

Independent regulation of plasma apolipoprotein C-II and C-III concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins

Ngoc-Anh Le,^{1,*} Joyce C. Gibson,[†] and Henry N. Ginsberg^{2,†}

Department of Biomathematics and Biostatistics* and Department of Medicine, Division of Arteriosclerosis and Metabolism,[†] Mount Sinai School of Medicine, New York, NY

Abstract Apolipoproteins C-II (apoC-II) and C-III (apoC-III) are distributed among all the major lipoprotein classes, particularly very low density (VLDL) and high density lipoproteins (HDL). We have determined concentrations of apoC-II and apoC-III in VLDL and HDL in subjects with a wide range of VLDL triglyceride and HDL cholesterol levels, and correlated these levels with fractional catabolic rates (FCR) of VLDL triglyceride and HDL apolipoprotein A-I (apoA-I). Both apoC-II and apoC-III levels increased in VLDL as VLDL apolipoprotein B (apoB) and triglyceride levels rose. The rate of rise of VLDL apoC-III, however, was approximately 3 times greater than that of apoC-II, and positive correlations were present between the ratio of VLDL apoC-III/apoC-II and both VLDL apoB ($r = 0.59$; $P < 0.01$) and VLDL triglyceride ($r = 0.70$; $P < 0.005$) levels. Univariate analysis demonstrated that the FCR for VLDL triglyceride was inversely related to the ratio of apoC-III/apoC-II in VLDL ($r = -0.58$; $P < 0.05$), although this relationship was not significant in a multivariate analysis. In HDL, concentrations of apoC-III and apoA-I were correlated ($r = 0.73$; $P < 0.005$) while no correlation was observed between apoC-II and apoA-I levels. Univariate analyses of HDL variables revealed inverse correlations between the concentration of apoC-III and the FCR for apoA-I ($r = -0.67$; $P < 0.005$) and between the ratio of apoC-III/apoA-I and the FCR for apoA-I ($r = -0.66$; $P < 0.005$). Multivariate analysis confirmed the latter relationship. These results indicate that concentrations of apoC-II and apoC-III in VLDL and HDL are independently regulated, and support roles for both apolipoproteins as regulators of VLDL triglyceride hydrolysis. Our results suggest that apoC-III may also regulate HDL metabolism. — **Le, N.-A., J. C. Gibson, and H. N. Ginsberg.** Independent regulation of plasma apolipoprotein C-II and C-III concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins. *J. Lipid Res.* 1988. **29**: 669–677.

Supplementary key words apoA-I • apoB • VLDL triglyceride • fractional catabolic rate

Apolipoprotein C-II (apoC-II) and apolipoprotein C-III (apoC-III) are small proteins with similar molecular

weights (approximately 8800), which were first isolated together and characterized by Brown, Levy, and Fredrickson (1). Because of their similar molecular weights, their coincident purification, and the observation that they are both associated to varying degrees with all the major lipoprotein classes, these two apolipoproteins (along with apolipoprotein C-I (apoC-I)) have frequently been portrayed as a family: the “C apolipoproteins.” A variety of in vitro studies have clearly indicated one or more specific functions for both apoC-II and apoC-III. The functions of apoC-I have been less well defined. The major role of apoC-II appears to be as a necessary activator for lipoprotein lipase (LPL) (2, 3), and its absence from in vitro assay systems containing LPL results in the absence of triglyceride hydrolysis. That this in vitro requirement is physiologically relevant in vivo has been demonstrated by the striking elevations of very low density lipoprotein (VLDL) triglycerides present in individuals who lack apoC-II (4). Serum from these individuals is unable to activate LPL in vitro (5), and infusion of normal plasma (4) or a synthetic fragment of apoC-II (6) into these patients corrects their hypertriglyceridemia. ApoC-II may also play an inhibitory role in hepatic uptake of triglyceride-rich lipoproteins (7).

Several groups have presented evidence that apoC-III inhibits the uptake of triglyceride-rich lipoproteins by the liver (7–9). The endocytosis of these triglyceride-rich

Abbreviations: FCR, fractional catabolic rate; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein.

¹Reprint requests should be addressed to Dr. Ngoc-Anh Le, George Hyman Research Building, Medlantic Research Foundation, 108 Irving Street NW, Washington, DC 20010.

²Present address: Department of Medicine, Columbia University College of Physicians and Surgeons, New York, NY.

lipoproteins by hepatocytes is thought to occur via the interaction of apolipoprotein E (apoE) with a receptor(s) present on these cells (10, 11), and the relative amounts of apoC-III and apoE present on a lipoprotein particle may regulate this process (12, 13). ApoC-III may also inhibit LPL activity (14, 15), raising the possibility that the apoC-III/C-II ratio of a triglyceride-rich lipoprotein plays a critical role in regulating triglyceride hydrolysis. A further role for apoC-III in the catabolism of triglyceride-rich lipoproteins derives from evidence supporting a role for apoC-III as an inhibitor of hepatic triglyceride lipase (HTGL) activity (16). This enzyme appears to be involved in the catabolism of small VLDL and intermediate density lipoproteins (17) and in the regulation of high density lipoprotein (HDL) concentrations (18). Studies of the metabolism of triglyceride-rich lipoproteins in two sisters who lack apoC-III (and apoA-I) supported the hypothesis that apoC-III normally inhibits VLDL triglyceride catabolism in vivo (19).

