7	0.14	0.58

HDL-PL, HDL-phospholipids; HDL-TG, HDL-triglycerides; LpA-I, HDL's that contain apoA-I but not apoA-II; LpA-I:A-II, HDL's that contain both apoA-I and apoA-II. Values shown are means ± SD.

[a] P value for the within-diet effects.

[b] P value for the between-diet effects (low-fat/high-CHO vs. high-MUFA).

[c] Values were log-transformed before statistical analyses.

attributable to a parallel 16.7% reduction in plasma HDL_3-C concentrations ($P < 0.0001$) with no change in HDL_2-C concentrations. As a result, the HDL_2-C/HDL_3-C ratio increased by 21.6% ($P < 0.01$). Significant reductions in HDL-phospholipid, plasma apoA-I, apoA-II, and LpA-I:A-II concentrations were also observed after the low-fat/high-CHO diet. HDL particle size, on the other hand, increased significantly with the low-fat/high-CHO diet ($P < 0.05$). The high-MUFA diet-induced reductions in plasma HDL-C (−3%) and HDL_3-C (−11.8%) concentrations were significantly less important than in the low-fat/high-CHO group ($P < 0.05$).

Among the subjects who participated in the kinetic studies, the magnitude of the change in waist circumference and in plasma lipid levels after the low-fat/high-CHO diet was similar to the change in the entire low-fat/high-CHO group (**Table 4**). Although the reduction in obesity indices tended to be more important in the sub-group involved in the kinetic studies within the high-MUFA dietary group, changes in lipid levels were similar in magnitude to those seen in the entire high-MUFA group.

Consumption of the low-fat/high-CHO diet resulted in a significant reduction in apoA-I PR ($P < 0.001$) along with parallel reductions in plasma apoA-I concentrations and pool sizes ($P < 0.05$ and $P < 0.01$, respectively; **Table 5**). A significant reduction in apoA-I FCR in the low-fat/high-CHO group was also observed ($P < 0.05$). The only significant difference in apoA-I kinetics found within the high-MUFA dietary group was a reduction in apoA-I pool size ($P < 0.05$). The diet-induced changes in apoA-I PR and FCR ($P < 0.01$ and $P < 0.05$, respectively) were significantly different between the two dietary groups (Table 5). ApoA-II plasma concentrations and pool size were significantly and similarly reduced by both dietary interventions (Table 5). In both dietary groups, these reductions appeared to be attributable to an increase in apoA-II FCR, as

TABLE 4. Effects of the low-fat/high-CHO and high-MUFA diets on lipid profiles and anthropometric characteristics in the kinetic subgroup

| Variable | Low-Fat/High-CHO Diet (n = 10) | | | | High-MUFA Diet (n = 8) | | | | |
	Pre	Post	Percent Change	P (Post vs. Pre)[a]	Pre	Post	Percent Change	P (Post vs. Pre)[a]	P (Between Diets)[b]
Body weight (kg)	88.7 ± 18.8	87.3 ± 17.1	−1.6	0.15	91.8 ± 14.2	88.1 ± 14.2	−4.0	<0.01	0.11
Body mass index (kg/m²)	29.1 ± 5.2	28.7 ± 4.7	−1.4	0.16	30.6 ± 4.0	29.3 ± 4.0	−4.2	<0.01	0.10
Waist circumference (cm)	100.1 ± 12.3	97.6 ± 11.8	−2.5	<0.01	104.3 ± 9.4	100.1 ± 11.0	−4.0	<0.0001	0.11
Total cholesterol (mmol/L)	4.73 ± 1.33	3.80 ± 0.90	−19.7	<0.001	5.46 ± 0.75	4.59 ± 0.92	−15.9	<0.001	0.85
LDL-C (mmol/L)	3.08 ± 1.15	2.27 ± 0.75	−35.7	<0.001	3.54 ± 0.69	2.84 ± 0.73	−19.8	<0.01	0.69
Triglycerides[c] (mmol/L)	1.43 ± 0.96	1.45 ± 1.14	+1.4	0.90	1.97 ± 0.95	1.60 ± 0.91	−18.8	<0.05	0.15
Total cholesterol/HDL-C ratio	4.46 ± 1.75	4.04 ± 1.39	−9.4	0.10	5.01 ± 1.29	4.24 ± 1.17	−15.4	<0.05	0.33
Plasma apoB-100 (g/L)	0.99 ± 0.33	0.79 ± 0.25	−20.2	<0.001	1.18 ± 0.22	0.93 ± 0.23	−21.2	<0.001	0.53
HDL-C (mmol/L)	1.13 ± 0.27	0.98 ± 0.17	−13.3	<0.05	1.13 ± 0.20	1.12 ± 0.24	−0.9	0.91	0.12
HDL_2-C (mmol/L)	0.37 ± 0.14	0.37 ± 0.10	0	0.92	0.36 ± 0.13	0.45 ± 0.21	+25.0	0.07	0.19
HDL_3-C (mmol/L)	0.76 ± 0.16	0.61 ± 0.09	−19.7	<0.01	0.77 ± 0.13	0.67 ± 0.09	−13.0	<0.05	0.33

