Effects of dietary carbohydrate and fat on plasma lipoproteins and apolipoproteins C-II and C-III in healthy men

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Abstract Effects of isocaloric changes in dietary fat and carbohydrate on plasma apolipoproteins (apo) C-II, C-III, and lipoproteins were assessed in nine healthy men. Carbohydrate and fat comprised 80% of total calories. After a 1-week basal diet (40% of calories from carbohydrate), the subjects received either a high (65% of calories) or low (15% of calories) carbohydrate diet for 3 weeks; subsequently the diets were switched, those initially on high carbohydrate going on to low carbohydrate, and vice versa, and the new diets were maintained for 3 weeks. ApoC-II, C-III, and triglycerides initially rose and then declined during the high carbohydrate diet period; high density lipoprotein cholesterol (HDL-C) decreased. Comparing results after 3 weeks of high carbohydrate diet to those after 3 weeks on low carbohydrate, we observed the following significant differences: *1*) total plasma apoC-II and C-III were higher; the apoC-III/C-II ratio in very low density lipoproteins (VLDL) and in the lighter HDL subfraction (HDL_2) was lower indicating net lipoprotein enrichment with apoC-II than with apoC-III; *2*) unsialylated $apoC\text{-}III_0$ comprised a higher percent of total VLDL apoC-III mass; *3*) HDL_2 and HDL_2/HDL_3 ratio were lower. Isocaloric changes in dietary carbohydrate and fat cause significant alterations in plasma levels of VLDL and HDL_2, the two major lipoproteins that transport apoC-III and apoC-II. Diet-induced changes in circulating apoC-III and C-II may, in part, play a role in regulation of plasma triglycerides in man.—**Kashyap, M. L., R. L. Barnhart, L. S. Srivastava, G. Perisutti, P. Vink, C. Allen, E. Hogg, D. Brady, C. J. Glueck, and R. L. Jackson.** Effects of dietary carbohydrate and fat on plasma lipoproteins and apolipoproteins C-II and C-III in healthy men. *J. Lipid Res.* 1982. **23:** 877–886.

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The protein moiety of plasma lipoproteins consists of different apolipoproteins which play important roles in the synthesis, transport, and catabolism of plasma lipids. In addition to binding and transporting lipid, the plasma apolipoproteins stimulate or inhibit lipolytic enzymes and mediate the interaction of lipoproteins with cell membrane receptors (1–3). Although many studies in the past have documented the fact that dietary manipulation has important effects on plasma levels of triglycerides and cholesterol, relatively little is known concerning the effects of diet on the apolipoproteins, particularly the C apolipoproteins, in relation to changes in plasma lipids.

ApoC-II and C-III are apolipoprotein constituents of triglyceride-rich lipoproteins and HDL and appear to have a central role in triglyceride metabolism. ApoC-II increases the activity of extrahepatic tissue lipoprotein lipase, the rate limiting enzyme in clearance of triglyceride-rich lipoproteins (4). Absence of apoC-II in humans results in severe hypertriglyceridemia (5–9) which can be corrected by infusion of normal apoC-II-containing plasma (5, 9). In vitro, apoC-III inhibits LPL activity (10–12) and decreases the uptake of triglyceride-rich particles by the hepatocyte (13–16). ApoC-II and C-III also inhibit human post-heparin plasma hepatic lipase (17). Abnormal plasma levels of total plasma apoC-III and its subspecies are associated with hypertriglyceridemia (18). The ratios of apoC-II and C-III subspecies are also abnormal in triglyceride-rich lipoprotein isolated from hypertriglyceridemic subjects (18, 19).

The specific aim of the present investigation was to assess the effects of a 6-week isocaloric alteration in dietary carbohydrate and fat on apoC-II, C-III (and its subspecies), triglycerides, cholesterol, VLDL, LDL, and HDL in the plasma of normal healthy men.

MATERIALS AND METHODS

Subjects

Nine healthy adult men were studied in the General Clinical Research Center (GCRC) at the University of

Abbreviations: CARB, carbohydrate; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; C, cholesterol; PL, phospholipid; TG, triglyceride; CE, cholesteryl ester; GLC, gas–liquid chromatography.

Cincinnati Medical Center. Their ages ranged from 19 to 36 years (mean ± SEM, 23.3 ± 1.8 years). They were all within 5% of normal weight, were normolipidemic, and were not on any medication. The subjects were housed within the General Clinical Research Center for the duration of the study, with physical activity constant throughout the study period.

Study design

The subjects received all of their meals from the metabolic kitchen of the GCRC; the food was presented in 2-day cycles. A 1-week basal diet (40% of total calories from carbohydrates) was followed by 3 weeks of either high CARB (65% total calories) or a low CARB (15% total calories) diet; protein was 20% of calories. The diets were then switched for an additional 3 weeks. The cholesterol content of the diets was held constant at approximately 400 mg/day and the P/S ratio of each invidiual diet was approximately 0.40 (20).

Five subjects (subjects 01, 02, 07, 08, and 09) received the high CARB diet first, followed by the low CARB diet. In four subjects (subjects 03, 04, 05, 06), the order of the diets was reversed. The mean (±SEM) weight of the nine subjects was 73.1 ± 2.4 kg (range 62.4–83.7 kg) on day 7 and was not significantly different at the end of 3 weeks (73.3 ± 2.4 kg on day 28) and at the end of 6 weeks (72.6 ± 2.4 kg). None of the subjects deviated by ±3.5% of baseline (day 7) weight during the study.

Blood (50 ml) was taken after 12 hr fasting at weekly intervals. In addition, at the end of each dietary protocol (days 28 and 49), 400 ml of plasma was obtained from each subject by isovolumetric plasmapheresis, with sterile plasmanate added back to the red cells and reinfused. Final concentrations of 0.01% EDTA, 0.01% sodium azide, and 10^{-3} M phenylmethyl sulfonyl fluoride were added immediately to the plasma and the lipoproteins were isolated within 2 weeks.