In the present study we have determined the concentration of apoC-II and apoC-III in VLDL and HDL in a group of subjects with a wide range of plasma VLDL triglyceride and HDL cholesterol levels. The relationship between the levels of these apolipoproteins and both the fractional rates of catabolism (FCR) of VLDL triglyceride and the FCR of HDL apoA-I was also examined. Our results demonstrate that the concentrations of apoC-II and apoC-III in these two lipoproteins are independently regulated. Hence, the ratio of apoC-III to apoC-II in VLDL increased significantly concomitant with increasing degrees of hypertriglyceridemia. In addition, the levels of apoC-III in HDL correlated with those of apoA-I, while no such correlation existed for apoC-II and apoA-I. Finally, the kinetic data suggested that the independent regulation of apoC-III and apoC-II concentrations may play a role in modulating the metabolism of VLDL and HDL.

METHODS

Subjects

Nineteen subjects, 15 males and 4 females, participated in these studies. Study subjects were chosen to generate a group with a wide range of plasma triglyceride and HDL cholesterol concentrations. All of these subjects were participating in comprehensive studies of lipoprotein metabolism that included measurements of the plasma turnovers of VLDL triglyceride and apoB; IDL and LDL apoB; and HDL apoA-I. Other results from some of those studies have been reported elsewhere (20). All subjects were free of diseases that might be associated with secondary hyperlipidemias and none were receiving any medications known to affect lipid metabolism. The protocol was ap-

proved by the Institutional Review Board at the Mount Sinai Medical Center, and informed consent was obtained from each subject.

Protocol

The studies were carried out while the subjects were inpatients on the General Clinical Research Center at our institution. In some studies, the final blood samples obtained during the last 5 days of the HDL apoA-I turnover study were collected while the individuals were outpatients. In these instances, the subjects were instructed as to the diet they were to consume at home.

Upon admission to the hospital, all subjects were given a diet of solid food consisting of 40% carbohydrate, 40% fat, and 20% protein. The ratio of polyunsaturated to saturated fat was 0.4 and each subject ingested 300 mg of cholesterol/day. Caloric intake was begun at 35 kcal/kg per day, and adjusted as necessary to maintain body weight. After 3 days, fasting blood was obtained for isolation of HDL by sequential ultracentrifugation as previously described (21). All procedures were carried out using sterile techniques. ^{125}I -labeled HDL was prepared by a modification of the method of MacFarlane (22) and passed through a 0.22 μm filter before injection. Four to 5 days after admission, 300 μCi of [^3H]glycerol was injected intravenously and 18 blood samples were withdrawn over the following 48 hr (23). During this time period, the subjects consumed a fat-free liquid formula designed to eliminate the intermittent entry of chylomicrons into the bloodstream. This diet allowed us to maintain steady-state levels of plasma triglycerides, cholesterol, and apolipoproteins equal to fasting levels present during consumption of the solid food diet prior to the 48-hr sampling period (24). At the end of the 48 hr, 75 μCi of ^{125}I -labeled HDL was injected and blood samples were taken at timed intervals over the next 2 weeks. The subjects returned to the solid food diet at the time of the HDL injection. Saturated solutions of potassium iodide were given to all study subjects for the duration of the tests.

Laboratory procedures

VLDL ($d < 1.006$ g/ml) was isolated from each of the 18 timed samples obtained after injection of tritiated glycerol by ultracentrifugation of 3 ml plasma in a 40.3 fixed-angle rotor at 39,000 rpm for 20 hr at 10°C (23). The VLDL were then used to determine the specific radioactivity of [^3H]triglyceride by the method of Grundy et al. (24). Separate samples of plasma, obtained at 0, 12, 24, 36, and 48 hr were used to isolate VLDL and HDL ($d 1.063$ – 1.210 g/ml) for measurement of lipoprotein triglyceride, cholesterol, and apolipoproteins. HDL was also isolated from 3 ml of plasma from each of the timed samples obtained during the 2 weeks after injection of ^{125}I -labeled HDL. The plasma was ultracentrifuged in a 40.3 rotor at 39,000

rpm for 24 hr at d 1.063 g/ml to isolate VLDL, intermediate density lipoproteins, and low density lipoproteins. Ultracentrifugation at d 1.063 g/ml was repeated before HDL was isolated by ultracentrifugation at 39,000 rpm for 48 hr at d < 1.121 g/ml. The HDL were then used to determine the specific radioactivity of ^{125}I -labeled apoA-I as previously described (21). Briefly, HDL were delipidated and the apolipoproteins were separated by high performance liquid chromatography using a TSK 3000SW gel permeation column (25). The major peak, representing apoA-I, was collected and used for measurement of radioactivity and protein mass.

Plasma and lipoprotein triglyceride and cholesterol concentrations were determined by enzymatic methods using the ABA-100 automated spectrophotometer (26, 27). ApoB, apoA-I, apoC-II, and apoC-III were all determined by specific radioimmunoassays developed in our laboratory (28).

The FCR of VLDL triglyceride and of HDL apoA-I were estimated as previously described (21, 23). Statistical analyses were performed with CLINFO and SAS.