Values shown are means ± SD.

[a] P value for the within-diet effects.

[b] P value for the between-diet effects (low-fat/high-CHO vs. high-MUFA).

[c] Values were log-transformed before statistical analyses.

TABLE 5. Effects of the low-fat/high-CHO and high-MUFA diets on apoA-I, apoA-II, and VLDL-apoB-100 kinetic parameters

Variable	Low-Fat/High-CHO Diet (n = 10)				High-MUFA Diet (n = 8)				P (Between Diets)[b]
	Pre	Post	Percent Change	P (Post vs. Pre)[a]	Pre	Post	Percent Change	P (Post vs. Pre)[a]	
ApoA-I kinetics									
apoA-I (g/l)	1.12 ± 0.18	0.99 ± 0.12	−11.6	<0.05	1.21 ± 0.18	1.13 ± 0.11	−6.6	0.16	0.47
Pool size (mg)	4,439 ± 914	3,828 ± 527	−13.8	<0.01	4,956 ± 890	4,458 ± 771	−10.0	<0.05	0.71
PR (mg/kg/day)	11.1 ± 1.5	7.6 ± 2.2	−31.5	<0.001	10.6 ± 1.4	11.0 ± 2.0	+3.8	0.63	<0.01
FCR (pools/day)	0.226 ± 0.052	0.171 ± 0.040	−24.3	<0.05	0.198 ± 0.034	0.217 ± 0.032	+9.6	0.44	<0.05
ApoA-II kinetics									
apoA-II (g/l)	0.311 ± 0.054	0.281 ± 0.048	−9.6	<0.05	0.350 ± 0.050	0.294 ± 0.029	−16.0	<0.01	0.21
Pool size (mg)	1,241 ± 302	1,098 ± 245	−11.5	<0.05[c]	1,429 ± 184	1,170 ± 259	−18.1	<0.001	0.20
PR (mg/kg/day)	2.83 ± 0.68	2.85 ± 0.81	+0.7	0.94	2.93 ± 0.76	3.47 ± 1.10	+18.4	0.14	0.28
FCR (pools/day)	0.210 ± 0.070	0.230 ± 0.073	+9.5	0.51	0.186 ± 0.039	0.262 ± 0.075	+40.9	<0.05[d]	0.24
VLDL-apoB-100 kinetics									
VLDL-apoB-100 (g/l)	0.111 ± 0.065	0.127 ± 0.093	+14.4	0.41	0.175 ± 0.062	0.141 ± 0.056	−19.4	0.13	0.10
Pool size (mg)	481 ± 354	548 ± 515	+13.9	0.43	711 ± 225	559 ± 236	−21.4	0.12	0.10
PR (mg/kg/day)	39.71 ± 21.08	33.42 ± 12.74	−15.8	0.59	58.80 ± 50.67	41.44 ± 16.22	−29.5	0.20	0.53
FCR (pools/day)	9.18 ± 4.22	7.58 ± 4.58	−17.4	0.38	7.68 ± 6.90	7.39 ± 3.70	−3.8	0.89	0.63

Values shown are means ± SD. FCR, fractional catabolic rate; PR, production rate.

[a] P for the within-diet effects.

[b] P for the between-diet effects (low-fat/high-CHO vs. high-MUFA).

[c] After Tukey adjustment for multicomparison in the PROC MIXED procedure (P = 0.10).

