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High density lipoprotein2. Relationship of the plasma levels of this lipoprotein species to its composition, to the magnitude of postprandial lipemia, and to the activities of lipoprotein lipase and hepatic lipase.

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Research Article

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High Density Lipoprotein$_2$

Relationship of the Plasma Levels of This Lipoprotein Species to Its Composition, to the Magnitude of Postprandial Lipemia, and to the Activities of Lipoprotein Lipase and Hepatic Lipase

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Abstract

Lipoprotein lipase (LPL) activity in postheparin plasma of 38 normolipidemic volunteers was related to the magnitude of postprandial lipemia after a fat meal, to triglyceride content of high density lipoprotein$_2$ (HDL_2), to hepatic lipase (HL) activity, and to HDL_2 levels. LPL activity correlated indirectly with lipemia, triglyceride content of HDL_2, HL activity, and levels of HDL_2 but not of HDL_3. HL activity correlated directly with lipemia and indirectly with HDL_2 levels.

Triglyceride content of HDL_2 correlated directly with lipemia and indirectly with HDL_2 levels. In HDL_2, abundance of apolipoprotein (apo) A-II and the apoA-I/apoA-II ratio varied widely. The latter correlated positively with LPL activity and HDL_2 levels, and, inversely, with HL activity, lipemia, and triglyceride content of HDL_2. The study suggests that HDL-cholesterol is not an independent parameter of lipid transport, but is strongly affected by triglyceride metabolism through lipolytic enzymes, as exemplified by postprandial lipemia that affect both composition and plasma levels of HDL_2.

Introduction

The negative association between coronary heart disease (CHD)[1] and high density lipoprotein (HDL)-cholesterol (1–4) has been found to be at least as powerful as correlations between CHD and all other known risk factors (5). Of the two major HDL subfractions (6, 7), the plasma levels of HDL_2, but not of HDL_3, correlate strongly with HDL-cholesterol (8, 9), which suggests that the epidemiological data that relate risk of CHD to HDL-cholesterol are correlated with the HDL_2 fraction.

The mechanism(s) underlying the negative association between CHD and HDL_2 is unknown. HDL_2 could directly interfere with the atherosclerotic process by playing a major role in the "reverse cholesterol transport" (10). Alternatively, HDL_2 could be an indicator for a state of lipid transport that protects against atherosclerosis. For instance, among all the lipid or lipoprotein variables in fasting plasma, HDL_2 is most reliable and sensitive in predicting a normolipidemic individual's capacity to clear triglyceride (TG)-rich lipoproteins from the plasma (8). This significant finding has raised these questions: (*i*) Do the differences in postprandial lipemia contribute to the large differences in HDL_2 levels, and (*ii*) can the pronounced differences in the magnitude of postprandial lipemia among normolipidemics arise from differences in lipase activities?

To answer these questions we formulated the hypothesis that lipoprotein lipase (LPL) activity limits the magnitude of postprandial lipemia, which determines the TG content of HDL_2 that, in turn, affects the levels of HDL_2 through the action of hepatic lipase (HL). To test this hypothesis, we administered an oral fat load to normolipidemic individuals as index of TG clearance, analyzed levels and composition of HDL_2, and determined the activities of LPL and HL in postheparin plasma. We report, consistent with our hypothesis, that the lower the activity of LPL, the greater is the magnitude of postprandial lipemia and the TG content of HDL_2, and consequently the lower the plasma concentration of HDL_2. Opposite associations were found with HL.

Methods

Study subjects. This study was approved by the Human Subject Review Committee of Baylor College of Medicine, and informed consent was obtained from participants. Volunteers were recruited by announcement at the institution campus and participation in the study was remunerated. 41 subjects were enlisted as outpatients for three visits within 3 wk. Each subject was instructed to adhere to his/her usual diet and alcohol intake for this time period. Blood was obtained in the morning between 7:30 and 9:30 a.m. in the postabsorptive state, i.e., ~ 14 h after ingestion of a light dinner and no more than 30 g of ethanol. All blood samples were collected in tubes containing 1.5 mg/ml EDTA. The plasma specimens from the first visit were analyzed for cholesterol, TG, and HDL-cholesterol. At this stage of the study, three individuals were excluded because of cholesterol and/or TG levels elevated above the 95th percentile of normal age- and sex-matched subjects (11). Of the remaining 38 individuals, 13 were females and 25 were males, ranging in age from 21 to 42 yr. During the second visit, an oral fat-tolerance test was performed. A third visit was scheduled within 2 wk after the second visit to obtain a blood sample in the postabsorptive state, repeat lipid analysis, and perform HDL subfraction and apoprotein quantification. During this visit, heparin was injected intravenously and, after 15 min, a second blood sample was obtained for determining the activities of LPL and HL in postheparin plasma.