Isolation of lipoproteins

Plasma lipoproteins were isolated by ultracentrifugation in salt solutions of KBr. Lipoproteins were isolated from the plasmapheresis samples specifically for assessment of lipid and apolipoprotein composition. VLDL isolated by ultracentrifugation at d 1.006 g/ml were further purified by a second ultracentrifugation at d 1.006 g/ml. LDL was isolated from a narrow density range of d 1.02–1.05 g/ml. LDL was further purified by chromatography on Sepharose CL-4B (Pharmacia) as described previously (21). LDL reacted only with antisera prepared against human apoB; no reactivity was detected against anti-human apoA-I or anti-human serum albumin. Total HDL (isolated by preparative ultracentrifugation between d 1.063–1.210 g/ml) was further fractionated into HDL_2 and HDL_3 by zonal ultracentrifugation in a salt gradient of NaBr (d 1.00–1.40 g/ml) as described by Patsch et al. (22). Zonal ultracentrifugation was performed in a Beckman Ti-14 zonal rotor using a Beckman L5-65B centrifuge. The lipoproteins were subjected to ultracentrifugation at 41,000 rpm for 24 hr at 10°C and were removed from the rotor with a Beckman gradient pump; 8-ml fractions were collected. Each lipoprotein fraction was dialyzed against a standard buffer containing 10 mM Tris-HCl, pH 7.4, 0.9% NaCl, 1mM EDTA, and 0.01% NaN_3.

Analytical methods

Plasma cholesterol (C), and triglycerides (TG), HDL-cholesterol (HDL-C), and LDL cholesterol (LDL-C) were determined in duplicate by standardized Autoanalyzer II procedures (23) at the University of Cincinnati Lipid Research Center Laboratory.

Lipids from isolated lipoproteins were extracted by the procedure of Folch, Lees, and Sloane Stanley (24) and were separated by thin-layer chromatography on Silica Gel 60 in a solvent system of glacial acetic acid–heptane–isopropyl ether 4:65:40. Lipids were extracted from thin-layer plates and their mass was determined by chemical methods or by gas–liquid chromatography (GLC); lipoprotein cholesterol, free and esterified, was determined by GLC (25), and phospholipid-phosphorus was determined by the method of Bartlett (26). The fatty acyl compositions of LDL-PL, TG, and CE were determined by GLC (27, 28). Protein was determined by the method of Lowry et al. (29). Plasma concentrations of apoC-II and apoC-III were determined by specific double-antibody radioimmunoassays (18, 30). All of the radioimmunoassays were performed in duplicate. The relative concentrations of apoC-II and apoC-III and of each of the apoC-III subspecies were determined by isoelectric focusing between pH 3.5–5.0 (19). VLDL (1 mg of protein), which was isolated from the plasmapheresis samples, was delipidated twice with 10 vol of acetone–ethanol 1:1 at −20°C and then once with diethyl ether. Then 0.5 ml of 0.01 M Tris-HCl, pH 8.0, containing 6 M urea was added to the lipid-free protein. After incubating overnight at 4°C, the urea-insoluble protein (mostly apoB) was removed by centrifugation. The concentration of urea-soluble proteins was determined by the Lowry procedure (29); the gels were loaded with 100 μg of protein in duplicate. After focusing for 18 hr, the gels were stained with Coomassie Blue, scanned on a Gilford Spectrophotometer at 550 nm, and the relative percentages of apoC-II, apoC-III_0 (no sialic acid), apoC-III_1 (1 mol sialic acid), and apoC-III_2 (2 mol of sialic acid) were determined as described previously (19).

HDL subfractions that were isolated by zonal ultracentrifugation were analyzed by gradient gel electrophoresis as described by Anderson et al. (31), using a Phar-

macia Electrophoresis Apparatus. Pre-made gradient gels PAA 4/30 were purchased from Pharmacia. Electrophoresis was performed at 150 V for 24 hr at 10°C in a buffer containing 0.09 M Tris-HCl, 0.08 M borate, 0.01% EDTA, 0.001% NaN_3, pH 8.35. Analytic ultracentrifugation was performed by Dr. Frank Lindgren at the Donner Laboratory, Berkeley, CA. Plasma from subjects 06–09 was shipped to the Donner Laboratory within 24 hr of blood drawing and was analyzed for HDL_2 and HDL_3 by established analytic ultracentrifugation procedures as described by Anderson et al. (32). Differential scanning calorimetry of LDL was performed as described previously (33) by Dr. Henry Pownall, Baylor College of Medicine, Houston, TX.

Statistical analysis

All of the data were analyzed by the University of Cincinnati CLINFO computer facility. Paired *t* and paired nonparametric tests of difference were used (34). Paired nonparametric tests of difference were used where indicated by the nature of the variables' distributions. For analyses, the data in all subjects on the high or low CARB diet were not pooled because the 3-week diet period was preceded by either the basal diet or one of the two 3-week diet periods. Inspection of the data also showed that the nature of the dietary sequence affected the magnitudes of plasma lipids and apolipoprotein responses.

RESULTS

Effects of diet on plasma lipids and apolipoproteins

The effects of dietary manipulation on the plasma levels of TG, C, LDL-C, and HDL-C are shown in **Table 1** and **Figs. 1 and 2.** On both dietary sequences, the high CARB diet was associated with an increase in plasma TG over baseline. In four of the five subjects who received the high CARB diet following the basal diet, plasma TG rose and was maximal at 2 weeks (day 21, Fig. 2A). In one subject (01), it was maximal at 1 week. After 3 weeks on the high CARB diet, on day 28, plasma TG had nearly returned to the basal diet values.