[d] After Tukey adjustment for multicomparison in the PROC MIXED procedure (P = 0.16).

reflected by the inverse correlations found between the diet-induced changes in plasma HDL$_3$-C and changes in apoA-II FCR observed within the high-MUFA group ($r = −0.90$, $P < 0.01$) and the low-fat/high-CHO group ($r = −0.71$, $P < 0.05$). However, the increase in apoA-II FCR was significant only in the high-MUFA group ($P < 0.05$).

Although the changes did not reach statistical significance, in the low-fat/high-CHO group, VLDL-apoB-100 PR and FCR tended to be reduced equally ($−15.8\%$ vs. $−17.4\%$, respectively), resulting in no change in plasma concentrations of TG and VLDL-apoB-100. The reduction in VLDL-apoB-100 and TG levels in the high-MUFA group appeared to be almost entirely attributable to a reduction in VLDL-apoB-100 PR ($−29.5\%$), although this reduction also did not reach statistical significance. However, variations in plasma VLDL-apoB-100 concentrations in the high-MUFA group were positively correlated with diet-induced variations in VLDL-apoB-100 PR ($r = 0.69$, $P = 0.06$) and showed no association with VLDL-apoB-100 FCR ($r = 0.28$, $P = 0.51$). Diet-induced changes in body weight did not correlate with variations in VLDL-apoB-100 kinetics in either dietary group.

DISCUSSION

The present study investigated the effects of a low-fat/high-CHO diet and a high-MUFA diet consumed under ad libitum conditions on apoA-I, apoA-II, and VLDL-apoB-100 kinetics in men. First, the reduction in plasma HDL-C concentrations induced by the low-fat/high-CHO diet was mainly attributable to concurrent reductions in plasma HDL$_3$-C concentrations. Second, the reduction in plasma HDL-C and HDL$_3$-C concentrations with the low-fat/high-CHO diet was attributable largely to a reduction in apoA-I PR. Third, the significant reduction in HDL$_3$-C concentra-

tions induced by the consumption of the high-MUFA diet was mediated by an increase in apoA-II FCR. Finally, no statistically significant diet effect on VLDL-apoB-100 kinetics was observed in the kinetic subgroup.

The evidence supporting the fact that low-fat/high-CHO diets are associated with a decrease of HDL-C concentrations and an increase of plasma TG concentrations under isocaloric conditions has been consistent. In a meta-analysis of 27 well-controlled trials conducted under isocaloric conditions published by Mensink and Katan (30), it was calculated that for each 10% of energy from CHO replaced by fat, HDL-C concentrations increased by 0.07 mmol/l (5–10%). However, because it is recognized that body weight and more specifically abdominal obesity are important determinants of HDL-C concentrations and metabolism (31), the extent to which conclusions drawn from studies performed under isocaloric conditions can be applied to the day-to-day management of obese patients' weight and risk profile has been questioned.

In a landmark study, Schaefer et al. (5) measured plasma HDL-C concentrations in 27 slightly overweight men and women during their usual, high-fat diet (35% fat, 49% CHO), during an isocaloric low-fat/high-CHO diet (15% and 68% of energy from fat and CHO, respectively) when weight remained stable for 5–6 weeks, and during an ad libitum low-fat/high-CHO diet lasting 12 weeks, during which participants spontaneously lost an average of 3.63 kg. Consumption of the low-fat diet under weight maintenance conditions was associated with a significant increase in plasma TG concentrations and a reciprocal 22% decrease in HDL-C concentrations compared with the usual high-fat diet. The low-fat ad libitum phase induced a significant reduction in plasma TG concentrations, whereas HDL-C concentrations did not decrease further relative to the low-fat, weight-maintenance diet period. Lichtenstein et al. (32) obtained similar re-

sults. In the present study, the low-fat/high-CHO diet was associated with a significant 10% reduction in plasma HDL-C concentrations. The magnitude of the change in plasma HDL-C concentrations was not related to the diet-induced reduction in body weight or body fat distribution (16).