Test meal. A standard liquid fatty meal, whose composition and preparation have been described in detail previously (8), was administered orally in the postabsorptive state. Each individual ingested 65 g fat/m^2 body surface. Blood samples were collected in the postabsorptive state and at 2, 4, 6, and 8 h after ingestion of the fat meal. The magnitude of postprandial lipemia was quantified as the area under the time-dependent TG-level curve, and is expressed as milligrams per deciliter plasma times 8-h TG area (8).

Quantification of HDL_2. Concentrations of HDL_2 and HDL_3 in postabsorptive plasma were quantified by two independent procedures.

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1. *Abbreviations used in this paper:* apo, apolipoprotein; CHD, coronary heart disease; HL, hepatic lipase; LPL, lipoprotein lipase; TG, triglyceride.

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The first procedure involved stepwise precipitation of apolipoprotein (apo) B–containing lipoproteins (12) followed by precipitation of HDL_2 (13). Cholesterol was quantified in the respective supernatants. In this way, HDL-cholesterol and HDL_3-cholesterol were measured, and HDL_2-cholesterol was estimated by subtracting the latter from the former (13). The second quantification of HDL subfractions used zonal ultracentrifugation (7). Rotor fractions under the HDL_2 and HDL_3 peaks were pooled and analyzed for lipid and protein components. Lipid phosphorus was determined by the procedure of Bartlett (14); TG, by an enzymatic method (Triglycerides GPO; Boehringer Mannheim, GmbH, Mannheim, FRG); cholesterol and cholesteryl esters, by the High Performance K enzymatic method (15) (Boehringer Mannheim GmbH); and protein was quantified by the method of Lowry et al. (16), using bovine serum albumin (BSA) as standard. In this way, the plasma levels of all major lipid components, and of HDL_2 and HDL_3 protein could be determined, from which the concentrations of the entire HDL_2 and HDL_3 particle mass were calculated (7). Cholesterol recovered from the two zonally isolated HDL fractions averaged 93.8% of HDL-cholesterol.

For determination of HDL_2-cholesterol, zonal ultracentrifugation (7) and sequential precipitation (13) gave satisfactory agreement. By using the former method we obtained mean levels for HDL_2-cholesterol of 13±10 mg/dl (range between 2 and 35 mg/dl), and by the latter method, mean levels of 15±12 mg/dl (range between 0 and 45 mg/dl). The correlation coefficient was 0.90, and $P < 0.0001$. Because of this agreement, all comparisons of HDL_2 levels with other parameters of lipid transport were very similar for both methods of HDL_2 quantification. Total HDL-cholesterol, for example, correlated with HDL_2 from zonal ultracentrifugation at $r = 0.83$ ($P < 0.0001$) and from precipitation at $r = 0.88$ ($P < 0.0001$). All correlations, presented in this report, are given for the entire set of study subjects. When analyzed for males and females separately, no major differences in the levels of significance were found.

Other laboratory procedures. Cholesterol and TG in plasma were measured by automated, enzymatic procedures (17, 18). ApoA-I, apoA-II, and apoB were measured in plasma and in zonally isolated HDL fractions by respective double-antibody radioimmunoassay procedures (19–21). For quantification of apoA-I, 0.35% Tween-20 was included in the borate-BSA buffer system for adequate exposure of apoA-I immunoreactivity (22). ApoA-I and apoA-II recovered from the zonally isolated HDL fractions averaged 91 and 94% of plasma apoA-I and apoA-II, respectively.