The increase in plasma TG was more dramatic in the four subjects who received the low CARB (high fat) diet prior to high CARB (Fig. 1). One week after shifting from the low to high CARB diet, mean TG levels were maximal and had risen twofold. After 2 weeks on high CARB, mean TG fell, but remained higher than on the low CARB diet. At week 3 on the high CARB diet, TG levels remained elevated, similar to levels at week 2.

Plasma cholesterol was significantly lower than baseline (day 7) on high CARB diet following basal diet. After a fall, plasma C levels tended towards baseline at

TABLE 1. Plasma lipids and apolipoproteins C-II and C-III following high and low carbohydrate diets

	Day of Study						
	7	14	21	28	35	42	49
High carbohydrate (5 subjects)							
Chol (mg/dl)	175.2 ± 9.35	154.6 ± 8.28[a]	149.8 ± 9.95[a]	157.6 ± 12.24[b]	147.0 ± 10.27[a]	152.4 ± 6.96[b]	152.8 ± 7.55[a]
Trig (mg/dl)	104.8 ± 21.22	156.6 ± 55.87	137.0 ± 20.3[a]	116.0 ± 27.21	82.8 ± 8.52	82.4 ± 11.05	80.4 ± 11.72
HDL-chol (mg/dl)	52.0 ± 6.09	43.8 ± 5.78[a]	40.0 ± 4.60[a]	43.2 ± 3.55[a]	41.8 ± 2.63	44.6 ± 5.26[b]	41.4 ± 6.55[a]
LDL-chol (mg/dl)	102.4 ± 9.88	79.6 ± 7.35[b]	82.4 ± 9.57[a]	92.4 ± 9.72[b]	89.0 ± 11.41[a]	91.8 ± 10.67	95.8 ± 12.25
ApoC-II (μg/ml)	26.0 ± 1.19	28.6 ± 2.13	26.29 ± 2.33	26.78 ± 2.26	19.91 ± 1.19[a,c]	19.46 ± 1.97[b,d]	18.89 ± 1.53[a,d]
ApoC-III (μg/ml)	123.4 ± 7.02	135.7 ± 13.63	140.6 ± 16.13[b]	121.7 ± 16.68	91.8 ± 6.41[a]	95.0 ± 8.95[d]	88.2 ± 7.11[a,d]
Low carbohydrate (4 subjects)							
Chol (mg/dl)	170.5 ± 17.53	170.0 ± 19.57	172.2 ± 19.57	176.5 ± 20.99	144.5 ± 13.38	154.5 ± 10.82[d]	161.5 ± 16.32
Trig (mg/dl)	71.0 ± 10.92	60.5 ± 6.56	57.0 ± 7.16[b]	65.5 ± 9.84	137.0 ± 35.85[c]	95.2 ± 25.17	96.0 ± 25.87
HDL-chol (mg/dl)	55.0 ± 7.12	52.7 ± 3.90	52.8 ± 2.17	59.2 ± 4.73	37.0 ± 1.82[d]	36.7 ± 1.38[d]	37.5 ± 1.26[d]
LDL-chol (mg/dl)	107.0 ± 15.61	105.0 ± 15.19	108.2 ± 18.83	104.0 ± 17.15	80.5 ± 7.50	99.0 ± 6.45	105.2 ± 10.87
ApoC-II (μg/ml)	21.6 ± 2.49	18.4 ± 2.71	20.36 ± 1.95	19.20 ± 3.29	23.77 ± 3.22[c]	21.24 ± 2.40[c]	21.37 ± 2.47[d]
ApoC-III (μg/ml)	108.0 ± 7.60	89.1 ± 9.8[a]	92.3 ± 8.71	94.6 ± 12.43	114.3 ± 12.61[c]	99.5 ± 9.70	98.7 ± 9.46

[a] Significance $P < 0.01$ vs. day 7.
[b] Significance $P < 0.05$ vs. day 7.
[c] Significance $P < 0.01$ vs. day 28.
[d] Significance $P < 0.05$ vs. day 28.
Chol, cholesterol; Trig, triglyceride; results are given as mean ± SEM.

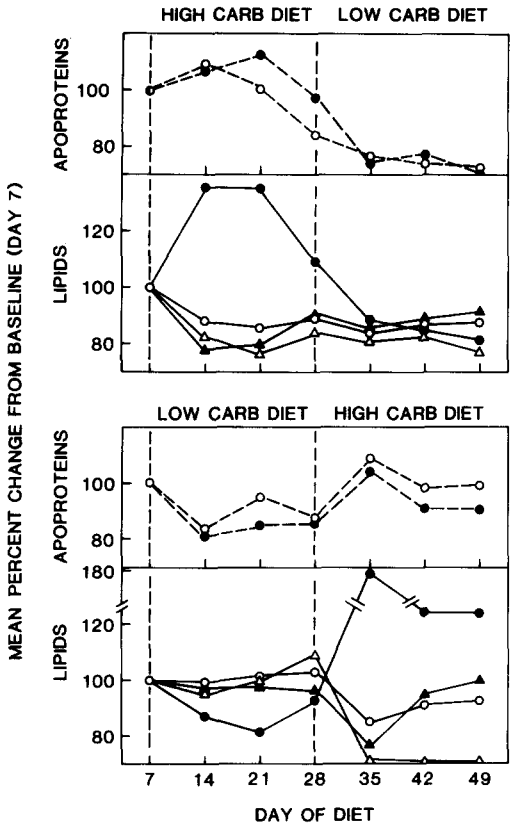

Fig. 1. Effect of high and low carbohydrate diets on plasma apoC-II (○ - - - ○), apoC-III (● - - - ●), cholesterol (○ —— ○), triglycerides (● —— ●), high density lipoprotein cholesterol (△ —— △), and low density lipoprotein cholesterol (▲ —— ▲). Nine subjects were admitted to the Clinical Research Center and given a basal diet. This was followed by 3 weeks of a high CARB diet followed by low CARB diet (n = 5; top panel). In four subjects, 3 weeks of low CARB diet preceded 3 weeks of high CARB diet (lower panel). Results are expressed as percent change from the baseline measurement (day 7).