The multivariate analysis for the best linear regression for the two dependent parameters, the FCR for VLDL triglyceride and the FCR for HDL apoA-I, in terms of other compositional parameters of the respective lipoproteins, was carried out using the STEPWISE program of SAS. The results presented here are based on the use of the maximum R_2 improvement (MAXR) option of the program. In brief, the program first finds the one-variable model producing the highest R^2 . A second variable, yield-

ing the greatest increase in R^2 is then included as an initial starting point for a two-variable model. Each of the variables in this initial two-variable model is then compared to all other variables not already in the model, and replacement of variables is continued until no further increases are achieved in R^2 . The two remaining variables define the "best" two-variable model. In a similar fashion, each time the number of independent parameters is increased, the program insures that the set of parameters producing the greatest increase in R^2 is determined.

For the characterization of the FCR of VLDL triglyceride, the parameters included in the multivariate analysis were VLDL triglyceride, apoB, apoC-II, apoC-III, and the ratios of apoB/triglyceride, apoC-II/apoB, apoC-II/apoB, and apoC-III/apoC-II within the VLDL density range. The same analysis was applied to the FCR of HDL apoA-I using as independent variables HDL cholesterol, apoA-I, apoC-II, apoC-III, and the ratios of apoA-I/cholesterol, apoC-II/apoA-I, apoC-III/apoA-I, and apoC-III/apoC-II in HDL.

RESULTS

Table 1 depicts the clinical characteristics of the study group. The mean ages of the males and females were 35.2 ± 12.7 and 49.0 ± 5.2 years, respectively. Only two of the subjects had relative weights greater than 30% above ideal. Plasma triglyceride concentrations ranged from 50 to 526 mg/dl while HDL cholesterol levels varied from 14 to 60 mg/dl. Although several subjects had low HDL cholesterol concentrations in association with normal plasma triglycerides, for the overall group there was the expected inverse relationship between these two variables, $r = -0.43$, $P = 0.056$. None of the subjects had a ratio of cholesterol to triglyceride in VLDL greater than 0.30.

The ranges for the apolipoproteins measured in VLDL and HDL are presented in **Table 2**. Assuming that there is a single apoB molecule on each VLDL particle, individuals with hypertriglyceridemia might have as many as 20 times the number of plasma VLDL particles as the subjects with the lowest concentration of apoB. In addition, the ratio of triglyceride to apoB in VLDL ranged from approximately 5 to 20 in the group, with higher ratios present in the hypertriglyceridemic individuals.

The concentration of apoC-II in VLDL also varied by 20-fold in this group of subjects suggesting that, on the average, the number of apoC-II molecules per VLDL particle remained constant. When the difference in molecular weights of apoB and apoC-II are taken into account, these data indicate that on the average, 15–20 molecules of apoC-II were present on VLDL particles regardless of the number of particles present. In contrast, apoC-III increased about 50-fold across the spectrum of

TABLE 1. Clinical characteristics

Subject	Age	Ideal Body Weight	TG	TC	LDL-C	HDL-C
1	35	1.00	64	162	87	60
2	30	0.97	66	170	102	48
3	36	1.00	59	155	91	44
4	30	1.26	191	205	131	30
5	27	0.88	55	178	121	54
6	30	1.18	78	129	92	23
7	64	1.02	66	161	93	31
8	53	1.47	114	164	97	42
9	45	1.19	264	175	111	25
10	44	1.12	215	159	81	21
11	56	1.40	526	236	94	19
12	50	1.26	289	170	64	18
13	30	0.95	312	132	36	16
14	30	0.92	313	150	61	29
15	54	0.98	55	99	76	14
16	24	1.00	50	110	87	22
17	23	1.14	132	134	62	32
18	20	1.03	130	104	71	26
19	43	0.93	79	244	181	15

TG, triglyceride; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

TABLE 2. Apolipoprotein levels^a

Lipoprotein	ApoB	ApoC-II	ApoC-III	ApoA-I
		<i>μg/ml</i>		
VLDL	14.9-350.4	5.5-96.5	7.2-322.8	
HDL		4.1-20.5	8.9-84.5	354.0-1691.1

^aApolipoprotein levels: range of values for each apolipoprotein in VLDL and HDL. Each subject's value was the mean of five samples obtained during the 48-hr turnover studies. Coefficients of variation for all measurements were less than 15%.

our subject population. Thus, at the lower end of the range of VLDL triglyceride concentrations, about 20-25 molecules of apoC-III were on each VLDL particle, while at the higher end of the VLDL triglyceride concentration range, an average of 50 apoC-III molecules were present on each particle.

As a result of these differences in apoC-II and apoC-III content per VLDL particle, the ratio of apoC-III/apoC-II in VLDL increased in parallel with the ratio of triglyceride to apoB in VLDL. The apoC-III/apoC-II ratio in VLDL ranged from 1.0 in normolipidemic subjects to 5.0 in individuals with the greatest degree of hypertriglyceridemia, and was closely related to the plasma concentration of VLDL, in terms of either the apoB or triglyceride content (Fig. 1). There were positive and significant correlations of the apoC-III/apoC-II ratio with the concentrations of both VLDL apoB and VLDL triglyceride ($r = 0.59$, $P < 0.01$ and $r = 0.70$, $P < 0.005$, respectively).

The FCR for VLDL triglyceride of individuals in our study group ranged from 0.09 to 0.60 hr⁻¹. This measure of VLDL degradation was inversely related to the concentrations of triglyceride, apoB, apoC-III and apoC-II in VLDL (Table 3). In addition, the VLDL triglyceride FCR was inversely and significantly correlated with the VLDL apoC-III/apoC-II ratio; $r = -0.58$, $P < 0.01$ (Fig. 2).