To release LPL and HL into the circulation, heparin sodium, U. S. Pharmacopeia from beef lung, was used (Upjohn Co., Kalamazoo, MI). A blood sample was drawn in the postabsorptive state (preheparin plasma), then 2,280 U/m^2 heparin was administered intravenously. A second blood sample (postheparin plasma) was obtained 15 min after injection of heparin. Plasma was immediately separated by centrifugation at 4°C and stored at −70°C. Activities of LPL and HL were determined according to Huttunen et al. (23), using a [^{14}C]triolein-labeled substrate and an antiserum directed against HL. For assay of LPL, samples were preincubated with antiserum against human HL sufficient to inhibit > 95% of HL activity. HL was assayed in 1 M NaCl. All samples were analyzed in triplicate within one assay. This analysis was repeated in a second assay using a second set of aliquots of frozen-plasma samples. Mean interassay coefficient of variation was 7.2% for LPL and 5.2% for HL.

Validation of HL assay. Before its application in this study, the assay was evaluated in the presence of greatly varying amounts of HDL_2 and HDL_3 in postheparin plasma. HL is a single enzyme molecule displaying TG lipase, monoglyceride lipase, and phospholipase activities (24). TG and phospholipids of HDL_2 serve as substrates for HL (25), and HDL_2 compete with native TG-rich lipoproteins and artificial TG-rich emulsions as substrate for the enzyme (26). Endogenous HDL_2, when present in high amounts in a given plasma specimen to be assayed, could potentially compete with the exogenous substrate and thereby reduce the observed lipase activity. Using postheparin plasma for the lipase assays could further aggravate such interference. Injection of heparin enhances clearance of TG-rich lipoproteins and transfer of their surface components, mainly phospholipids, to HDL (27), thereby increasing HDL components that are able to compete for the enzyme activity. Such interference has indeed been suggested by some authors (28), but dismissed by others (29, 30). To resolve this issue, we isolated and quantified HDL_2 and HDL_3 from the pre- and postheparin plasma of two individuals (7), one male and one female. Comparison of preheparin to postheparin plasma showed that HDL_2 levels rose by 2 and 6%, and HDL_3 levels were altered by −2 and 3%, respectively. These HDL preparations were titrated to HL-assay mixtures using the postheparin plasma of a male representative of our study subjects to determine the inhibitory effect of HDL fractions on apparent HL activity against the artificial [^{14}C]triolein substrate. As summarized in Fig. 1, addition of HDL_2 and HDL_3 indeed reduced the observed activity of HL. However, a 5% reduction of HL activity required the addition of HDL_2 in concentrations equivalent to ~ 150 mg HDL_2 protein/dl plasma. The levels of HDL_2 protein in postabsorptive plasma of our study subjects were much lower, ranging from 2.5 to 44.5 mg/dl of plasma. Heparin altered the plasma levels of HDL_2 and HDL_3 by < 10%. Also, HDL_2 or HDL_3 purified from postheparin plasma did not differ noticeably from their counterparts, preheparin plasma, in their ability to inhibit lipase activities. We conclude, therefore, that low HL activities observed using the described assay (23) are not due to high levels of HDL_2 in a given plasma specimen.

Results

Lipid and apo levels in postabsorptive plasma, the triglyceridemic response to the standardized oral fat load, and the lipase activities in postheparin plasma from the 38 study subjects are given in Table I. Both HDL_2-cholesterol and triglyceridemic responses displayed by far the greatest interindividual variabilities, followed by the activity of LPL as a distant third, but still greater than the remaining parameters. Thus, our study subjects, all normolipidemic in the postabsorptive state, displayed the largest differences both in HDL_2 concentrations in postabsorptive plasma and in the TG levels after ingestion of the fatty test meal.