3 weeks. LDL-C levels moved in the same direction as total plasma C concentrations. High CARB diets were uniformly associated with decreased HDL-C. The decline was greater when the high CARB diet followed a 3-week period of a low CARB diet. As shown in Table 1, (lower panel), mean HDL-C was 59.2 ± 4.73 mg/dl at the end of 3 weeks of low CARB diet and decreased to 37.0 ± 1.82 mg/dl after 1 week of high CARB diet.

On high CARB, irrespective of dietary sequence, TG rose and then gradually fell, while HDL-C fell and remained stable at the lower levels. Thus, the change in plasma TG on high CARB was dissociated from the change in HDL-C.

Total plasma apoC-II was higher after 1 week of high CARB following the basal or low CARB diet (i.e., day 7 versus day 14, or day 28 versus day 35; Fig. 2B) in all subjects except subject 08. On at least one time point during the high CARB diet, irrespective of dietary sequence, total plasma apoC-III was higher than at day 7 or day 28 for all nine subjects. When high CARB followed low CARB, mean absolute plasma concentration of apoC-II was significantly elevated at day 35 over levels at day 28 (Table 1). On high CARB diets, at day 21, (Table 1, upper panel) mean plasma apoC-III level was significantly elevated above baseline levels (day 7); on day 35 (Table 1, lower panel) mean apoC-III was higher than on day 28.

When high CARB diets were preceded by basal diets and followed by low CARB diets (Table 1, upper panel), mean plasma apoC-II and apoC-III levels were significantly higher at the end of the high CARB (26.78 ± 2.26 and 121.70 ± 16.68 μg/ml, respectively) than at the end of the low CARB periods (18.89 ± 1.53 and 88.20 ± 7.11 μg/ml, respectively; $P < 0.01$). The mean (± SEM) ratio of apoC-III to apoC-II was lower following 3 weeks of high CARB diet (4.48 ± 0.25) compared to the low CARB diet (4.73 ± 0.38); the difference did not reach statistical significance. The ratio of plasma apoC-III to apoC-II was lower during the high CARB diet (4.66 ± 0.21) than when it followed the low CARB diet (5.05 ± 0.27).

As shown in Fig. 1, on high CARB diets, compared to baseline, mean percent change in plasma TG tended to parallel mean percent change in apoC-II and apoC-III. When high CARB diets followed low CARB diets (Fig. 1, lower panel), the magnitude of plasma apoC-II and apoC-III was higher during the high CARB periods than the reverse sequence (Fig. 1, upper panel).

Characterization of VLDL and LDL

At the end of each 3-week diet period, on days 28 and 49, plasma was obtained by plasmapheresis. VLDL and LDL were isolated and their lipid and protein compositions were determined. The mean ± SEM weight percent (of total mass) of protein, phospholipid, TG, and C in VLDL isolated from five subjects at the end of 3 weeks of high CARB diet preceded by 1 week of basal diet was 13.7 ± 1.8, 17.7 ± 0.6, 57.5 ± 2.0, and 11.1 ± 0.8, respectively. No significant difference in the composition of VLDL was noted in the group of subjects who received the high CARB diet after 3 weeks of the low CARB diet. VLDL isolated after 3 weeks of low CARB diet was not significantly different in gross composition from VLDL isolated after 3 weeks of high CARB diet. The weight percent (mean ± SEM) composition of LDL following 3 weeks of high CARB diet preceding the basal diet of protein, phospholipid, TG, and C was 24.3 ± 2.5, 26.8 ± 1.2, 7.7 ± 0.7, and 41.4 ± 1.9, respectively. The ratio of cholesteryl ester (CE) to CE + TG in LDL was 0.85 ± 0.02 in this group. The compositional values in LDL obtained after 3 weeks of low CARB diet were similar to that after high CARB, except for a significant ($P < 0.05$) reduction in the weight