Because all of these molecules comprise the VLDL particle, strong correlations were expected between many of these parameters. Multivariate analysis was performed, therefore, to determine which parameters best predicted the FCR of VLDL triglyceride. In this analysis (see Methods), all of the available parameters were used to determine the sets which result in the maximum R², but only the parameter with the most significant contribution was added at each level of increasing complexity. This analysis indicated that the FCR of VLDL triglyceride could best be predicted by a linear regression which included VLDL triglyceride concentration, the ratio of apoC-II/apoB in VLDL and the concentration of apoC-II in VLDL. The equation depicting these relationships was:

$$\text{VLDL-TG FCR} = 0.510 - 0.001 (\text{VLDL-TG}) - 0.17 (\text{apoC-II/apoB}) + 0.001 (\text{apoC-II}); R^2 = 0.49.$$

The addition of the apoC-III/apoC-II ratio to this equation did not increase the R² value significantly.

In HDL, there was a 5-fold increase in apoA-I, a 5-fold increase in apoC-II, and a 10-fold increase in apoC-III concentrations across the range of the study group (Table 2). Because the number of apoA-I molecules per HDL particle does not appear to be fixed, we cannot estimate the number of apoC-II or apoC-III molecules per HDL particle. However, we can suggest that, on the average, HDL particles became enriched in apoC-III relative to apoC-II as the concentration of HDL apoA-I and HDL cholesterol (and probably HDL particle number) increased. The lack of a straightforward relationship between apoC-III and apoC-II and the other major components of HDL is depicted in Table 4. While there were strong positive correlations between both apoC-III and apoC-II and the concentration of HDL cholesterol, there was no correlation between apoC-II and HDL apoA-I. In contrast, there was an extremely strong correlation between apoC-III and apoA-I in HDL.

The FCR of apoA-I in HDL ranged from 0.15 to 0.50 day⁻¹ in the total study group. These rates were inversely related to the concentration of HDL cholesterol and HDL apoA-I (Table 5). HDL apoC-III concentration was also inversely correlated with the apoA-I FCR ($r = -0.67$, $P < 0.005$). In addition, the apoC-III/apoA-I ratio was significantly and inversely related to the apoA-I FCR ($r = -0.66$, $P < 0.005$) (Fig. 3). In contrast, there were no significant correlations between the FCR of HDL apoA-I and the concentration of apoC-II, the ratio of apoC-II/apoA-I, or the ratio of apoC-III/apoC-II.

Multivariate analysis of the HDL data was performed as described for VLDL. We found that the apoA-I FCR could best be predicted by a linear regression which included the concentration of HDL cholesterol, the ratio of

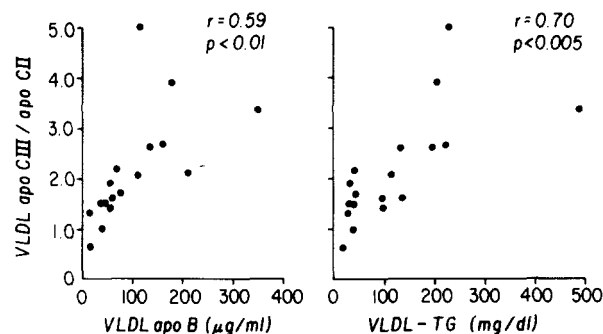


Fig. 1. Relationship between the ratio of apoC-III to apoC-II in VLDL and the concentration of VLDL apoB (left panel) and VLDL triglyceride (right panel). Overall, as the concentration of VLDL increased, the ratio of apoC-III to apoC-II increased, indicating an enrichment of VLDL with apoC-III.

TABLE 3. Relationships between VLDL catabolism and composition^a

	VLDL-TG	VLDL-B	VLDL-C-III	VLDL-C-II	VLDL C-III/C-II
VLDL-TG FCR ^b	-0.70	-0.63	-0.69	-0.58	-0.58
P Value	<0.005	<0.01	<0.005	<0.01	<0.01

^aValues are correlation coefficients between VLDL-TG FCR and VLDL triglyceride, apoB, apoC-III, or apoC-II concentrations and VLDL apoC-III/C-II.

^bFractional catabolic rate of VLDL triglyceride determined by analysis of VLDL-[³H]triglyceride kinetics.

apoC-III/apoA-I in HDL, and the HDL apoC-II concentration:

$$\text{apoA-I FCR} = 0.492 - 0.006 (\text{HDL Chol}) - 2.171 (\text{apoC-III/apoA-I}) + 0.007 (\text{apoC-II}); R^2 = 0.74.$$