We have further examined the impact of both diets on various subclasses of HDL characterized on the basis of their composition and size. In general, HDLs that contain apoA-I but not apoA-II (LpA-I) are found within particles of larger size and lower density (HDL_2), whereas LpA-I:A-II (HDLs that contain both apoA-I and apoA-II) are generally associated with smaller HDL_3 particles (33). Although earlier data tended to suggest that most of the cardioprotective properties attributed to HDL would reside within LpA-I particles and HDL_2 rather than within LpA-I:A-II or HDL_3 (33, 34), recent data from the large Prospective Epidemiological Study of Myocardial Infarction revealed that both LpA-I and LpA-I:A-II were inversely related to the incidence of CHD (35). Our knowledge of the low-fat diet-induced changes in the concentrations of specific HDL subfractions is rather limited. It was reported that compared with a diet high in saturated fat and cholesterol, consumption of a National Cholesterol Education Program (NCEP) Step 2 diet for 6 months under isocaloric conditions resulted in significant reductions in HDL particles that do and do not contain apoA-II (LpA-I:A-II and LpA-I, respectively) (36). Isoenergetic replacement of dietary fat with CHO has also been associated with reductions in HDL_{3a}, HDL_{2a}, HDL_{2b}, and HDL particle size (37). In the present study, the low-fat/high-CHO diet was associated with a significant reduction in plasma HDL_3-C and LpA-I:A-II concentrations but with no change in HDL_2-C and LpA-I concentrations. As a result, the HDL_2-C/HDL_3-C ratio and HDL particle size increased after the low-fat/high-CHO diet. The increased HDL_2-C/HDL_3-C ratio and HDL size and the lack of change in HDL_2-C concentrations after the low-fat/high-CHO diet would suggest a beneficial modification in terms of cardiovascular risk (33, 34). Consumption of the high-MUFA diet yielded similar results, with the exception of a smaller reduction in plasma HDL-C and HDL_3-C concentrations compared with the low-fat/high-CHO diet.

Few studies have reported the effects of dietary changes on plasma apoA-I and apoA-II kinetics, and to our knowledge the present study is the first to compare the impact of an unrestricted low-fat/high-CHO diet vs. a high-MUFA diet on HDL kinetics. Among those, Blum et al. (11) studied three women in whom a 39.1% increase in apoA-I FCR was observed when an isocaloric CHO-rich diet (80% CHO, <5 g of fat) was compared with a normal diet (40% fat, 40% CHO). Brinton, Eisenberg, and Breslow (10) showed a significant reduction in apoA-I PR and an increase in apoA-I FCR after an isocaloric very-low-fat/high-CHO diet (9% fat, 76% CHO, 16% protein) compared with a baseline diet (42% fat, 43% CHO, 15% protein). More recently, Vélez-Carrasco et al. (12) also reported in an isocaloric study that a low-saturated-fat diet (25% fat, 60% CHO, 15% protein) induced a significant decrease

in apoA-I PR and no change in apoA-I FCR, apoA-II PR, and apoA-II FCR compared with a baseline diet (36% fat, 49% CHO, 15% protein). Interpretations of the study by Blum et al. (11) are limited by the small number of subjects investigated. However, consistent with data from the present study, two previous studies have found that low-fat/high-CHO diets reduced apoA-I PR, which in turn explained the decreased plasma HDL-C concentrations.

The effects of low-fat/high-CHO diets on the apoA-I FCR appear to be less consistent. The increase in apoA-I FCR after a low-fat diet observed by Brinton, Eisenberg, and Breslow (10) and Blum et al. (11) was paralleled by an increase in plasma TG concentrations in both studies. On the other hand, Vélez-Carrasco et al. (12) did not observe differences in plasma TG concentrations when a low-fat/high-CHO Step 2 diet was substituted for a baseline diet, and no change in apoA-I FCR was reported. In the present study, the low-fat/high-CHO diet was associated with a significant reduction in apoA-I FCR in the absence of change in plasma TG concentrations. We hypothesize that the lack of deleterious change in plasma TG concentrations combined with moderate weight loss and increased HDL particle size in the low-fat/high-CHO group may have favorably reduced the HDL catabolic rate. Interestingly, the reduction in HDL_3-C concentrations in the high-MUFA diet appears to be attributable to an enhanced catabolism of apoA-II. Because of the significantly greater reduction in HDL_3-C concentrations observed with the low-fat/high-CHO diet compared with the high-MUFA diet, it may have been interesting to perform kinetic analyses on the HDL_3 subclass to address this issue further. However, a previous study by Frenais et al. (38) indicated that HDL_2 and HDL_3 had similar kinetics when measured using the primed-infusion methodology. It must be stressed that in our study, apoA-I and apoA-II were isolated from the plasma d > 1.25 g/ml fraction by ultracentrifugation, a process that may provoke the dissociation of the "loosely bound" apolipoproteins from lipoproteins. Horowitz et al. (39) have shown that a low-HDL condition was associated with a greater proportion of apoA-I being dissociated from HDL upon ultracentrifugation. Because on-diet HDL-C concentrations were the same in the high-CHO and the high-MUFA groups (Table 3), we hypothesize that diet per se may not have affected the pool of "dissociable" apoA-I.