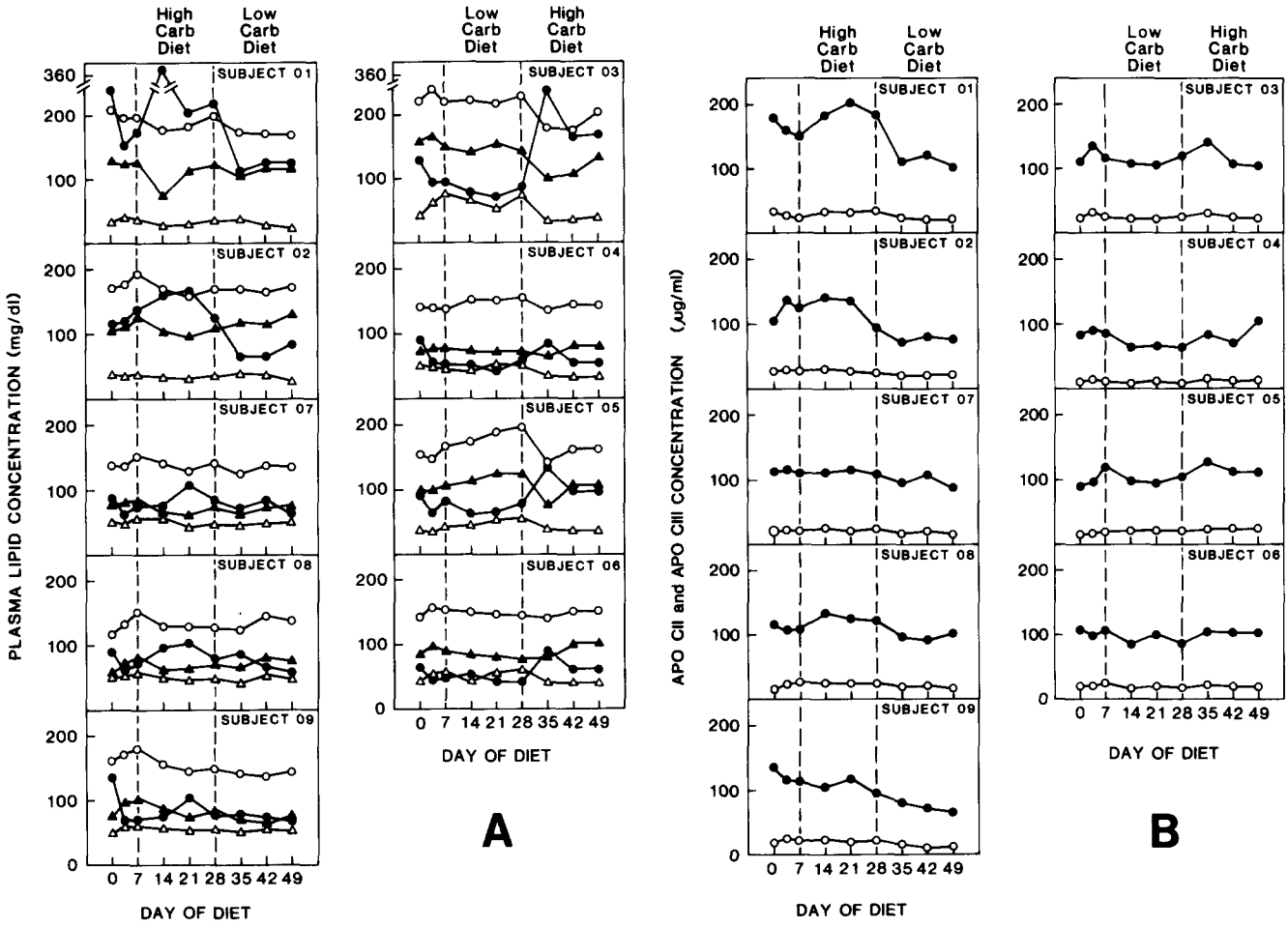

Fig. 2. A, Effect of dietary modification on plasma cholesterol (— ○ —), triglycerides (— ● —), HDL-cholesterol (— △ —), and LDL-cholesterol (— ▲ —). B, Effect of dietary modification on plasma apoC-II (— ○ —) and apoC-III (— ● —).

content of TG to 6.8 ± 0.6% vs. 7.7 ± 0.7% of total LDL mass, respectively. In the group of four subjects who underwent the other diet sequence, LDL composition was similar after both diet periods. The fatty acyl composition (C 16:0, C 18:0, C 18:1, and C 18:2 fatty acids) of LDL-lipids (cholesteryl esters, phospholipid, and TG) were not significantly different at the end of either dietary sequence. Differential scanning calorimetry revealed little difference between the melting behavior of the LDL isolated after each diet. On the high and low CARB diets, the mean transition temperatures (peak temperature) were 28.9 ± 3.0°C and 29.6 ± 2.8°C, respectively.

To determine whether the diets caused a change in the distribution of VLDL apoC-II and apoC-III and each of the apoC-III subspecies, the VLDL fractions obtained from plasmapheresis were delipidated and the urea-soluble proteins were subjected to isoelectric focusing. All of the nine subjects had a VLDL apoC-III to apoC-II ratio which was lower on the high CARB diet as compared to the low CARB diet (**Fig. 3A**). The mean (±SEM) ratio of apoC-III to apoC-II for the five subjects whose high CARB diets followed baseline (subjects 01, 02, 07, 08, and 09) was 3.04 ± 0.35 compared to 4.49 ± 0.48 on their subsequent low CARB diets ($P < 0.05$). The mean ratio of apoC-III to apoC-II for the four subjects whose high CARB diets followed low CARB diets (subjects 03, 04, 05, and 06) was 3.92 ± 0.96 compared to 5.23 ± 1.52, respectively.

The percent of total apoC-III comprised by apoC-III_0, apoC-III_1, and apoC-III_2 was determined by scanning of the isoelectric focusing gels (Fig. 3B). The percent of total apoC-III as apoC-III_0 was increased in all but one subject, subject 08 being the exception, on the high CARB diet (Fig. 3B). The mean VLDL apoC-III_0 as percent of total apoC-III for the five subjects whose high CARB diets followed baseline was 9.72 ± 1.48%, compared to 5.78 ± 0.87%, on their subsequent low CARB diets ($P < 0.05$). For the four subjects whose high CARB diets followed low CARB diets, the mean percent of total apoC-III comprised by C-III_0 was 13.99 ± 4.28% on the high CARB versus 9.16 ± 2.45% on the low CARB. There were no consistent differences in the percent of