DISCUSSION

ApoC-II and apoC-III are apolipoproteins with similar, but not necessarily identical, dynamic and steady-state distributions across the spectrum of plasma lipoproteins. Because of these similarities, their close molecular weights, and the fact that they were initially isolated and characterized together (1), they are often referred to as the "C apolipoproteins." The present study, however, together with those by previous investigators, suggests distinct differences in their pattern of association with plasma lipoprotein particles. As expected, concentrations of both apoC-II and apoC-III increased in parallel with increasing VLDL triglyceride and apoB levels. However, the slope of the relationship between concentrations of VLDL apoC-III and VLDL apoB was two to three times that of VLDL apoC-II and VLDL apoB in a group of 19 individuals with a wide range of VLDL triglyceride concentrations. This discordance resulted in an average twofold enrichment of apoC-III molecules per VLDL particle relative to apoC-II in subjects with hypertriglyceridemia. Carlson and Ballantyne (29) also demonstrated that the ratio of apoC-III to apoC-II was greater in hypertriglyceridemic subjects compared with normal individuals. Schonfeld and co-workers (30) presented data demonstrating that, although both VLDL apoC-II and apoC-III rose as triglyceride concentration increased in a large group of subjects, the rate of rise of apoC-III was greater than that of apoC-II. These results suggested that the apoC-III/apoC-II ratio rose across the range of triglyceride concentrations as well (30). Finally, Saku et al. (31) recently reported that reductions in plasma triglyceride levels during treatment with gemfibrozil were associated with significant reductions in the ratio of total apoC-III to apoC-II in six patients with severe hypertriglyceridemia.

VLDL are a heterogeneous spectrum of particles varying in size, density, and composition. A possible explanation for the discordant associations of apoC-II and apoC-III

with VLDL as plasma triglycerides increase is a preferential binding of apoC-III (or a reduced binding capacity of apoC-II) to a particular subpopulation of VLDL that was increased in the hypertriglyceridemic subjects. Both Carlson and Ballantyne (29), using gradient ultracentrifugation, and Kane et al. (32), utilizing gel permeation chromatography, observed relative increases in apoC-III compared to apoC-II in more dense, smaller VLDL. Severe hypertriglyceridemia would likely be associated with a shift in VLDL toward less dense, larger particles which, based on these previous reports (29, 30), would tend to reduce the overall apoC-III/apoC-II ratio in VLDL. Moderate elevations in VLDL triglyceride concentrations of the degree present in our subjects is, however, usually associated with increased numbers of VLDL particles and a continued predominance of more dense, smaller VLDL subpopulations. The significant correlation between VLDL apoB and triglyceride levels in our subjects supports this view. In addition, in preliminary studies of a group of six subjects with plasma triglyceride levels ranging from 60 to 500 mg/dl, the ratio of apoC-III/apoC-II in VLDL was elevated in the S_f 100-400, S_f 60-100, and S_f 60-20 subclasses in the hypertriglyceridemic subjects (H. Ginsberg, unpublished data). Hence, we believe that apoC-III has an increased affinity for VLDL of all sizes, relative to apoC-II, although this difference in binding capacity may

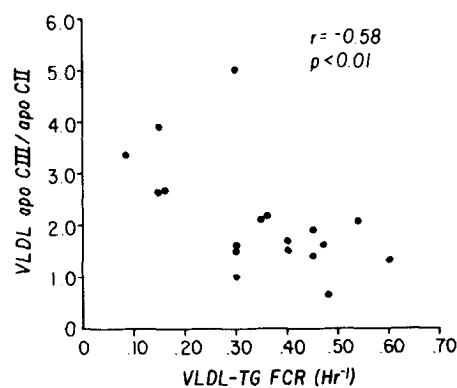


Fig. 2. Relationship between the ratio of apoC-III to apoC-II in VLDL and the fractional catabolic rate (FCR) of VLDL triglyceride. As the ratio of apoC-III to apoC-II in VLDL increased, the VLDL-TG FCR fell, suggesting the apoC-III-enriched VLDL were catabolized less efficiently.

TABLE 4. Relationships between components of HDL

	HDL-C	HDL ApoA-I
HDL ApoC-III	0.75 ^a <i>P</i> < 0.005	0.73 <i>P</i> < 0.005
HDL ApoC-II	0.58 <i>P</i> < 0.01	0.15 NS ^b

^aCorrelation coefficient.^bNot significant.

be most marked in smaller, more dense VLDL particles. An alternative basis for our findings is an increased rate of production of apoC-III in hypertriglyceridemic subjects.

The mechanisms proposed above seem, at first glance, to be inconsistent with prior studies showing that consumption of high carbohydrate diets, which cause elevations in plasma triglyceride levels, is associated with greater increases in VLDL apoC-II compared to VLDL apoC-III (33, 34). The reduced apoC-III/C-II ratios observed during carbohydrate-induced hypertriglyceridemia in the latter studies are in contrast to the increased ratios present in hyperlipidemic subjects consuming diets of normal composition presented in this and previous reports (29, 30). However, high carbohydrate diets appear to increase plasma VLDL triglycerides by increasing the quantity of triglyceride per particle (35, 36), and therefore increasing the size of the particles (36) without altering the number of VLDL particles in plasma (35). Hence, the reduced apoC-III/C-II ratios present during carbohydrate-induced hypertriglyceridemia are in accord with the observation that larger, less dense VLDL may be relatively enriched in apoC-II (29, 32). This model of hypertriglyceridemia may not, therefore, be directly relevant to considerations of the VLDL apoC-III/C-II ratio in endogenous hypertriglyceridemia. Direct effects of high carbohydrate diets on the synthesis of apoC-II, and/or apoC-III, that contribute to these contrasting data must be considered as well.