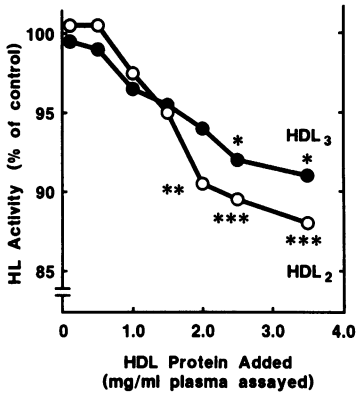

Figure 1. Effect of HDL_2 and HDL_3 on observed activity of HL. HDL_2 and HDL_3 were isolated from one female (31 mg/dl HDL_2-cholesterol, 32 mg/dl HDL_3-cholesterol) and one male (9 mg/dl HDL_2-cholesterol, 35 mg/dl HDL_3-cholesterol) before and 15 min after injection of heparin. Increasing amounts of each preparation (two preheparin and two postheparin) of HDL_2 ($n = 4$) and HDL_3 ($n = 4$) were added to assay mixtures for HL activity. Each mixture contained the same postheparin plasma from a male individual with low HDL_2 plasma levels (HDL_2 protein was 0.15 mg/ml; HDL_3 protein, 1.0 mg/ml plasma; and HDL-cholesterol, 51 mg/dl). As can be seen, ~ 1.5 mg HDL_2 protein/ml plasma (equivalent to 150 mg HDL_2 protein/dl) was required to reduce apparent activity of HL by 5%. HDL_2 protein in our study subjects ranged from 2.5 to 44.5 mg/dl plasma. Statistically, significant differences from control value (100%): * $P < 0.02$, ** $P < 0.01$, *** $P < 0.001$; $n = 4$. Results obtained with HDL_2 and HDL_3 from pre- ($n = 2$) and postheparin ($n = 2$) plasma were pooled ($n = 4$) because effects on lipase activity were identical.

Table I. Lipid and Apo Levels in Postabsorptive Plasma, Triglyceridemic Response, and Lipase Activities in Postheparin Plasma of 38 Study Subjects

	Chol	TG	HDL-Ch	HDL_2-Ch	HDL_3-Ch	ApoA-I	ApoA-II	ApoB	Triglyceridemic response*	Activity in postheparin plasma‡	
										LPL	HL
	mg/dl§	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*			
Mean	184	85	54	17	37	155	34	83	501	12	23
±1 SD	25	31	14	12	7	33	7	22	314	7	9
Range	131–235	28–159	24–81	0–45	24–52	90–215	21–50	47–145	58–1,338	4–34	9–41

Chol, total cholesterol; HDL-Ch, HDL_2-Ch, or HDL_3-Ch, cholesterol associated with total HDL, HDL_2, or HDL_3, respectively. * Triglyceridemic response expressed as milligrams per deciliter times 8-h TG area (8). ‡ Activity expressed as micromoles of free fatty acids released per milliliter plasma per hour at 28°C. § Milligrams per deciliter of postabsorptive plasma.

The magnitude of postprandial lipemia and the levels of HDL_2 in postabsorptive plasma showed a significant negative association (Table II). The association was virtually identical using either the entire HDL_2 lipoprotein values obtained by zonal ultracentrifugation or the HDL_2-cholesterol values, by the precipitation procedure. Also, this negative association persisted when HDL_2-cholesterol was replaced by other HDL_2 components such as HDL_2-protein, HDL_2-phospholipid, or HDL_2-TG. HDL_2-TG, however, displayed a much weaker association than did the other HDL_2 components. No correlation existed between the magnitude of postprandial lipemia and the plasma levels of HDL_3 or any of its components.

Because we used zonal ultracentrifugation, in addition to the precipitation technique, we were able not only to quantitate HDL_2 and HDL_3, but also to characterize these HDL subfractions (Table III). With a range of 4–115 mg/dl, the interindividual variation in plasma levels of HDL_2 was much larger than that of HDL_3, 170–312 mg/dl. In HDL_2, the percentage contributions from protein, phospholipid, and unesterified cholesterol were fairly constant. A higher degree of variability was seen with cholesteryl ester content. By far the highest degree of compositional variation was with the content of TG in HDL_2, which varied as much as 7.8-fold among individuals. Quite similar to HDL_2, the TG content of HDL_3 varied also greatly among individuals. The TG content of HDL_2 and HDL_3 changed in a parallel fashion ($r = 0.81$, $P < 0.0001$).