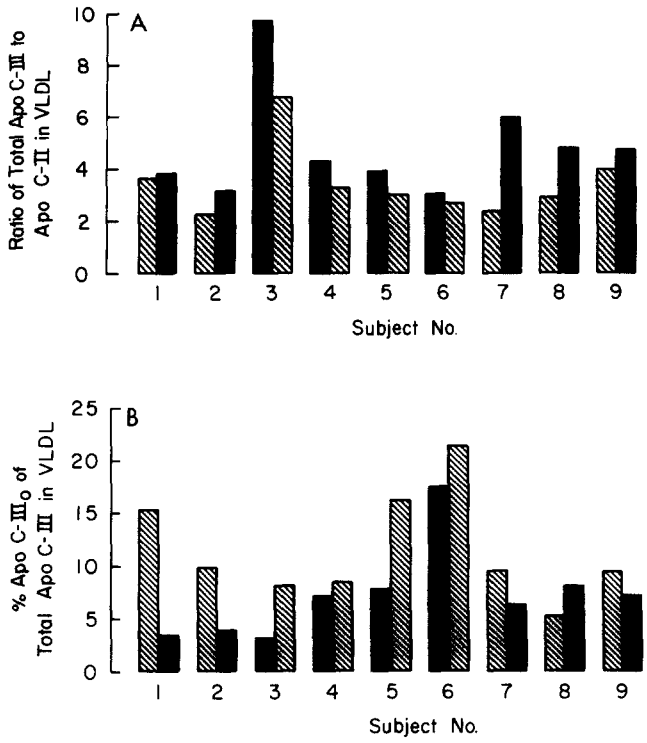

Fig. 3. A, ApoC-III to apoC-II ratio in VLDL isolated at the end of each 3-week dietary period. B, ApoC-III$_0$ as percent of total apoC-III in VLDL isolated at the end of each 3-week dietary period. High CARB diet ▧; low CARB diet ■. The dietary sequence is represented by the order of the bars.

apoC-III$_1$ or apoC-III$_2$ as percent of total apoC-III on the two diets.

Characterization of HDL subfractions

Analytic ultracentrifugation was used to quantitate the amount of plasma HDL_2 and HDL_3 in the last three of the nine study subjects (**Table 2**). In these subjects whose high CARB diets followed the basal diet (subjects 07, 08, and 09), mean total HDL (282.3 ± 9.9 mg/dl) was not statistically different after high CARB than after low CARB (298.3 ± 36.4 mg/dl). However, on the high CARB diet, HDL_2 and the HDL_2/HDL_3 ratio was lower in all subjects than on the low CARB diet. Thus, HDL_2 comprised a consistently smaller percent of total HDL at the end of the high CARB diet in every subject.

Total plasma HDL that was isolated between d 1.063–1.210 g/ml was subjected to zonal ultracentrifugation as shown in **Fig. 4.** In all four subjects studied (subjects 06, 07, 08 and 09), high CARB produced a decrease in HDL_2 relative to HDL_3.

The lipid and protein compositions of the isolated HDL subfractions are displayed in **Table 3** and **Table 4.** There were no significant differences between the high and low CARB dietary periods in the two dietary protocols for total protein, PL, TG, or total C composition in HDL_2 or HDL_3. Furthermore, the ratio of protein to phospholipid was not different. The amount of apoC-II and C-III as a percent of total protein was approximately two to three times higher in HDL_2 than HDL_3 on both diets (Table 4). In addition, there was a significantly higher amount of apoC-II in HDL_2 protein on the high CARB diet than on the low CARB diet in both dietary sequences (Table 4). ApoC-III as a percent of total protein in HDL_2 and HDL_3 did not differ appreciably between the two dietary periods. Thus, the high CARB diet was associated with a greater enrichment of apoC-II than apoC-III in HDL_2, but not in HDL_3. This resulted in a lower ratio of HDL_2 apoC-III to apoC-II on the high CARB diet versus low CARB diet (3.77 ± 0.35 versus 5.83 ± 1.03, respectively). In HDL_3, the ratios of apoC-III to apoC-II on the high CARB diet and low CARB diets were similar (5.35 ± 0.29 versus 5.76 ± 0.54, respectively).

DISCUSSION

The effects of isocaloric diets high and low in carbohydrate with fixed dietary cholesterol and P/S ratios, on plasma lipoproteins, lipids, and apolipoproteins C-II and C-III have been assessed over two 3-week periods in healthy men. The nature of the dietary stimulus and the experimental design is different from several previous studies (35–40) in two important aspects. First, the isocaloric dietary alteration in CARB and fat intake more

TABLE 2. Effects of high and low carbohydrate diets on HDL subfractions[a] HDL_2 and HDL_3

Subject Number	High CARB Diet			HDL_2/HDL_3 Ratio	Low CARB Diet			HDL_2/HDL_3 Ratio
	Total HDL	HDL_2	HDL_3		Total HDL	HDL_2	HDL_3	
07	285	70	215	0.33	253	74	178	0.42
08	264	74	190	0.39	273	99	174	0.57
09	298	125	173	0.72	370	195	175	1.11
Mean ± SEM	282.3 ± 9.9	89.6 ± 17.7	192.6 ± 12.2	0.48 ± 0.12	298.3 ± 36.4	122.7 ± 36.9	175.7 ± 1.2	0.70 ± 0.21

[a] Measured by analytic ultracentrifugation and expressed in mg/dl.

closely simulated the range of normal daily variation, and secondly, serial measurements over a 3-week period allowed temporal assessment of initial and subsequent changes resulting from a sustained dietary modification.

In the present study, the high CARB diet was associated with a rise and a subsequent decline towards baseline in plasma TG by the third week. The early rise is probably the result of increased lipogenesis mainly from glucose oxidation. This interpretation is supported by in vivo human studies in which splanchnic TG synthesis has been measured following glucose administration (41). The mechanisms for the subsequent 'adaptive' decline toward baseline of plasma TG are unknown. Previous studies also have shown that after acute induction of hypertriglyceridemia, plasma TG levels return to baseline within 1–6 months when high CARB diets are habitually consumed (38, 40). However as discussed in detail below, the rise and fall in apoC-II and C-III in plasma, decreased VLDL and HDL apoC-III to apoC-II ratios, and an increase in VLDL apoC-III_0 mass during the high CARB diets suggest that these apolipoproteins may play an important role in regulating TG catabolism.