There are fewer data concerning the relative concentrations of apoC-III and apoC-II in HDL. In the present study, HDL apoC-III was highly correlated with HDL apoA-I levels while no such relationship was present between apoC-II and apoA-I. Alaupovic (37) also ob-

served a significant correlation between levels of apoC-III and cholesterol in HDL, but did not comment upon apoC-II. In addition, the ratio of apoC-III to apoA-I increased as HDL cholesterol and apoA-I increased, indicating enrichment of HDL particles with apoC-III in subjects with more HDL particles in the plasma. No such enrichment of HDL with apoC-II was evident. Sasaki et al. (38) demonstrated that high carbohydrate feedings, which result in decreased HDL cholesterol and apoA-I concentrations, were associated with reduced levels of apoC-III but increased concentrations of apoC-II in HDL₂. In view of the relationships between VLDL apoC-III and apoC-II described above, one may propose that, as VLDL triglyceride levels increase across a study population, the transfer of apoC-III to VLDL from HDL is quantitatively greater than the transfer of apoC-II. Conversely, as HDL levels rise, apoC-III movement to that lipoprotein class from VLDL is greater than the shift in apoC-II. In support of this hypothesis is the finding by Windler and Havel (13) that when apoC-II and apoC-III were incubated with VLDL and chylomicron remnants, these particles acquired significant quantities of apoC-III but essentially no apoC-II. In addition, we have previously demonstrated that there exist both rapidly equilibrating and nonequilibrating pools of apoC-III in the various lipoproteins (39). While this heterogeneity in the physical properties of apoC-III is likely to extend to apoC-II, differences in the relative mobility of apoC-III and apoC-II between lipoprotein classes could account for many of our observations.

The physiologic implications of our observations can, of course, only be inferred from correlational data such as these. In addition, the close physical interrelationships between the apolipoprotein and lipid components of VLDL and HDL make interpretation of any significant correlations more difficult. Hence, while simple univariate analysis demonstrated the interesting relationship between the VLDL apoC-III/apoC-II ratio and the FCR of VLDL triglyceride, this correlation did not appear in the multivariate linear regression equation. In the latter analysis, VLDL triglyceride concentration and the apoC-II/apoB ratio in VLDL were inversely related, while the absolute apoC-II concentration was positively correlated to VLDL triglyceride fractional catabolism. Despite the

TABLE 5. Relationships between HDL catabolism and composition^a

	HDL-C	HDL ApoA-I	HDL ApoC-III	HDL ApoC-III/A-I
HDL-ApoA-I-FCR ^b	-0.77	-0.50	-0.67	-0.66
<i>P</i> Value	<0.005	<0.05	<0.005	<0.005

^aValues are correlation coefficients between HDL-apoA-I FCR and HDL-C, HDL apoA-I, HDL apoC-III, and HDL apoC-III/A-I.^bFractional catabolic rate of HDL-apoA-I determined by analysis of ¹²⁵I-labeled HDL-apoA-I kinetics.

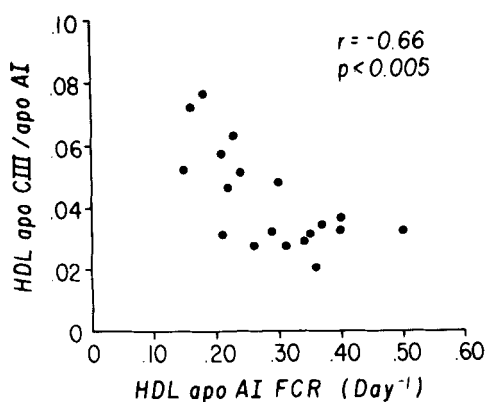


Fig. 3. Relationship between the ratio of apoC-III to apoA-I in HDL and the fractional catabolic rate (FCR) of HDL apoA-I. As the ratio of apoC-III to apoA-I increased, the HDL-apoA-I FCR fell. Lower fractional rates of apoA-I catabolism were associated with high plasma concentrations of HDL cholesterol and HDL apoA-I.

difficulties inherent in extrapolating from complex statistical analyses such as these, the clearly defined roles of apoC-II as a required activator of LPL (2, 3), and the convincing evidence that apoC-III is an inhibitor of LPL (14, 15), suggest that their independent regulation as apolipoprotein components of VLDL might be relevant in vivo. Our recent in vivo investigations of VLDL metabolism in two sisters with absence of apoC-III (19) as well as in vitro studies of LPL activation by Breckenridge et al. (5) using sera from subjects heterozygous for apoC-II deficiency also support the concept that the ratio of VLDL apoC-III/apoC-II plays a role in the regulation of VLDL triglyceride catabolism.

Our results suggesting a role for HDL apoC-III in the regulation of HDL metabolism, though unexpected, were more convincing than those we observed for VLDL apoC-III and VLDL metabolism. The concentration of HDL apoC-III and the apoC-III/apoA-I ratio in HDL were both inversely related to the FCR of apoA-I in our study group by univariate analysis. This analysis did not demonstrate significant correlations between the apoA-I FCR and either the HDL apoC-II level or the ratio of apoC-II/apoA-I. Multivariate analysis confirmed the relationship between the apoC-III/apoA-I ratio and the FCR for HDL apoA-I, with the absolute levels of HDL apoC-II and HDL cholesterol as the only other variables of significance. We would infer from these results that apoC-III-enriched HDL particles might have reduced rates of fractional catabolism because of the inhibition of hepatic triglyceride lipase by apoC-III (16). Regardless of the mechanism, however, our data support previous studies implicating apoC-III as a regulator of the fractional rate of catabolism of plasma HDL (18, 40). The significance of apoC-II is less clear, although the positive relationship might be indicative of efficient VLDL catabolism and transfer of surface components to HDL. Of course, the

rate of HDL, or more specifically, apoA-I production, may also play an important role in the regulation of plasma HDL concentrations. Indeed, we have also noted reduced rates of apoA-I production in a subgroup of our subjects with low HDL cholesterol and normal plasma triglyceride concentrations (20).