Several studies have suggested that low-fat diets provoke an increase in the PR rate of VLDL from the liver, although this is not a consistent observation (40). In the present study, ad libitum consumption of a low-fat/high-CHO diet consisting of solid foods, when accompanied by small but significant weight loss, was not associated with increased plasma TG and VLDL-apoB-100 levels. Consistently, the low-fat/high-CHO diet had no effect on VLDL-apoB-100 PR or FCR. The extent to which changes in body weight may have modulated the impact of the low-fat/high-CHO diet on VLDL-apoB-100 kinetics is unclear, but there was no correlation between diet-induced changes in body weight or waist circumference and variations in VLDL-apoB-100 kinetics. In the high-MUFA group, the re-

duction in VLDL-apoB-100 PR (-29.5%), although not significant, appeared to be proportionately greater than changes in VLDL-apoB-100 FCR (-3.8%). There was also a positive correlation between diet-induced changes in plasma VLDL-apoB-100 concentrations and VLDL-apoB-100 PR. These data suggest that the changes in the PR of VLDL-apoB-100 may be largely responsible for the observed reductions in plasma VLDL-apoB-100 concentrations associated with the high-MUFA diet. Very few studies have investigated the impact of diets rich in MUFA on VLDL kinetics. Replacing dietary saturated fatty acids with MUFA under isocaloric conditions had no impact on plasma TG levels and did not affect VLDL$_1$ and VLDL$_2$ plasma concentrations or kinetics (15).

Despite a greater reduction in plasma TG and VLDL-apoB-100 concentrations with the high-MUFA diet compared with the low-fat/high-CHO diet in the entire group of subjects, our study was not able to reveal significant between-diet differences in VLDL-apoB-100 kinetic parameters, although VLDL-apoB-100 PR was reduced by $\sim$2-fold in the high-MUFA group compared with the low-fat/high-CHO group. One of the study subjects in the high-MUFA group had a markedly increased VLDL-apoB-100 PR, particularly at baseline, thus explaining the high SD in that group. Excluding that subject from statistical analyses performed on VLDL-apoB-100 kinetic parameters did not affect our results.

Limitations of the present study must be addressed. First, it must be acknowledged that a crossover study design may have allowed us to better appreciate the between-diet differences in the kinetics of the various apolipoproteins, mainly because of the increased statistical power it provides. However, the ad libitum nature of the study, which led to significant variations in body weight, would have made such a design rather complex. Second, the age difference among subjects allocated to the low-fat/high-CHO diet and the high-MUFA diet in the kinetic subgroup is an issue that deserved to be investigated further. Adjustment for age had very little impact on the between-diet comparison of kinetic data, with the exception of the difference in apoA-I FCR, which was attenuated ($P = 0.13$; data not shown). Finally, although our study sample was heterogeneous in terms of obesity levels, it cannot be excluded that our results may have been influenced by the body composition status of the subjects who were overweight as a group. Because of the small number of subjects in the kinetic substudies, it was impossible to assess the impact of the experimental diets on kinetic parameters within subgroups of lean and obese individuals.

In conclusion, our results suggest that a reduction in apoA-I PR appears to explain the consistent decrease of HDL-C concentrations attributable to low-fat diets. However, the decrease in apoA-I FCR possibly attributable to body weight reduction and increased HDL particle size with the low-fat diet consumed under ad libitum conditions may have prevented further undesirable decreases of plasma HDL-C levels. Our data also indicated that the reduction in HDL$_3$-C concentrations in the high-MUFA

group was mediated by an increase in apoA-II FCR, suggesting that low-fat/high-CHO and high-MUFA diets may exert their effects on HDL through different mechanisms. Using a parallel study design, we were not able to reveal any difference in VLDL-apoB-100 kinetics between a low-fat/high-CHO diet and a high-MUFA diet consumed ad libitum and associated with moderate weight loss.

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