Falko et al. (35) measured apoC-II and C-III 1 week after a fat-free, high CARB (85% total calories) diet. In five normal subjects no change in mean total plasma C-III was found; mean total plasma apoC-II increased in some subjects but not significantly. In the present study, plasma apoC-II and C-III both increased after a high CARB diet. The effects were most marked and significant when the high CARB diet followed a 3-week period of low CARB diet (Table 1). After reaching a peak at week 1 or 2 on the high CARB diet, plasma levels of apoC-II and C-III then declined toward the pre-diet period. A low CARB diet following a basal diet was associated with an initial decline in apoCs followed by a return toward baseline. The effect of a low CARB diet was more marked when it was preceded by 3 weeks of a high CARB diet. In general, apoC-II and C-III paralleled plasma TG concentrations and not HDL-C. These observations are in accord with previous findings showing that the C apolipoproteins are more intimately linked with TG metabolism than with HDL (30, 42). However, as discussed below, high CARB diets did result in enrichment of apoC-II and C-III in the HDL_2 subfraction despite a decline in the total concentrations of HDL-C.

Although total plasma apoC-II and C-III moved in the same direction as total plasma TG, changes in apoC-II were not always parallel to that of apoC-III (Fig. 1). Thus, the same high CARB stimulus had different effects on these two apoproteins. This observation suggests that these two apoproteins do not necessarily behave as a single unit metabolically. This concept is also supported

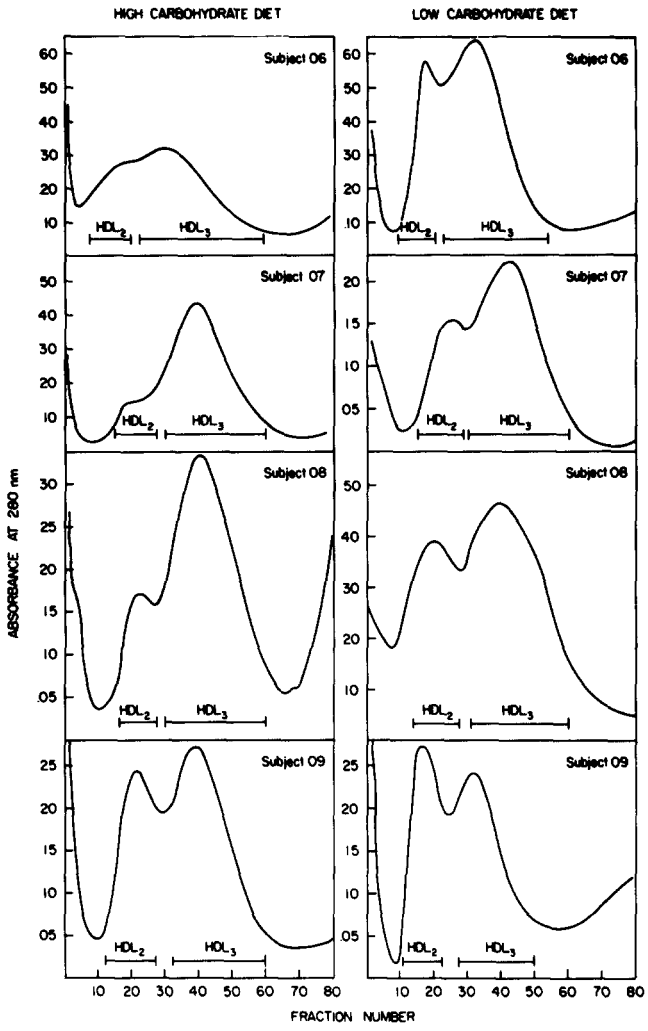

Fig. 4. Zonal ultracentrifugation profiles of HDL isolated at the end of each 3-week dietary period. Total HDL was isolated by ultracentrifugation between d 1.063–1.210 g/ml as described in Methods. The total HDL fraction was then subjected to zonal ultracentrifugation. The dietary sequence for each subject is as shown in Fig. 2.

by the observation that patients with an inherited absolute deficiency of apoC-II have plasma levels of total plasma apoC-III similar to hypertriglyceridemic subjects without absolute apoC-II deficiency (43).

As percent of total protein, HDL_2 contained approximately two to three times as much apoC-II and C-III as HDL_3. In this study, HDL subfractions were obtained by zonal ultracentrifugation and the results are similar to those found in HDL subfractions isolated by preparative ultracentrifugation (18, 30).

Three weeks of the high CARB diet was associated with decreased total apoC-III to C-II ratio in both VLDL (Fig. 3A) and HDL_2 (Table 4), compared to the ratio obtained after 3 weeks of low CARB diet. This indicates that high CARB diets resulted in a preferential enrichment of apoC-II in VLDL and HDL_2. These

TABLE 3. Effects of high and low carbohydrate diets on the protein and lipid composition[a] of high density lipoprotein subfractions