In summary, the lipoprotein concentrations of apoC-II and apoC-III, two proteins with very different and potentially counterbalancing functions, are independently regulated in VLDL and HDL. This independence leads to varying ratios of the two in both lipoproteins, depending on the total concentration and the other physicochemical characteristics of particles in each density class. This ratio in VLDL may be a critical factor in the regulation of triglyceride hydrolysis and, therefore, in the conversion of VLDL to lipoproteins of higher density. The discordant association of apoC-III and apoC-II in HDL (or HDL subpopulations) may regulate the catabolism of this lipoprotein as well. Further studies of the in vivo metabolism of subclasses of VLDL and HDL in which the relative quantities of apoC-II and apoC-III differ should be informative in this regard. ■

The authors would like to thank the staff of the General Clinical Research Center at Mount Sinai for their assistance in these studies. We also thank Ms. N. Ngai, Mrs. J. Lee, Mr. S. Wong, Ms. T. Han, and Mrs. R. Veiss for excellent technical support, and Ms. D. Shuler and Mrs. M. Anderson for preparation of this manuscript. This work was supported by the following grants from the USPHS-NIH: HL 23077, HL 36000, HL 21006, and RR-71. Dr. Ginsberg was a recipient of a Research Career Development Award, HL 00949. Dr. Le was a recipient of a New Career Investigator Award, HL 27170.

Manuscript received 24 September 1987 and in revised form 24 November 1987.

REFERENCES

1. Brown, W. V., R. I. Levy, and D. S. Fredrickson. 1969. Studies of the proteins in human plasma very low density lipoproteins. *J. Biol. Chem.* **244**: 5687-5694.
2. La Rose, J. C., R. I. Levy, P. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. A specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41**: 57-62.
3. Ekman, R., and P. Nilsson-Ehle. 1975. Effects of apolipoproteins on lipoprotein lipase activity of human adipose tissue. *Clin. Chim. Acta.* **63**: 29-35.
4. Breckenridge, W. C., J. A. Little, G. Steiner, A. Chow, and M. Poapst. 1978. Hyperlipidemia associated with a deficiency of apolipoprotein C-II. *N. Engl. J. Med.* **298**: 1265-1273.
5. Breckenridge, W. C., P. Alaupovic, D. W. Cox, and J. A. Little. 1982. Apolipoprotein and lipoprotein concentrations in familial apolipoprotein C-II deficiency. *Atherosclerosis.* **44**: 223-235.
6. Baggio, G., E. Manzato, C. Gabelli, R. Fellin, S. Martini, G. Baldo Enzi, F. Veriato, M. R. Baiocchi, D. L. Sprecher, M. L. Kashyap, H. B. Brewer, and G. Crepaldi. 1986. Apolipoprotein C-II deficiency syndrome. Clinical fea-

- tures, lipoprotein characterization, lipase activity, and correction of hypertriglyceridemia after apolipoprotein C-II administration to two affected patients. *J. Clin. Invest.* **77**: 520-527.
7. Windler, E., Y. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255**: 5475-5480.
 8. Shelburne, F., J. Hanks, W. Meyers, and S. A. Quarfordt. 1980. Effect of apoproteins on hepatic uptake of triglyceride emulsions in the rat. *J. Clin. Invest.* **65**: 652-658.
 9. Pattnaik, N. M., and D. B. Zilversmit. 1980. Effect of size and competition by lipoproteins and apolipoproteins on the uptake of chylomicrons and chylomicron remnants by hepatoma cells in culture. *Biochim. Biophys. Acta.* **617**: 335-346.
 10. Havel, R. J., Y. S. Chao, E. E. Windler, L. Kotite, and L. S. S. Guo. 1980. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **77**: 4349-4353.
 11. Hui, D. Y., T. L. Innerarity, and R. W. Mahley. 1981. Defective hepatic lipoprotein receptor binding of beta-very low density lipoproteins from type III hyperlipoproteinemic patients. Importance of apolipoprotein E. *J. Biol. Chem.* **256**: 860-869.
 12. Quarfordt, S. H., G. Michalopoulos, and B. Schirmer. 1982. The effect of human C apolipoproteins on the in vitro hepatic metabolism of triglyceride emulsions in the rat. *J. Biol. Chem.* **257**: 14642-14647.
 13. Windler, E., and R. J. Havel. 1985. Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J. Lipid Res.* **26**: 556-563.
 14. Havel, R. J., V. G. Shore, B. Shore, and D. M. Bier. 1970. Role of specific glycopeptides of human serum lipoproteins in the activation of lipoprotein lipase. *Circ. Res.* **27**: 595-600.
 15. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46**: 375-381.
 16. Kinnunen, P. K. J., and C. Enholm. 1976. Effect of serum and C-apoproteins from very low density lipoproteins on human post-heparin plasma hepatic lipase. *FEBS Lett.* **65**: 354-357.
 17. Goldberg, I. J., N-A. Le, J. R. Paterniti, H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70**: 1184-1192.
 18. Kuusi, T., P. Saarinen, and E. A. Nikkilä. 1980. Evidence for the role of hepatic endothelial lipase in the metabolism of plasma high density lipoprotein 2 in man. *Atherosclerosis.* **36**: 589-593.
 19. Ginsberg, H. N., N-A. Le, I. J. Goldberg, J. C. Gibson, A. Rubinstein, P. Wang-Iverson, R. Norum, and W. V. Brown. 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI: Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J. Clin. Invest.* **78**: 1287-1295.
 20. Le, N-A., and H. N. Ginsberg. 1988. Heterogeneity of apolipoprotein A-I turnover in subjects with reduced concentrations of plasma high density lipoprotein cholesterol. *Metabolism. In press.*
 21. Ginsberg, H. N., N-A. Le, and H. S. Gilbert. 1986. Altered high density lipoprotein metabolism in patients with myeloproliferative diseases and hypercholesterolemia. *Metabolism.* **35**: 878-882.
 22. McFarlane, A. S. 1958. Efficient trace labeling of proteins with iodine. *Nature (London)* **182**: 153-157.
 23. Ginsberg, H. N., N-A. Le, and J. C. Gibson. 1985. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. Effects of weight loss. *J. Clin. Invest.* **75**: 614-623.
 24. Grundy, S. M., H. Y. I. Mok, L. Zech, D. Steinberg, and M. Berman. 1979. Transport of very low density lipoprotein triglycerides in varying degrees of obesity and hypertriglyceridemia. *J. Clin. Invest.* **63**: 1274-1283.
 25. Polacek, D., C. Edelstein, and A. M. Scanu. 1981. Rapid fractionation of human high density apolipoproteins by high performance liquid chromatography. *Lipids.* **16**: 927-929.
 26. Allain, C. C., L. S. Poon, S. C. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20**: 470-475.
 27. Bucolo, G., and H. David. 1973. Quantitative determinations of serum triglycerides by use of enzymes. *Clin. Chem.* **19**: 476-482.
 28. Gibson, J. C., A. Rubinstein, P. R. Bukberg, and W. V. Brown. 1983. Apolipoprotein E-enriched lipoprotein subclasses in normolipidemic subjects. *J. Lipid Res.* **24**: 886-898.
 29. Carlson, L. A., and D. Ballantyne. 1976. Changing relative proportions of apolipoproteins C-II and C-III of very low density lipoproteins in hypertriglyceridemia. *Atherosclerosis.* **23**: 563-568.
 30. Schonfeld, G., P. K. George, J. Miller, P. Reilly, and J. Witztum. 1979. Apolipoprotein C-II and C-III levels in hyperlipoproteinemia. *Metabolism.* **28**: 1001-1010.
 31. Saku, K., P. S. Gartside, B. A. Hynd, and M. L. Kashyap. 1985. Mechanism of action of gemfibrozil on lipoprotein metabolism. *J. Clin. Invest.* **75**: 1702-1712.
 32. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56**: 1622-1634.
 33. Kashyap, M. L., R. L. Barnhart, L. S. Srivastava, G. Perisutti, P. Vink, C. Allen, E. Hogg, D. Brady, C. S. Glueck, and R. L. Jackson. 1982. Effects of dietary carbohydrate and fat on plasma lipoproteins and apolipoproteins C-II and C-III in healthy men. *J. Lipid Res.* **23**: 877-886.
 34. Falko, J. M., G. Schonfeld, J. L. Witztum, J. B. Kolar, and P. Salmon. 1980. Effects of short-term high carbohydrate, fat-free diet on plasma levels of apoC-II and apoC-III and on the apoC subspecies in human plasma lipoproteins. *Metabolism.* **29**: 654-661.
 35. Melish, J., N-A. Le, H. Ginsberg, D. Steinberg, and W. V. Brown. 1980. Dissociation of apolipoprotein B and triglyceride production in very-low-density lipoproteins. *Am. J. Physiol.* **239**: E354-E362.
 36. Ruderman, N. B., A. L. Jones, R. M. Kraus, and E. Shafir. 1971. A biochemical and morphologic study of very low density lipoproteins in carbohydrate-induced hypertriglyceridemia. *J. Clin. Invest.* **50**: 1355-1368.
 37. Alaupovic, P. 1981. David Rubenstein Memorial Lecture: The biochemical and clinical significance of the interrelationship between very low density and high density lipoproteins. *Can. J. Biochem.* **59**: 565-579.

38. Sasaki, N., G. Holdworth, R. L. Barnhart, L. S. Srivastava, C. S. Glueck, M. L. Kashyap, and R. L. Jackson. 1983. Effect of a high carbohydrate diet on the content of apolipoproteins C-II, C-III and E in human plasma high density lipoprotein subfractions. *Atherosclerosis* **46**: 341-352.
39. Bukberg, P. R., N-A. Le, H. N. Ginsberg, J. C. Gibson, A. Rubinstein, and W. V. Brown. 1985. Evidence for non-equilibrating pools of apolipoprotein C-III in plasma lipoproteins. *J. Lipid Res.* **26**: 1047-1057.
40. Hazzard, W. R., S. M. Haffner, R. S. Kushwaha, D. Applebaum-Bowden, and D. M. Foster. 1984. Preliminary report: Kinetic studies on the modulation of high-density lipoprotein, apolipoprotein and subfraction metabolism by sex steroids in a post-menopausal woman. *Metabolism* **33**: 779-784.