Subject Number[b]	HDL Subclass	Protein		Phospholipid		Triglyceride		Total Cholesterol		Protein/Phospholipid	
		High CARB	Low CARB	High CARB	Low CARB	High CARB	Low CARB	High CARB	Low CARB	High CARB	Low CARB
07	HDL_2	41.9	41.4	30.8	27.3	8.4	8.1	18.9	23.2	1.36	1.51
08	HDL_2	38.4	40.0	26.7	28.6	16.4	10.3	18.5	21.1	1.43	1.40
09	HDL_2	41.3	41.1	29.2	28.0	8.2	9.3	21.3	21.6	1.42	1.47
Mean	HDL_2	40.5	40.8	28.9	27.9	11.0	9.2	19.6	21.9	1.40	1.46
± SEM		± 1.1	± 0.4	± 1.2	± 0.4	± 2.7	± 0.6	± 0.9	± 0.6	± 0.02	± 0.04
07	HDL_3	55.9	54.9	25.5	22.3	4.0	6.6	14.6	16.2	2.20	2.44
08	HDL_3	55.3	55.4	22.5	21.8	5.9	6.7	16.3	16.1	2.47	2.56
09	HDL_3	55.3	57.0	22.1	19.3	8.1	7.9	14.5	15.8	2.51	2.94
Mean	HDL_3	55.5	55.8	23.4	21.1	6.0	7.1	15.3	16.0	2.40	2.65
± SEM		± 0.2	± 0.6	± 1.1	± 0.9	± 1.2	± 0.4	± 0.6	± 3.6	± 0.10	± 0.15
06	HDL_2	40.5	32.2	31.6	35.1	6.3	5.7	21.6	27.0	1.28	0.92
06	HDL_3	47.6	49.7	30.4	26.5	5.5	3.5	16.5	20.3	1.61	1.87

[a] Percent of total mass.
[b] For subjects 07, 08, and 09, the basal diet was followed by high CARB, followed by low CARB; vice versa for subject 06.

differences in the amounts of apoC-III and C-II in HDL subfractions may be due to different binding affinities of these apoproteins for HDL_2 and HDL_3.

High CARB diets were also associated with enrichment of VLDL with unsialylated apoC-III_0, suggesting that apoC-III subspecies may also be under differing metabolic control. Using a fat-free high carbohydrate diet for 1 week, Falko et al. (35) also reported an increased content of apoC-III_0 in VLDL in normal subjects. The explanations for these changes in apoC-III subspecies are not readily apparent.

Although the mass of plasma total HDL (analytic ultracentrifugation) was not different on the two diets, the ratio of HDL_2 to HDL_3 was lower on high CARB diet than on low CARB diet (Table 2) indicating that dietary carbohydrates affect HDL_2 to a greater extent

TABLE 4. Effects of high and low carbohydrate diets on the apoC-II and apoC-III content in HDL subfractions[a]

Subject Number	HDL Subclass	ApoC-II		ApoC-III		Ratio ApoC-III/ApoC-II	
		High CARB	Low CARB	High CARB	Low CARB	High CARB	Low CARB
Basal diet followed by high CARB followed by low CARB							
07	HDL_2	1.96	1.32	6.55	5.95	3.34	4.51
08	HDL_2	1.73	1.29	7.74	10.13	4.47	7.85
09	HDL_2	1.51	0.95	5.29	4.87	3.50	5.13
Mean	HDL_2	1.73[b]	1.19	6.53	6.98	3.77[c]	5.83
± SEM		±0.13	±0.12	±0.71	±1.61	±0.35	±1.03
07	HDL_3	0.47	0.42	2.69	2.07	5.72	4.93
08	HDL_3	0.63	0.48	3.02	2.68	4.79	5.58
09	HDL_3	0.42	0.18	2.33	1.22	5.55	6.78
Mean	HDL_3	0.51	0.36	2.68	1.99	5.35	5.76
± SEM		±0.06	±0.09	±0.21	±0.42	±0.29	±0.54
Basal diet followed by low CARB followed by high CARB							
06	HDL_2	1.73	0.86	6.38	8.74	3.69	10.16
06	HDL_3	0.57	0.41	3.67	3.22	6.44	7.85

[a] The values represent the weight percent of total protein from HDL_2 and HDL_3 isolated on days 28 and 49 by zonal ultracentrifugation (Fig. 4).
[b] $P < 0.01$ (high CARB vs. low CARB).
[c] $P < 0.05$ (high CARB vs. low CARB).

than HDL_3. The results confirm previous observations on the effects of high CARB diets on HDL-C (36–39) and extend the results to total HDL and its subfractions. The effects of high CARB diet on plasma HDL-C appeared to be persistent, since, by the third week, HDL-C levels had not returned to baseline levels (Table 1, Fig. 1). Because HDL metabolism is complex, any attempt to explain these observations would be incomplete.

The overall lipid and protein composition of VLDL, LDL, and HDL was not significantly changed after 3 weeks of each diet. There was a slight TG enrichment of VLDL, LDL, and HDL on the high CARB diet that has also been observed previously for VLDL (44, 45). These observations indicate that, in healthy subjects, overall lipoprotein composition is kept fairly constant despite considerable alterations in fat and CARB in the isocaloric state. These results do not exclude the possibility that narrower subfractions of these broadly defined lipoprotein classes may have had altered compositions.

Abnormal concentrations of apoC-II, C-III and its subspecies, and low HDL levels have been found in patients with hypertriglyceridemia (18, 19, 35, 37, 46–48). In patients with severe hypertriglyceridemia, the ratio of TG-rich lipoprotein unsialylated $apoC\text{-}III_0$ to total apoC-III, and $apoC\text{-}III_0$ to apoC-II, is subnormal while the ratio of $apoC\text{-}III_1$ to total apoC-III and $apoC\text{-}III_1$ to C-II is increased (18, 19). In some patients an abnormal preponderance of $apoC\text{-}III_2$ relative to total apoC-III has been found (49). Familial elevations in $apoC\text{-}III_0$ relative to sialylated apoC-III are not associated with hypertriglyceridemia (50). These observations raise the possibility that lipoprotein and apolipoprotein homeostasis may be abnormal in some individuals who develop abnormal lipoprotein and apolipoprotein profiles on diets similar to those used in this study. Because apoC-II and C-III (along with other apolipoproteins) are important in lipoprotein metabolism, it is possible that an abnormal regulation of these apolipoproteins may be responsible for disordered lipid transport in man.

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