Table II. Pearson Correlation Coefficients between the Magnitude of Postprandial Lipemia and HDL_2 Component Levels‡ in Postabsorptive Plasma*

	r	*P*
Total HDL_2	−0.65	0.0001
HDL_2-cholesterol§	−0.63	0.0001
HDL_2-cholesterol	−0.61	0.0001
HDL_2-phospholipid	−0.56	0.0008
HDL_2-protein	−0.53	0.0018
HDL_2-triglyceride	−0.39	0.0278

* Milligrams per deciliter times 8-h TG area (8).
‡ Milligrams per deciliter plasma.
§ Obtained by precipitation method (13). All others obtained by zonal ultracentrifugation (7).

The correlations of LPL activity with the magnitude of postprandial lipemia, TG content of HDL_2, plasma levels of HDL_2, and total HDL-cholesterol are illustrated in Fig. 2 *A*. LPL activity was inversely correlated with alimentary lipemia and TG content of HDL_2, but positively correlated with plasma concentrations of HDL_2 and with HDL-cholesterol. Fig. 2 *B* compares the activity of HL with the same parameters. Among these, the inverse correlation between HL and HDL_2 levels was strongest, followed by that of total HDL-cholesterol. Positive associations existed between HL activity and both, magnitude of postprandial lipemia and TG content of HDL_2. The latter, however, did not reach the level of statistical significance (Fig. 2 *B*).

Fig. 3 compares magnitude of postprandial lipemia with TG content of HDL_2, plasma levels of HDL_2, and HDL-cholesterol. Lipemia displayed a strong positive association with TG content of HDL_2, and an equally strong negative association with plasma levels of HDL_2 ($r = 0.69$ vs. $r = -0.65$). TG content of HDL_2, as the percent TG of HDL_2 mass, was inversely associated with HDL_2 levels in plasma ($r = -0.56$, $P < 0.0005$). In addition to these associations, postprandial lipemia displayed positive correlations with fasting levels of TG ($r = 0.59$, $P < 0.0001$) and apoB ($r = 0.50$, $P < 0.001$) as well as a negative correlation with plasma levels of apoA-I ($r = -0.34$, $P < 0.05$). Consistent with a previous report (8), these associations, however, were much weaker than that with HDL_2 levels. The activities of the two enzymes, LPL and HL, were inversely associated both in males ($r = -0.61$, $P < 0.0001$) and females ($r = -0.78$, $P < 0.0001$) as well as in the entire set of subjects ($r = -0.66$, $P < 0.0001$).

The abundance of apoA-I and apoA-II in HDL_2 and HDL_3 is summarized in Table IV. In HDL_2, apoA-I represented ~ 80% of the HDL_2 protein. ApoA-II averaged 12% of the protein, but varied widely, ranging from 4.5 to 29.5% of the HDL_2 protein. This resulted in an apoA-I/apoA-II molar ratio of 4.6±1.5, ranging from 1.4 to 7.3. In HDL_3, the amount of apoA-II was more than that in HDL_2 and exhibited a much lesser degree of variation, which resulted in an apoA-I/apoA-II molar ratio of 1.6±0.2, ranging from 1.2 to 2.1.

Table V compares abundance of apoA-I and apoA-II in HDL_2 with activity of LPL, magnitude of lipemia, TG content of HDL_2, HL activity, and plasma levels of HDL_2. Abundance of apoA-I did not correlate with any of the listed parameters. However, apoA-II in HDL_2 correlated positively with TG content of HDL_2 and with HL activity, and, negatively with HDL_2 levels in plasma. The apoA-I/apoA-II molar ratio in HDL_2 correlated

Table III. Plasma Levels and Weight-Percentage Composition of HDL_2 and HDL_3 from 38 Subjects

	Total lipoprotein	Protein	PL	UC	CE	TG
	mg/dl plasma	%*	%*	%*	%*	%*
HDL_2						
Mean	41	38.6	32.5	5.2	19.3	4.5
±SD	31	1.5	1.6	0.5	1.7	2.3
Range	4–115	36.1–41.3	30.1–34.6	4.2–6.4	11.8–22.1	1.8–14.0
HDL_3						
Mean	219	50.9	29.0	2.5	15.1	2.6
±SD	37	2.5	2.4	0.3	1.9	1.4
Range	170–312	46.6–56.4	23.5–35.6	1.8–2.9	8.6–20.6	1.3–9.1

PL, phospholipid; UC, unesterified cholesterol (387 mol wt); CE, cholesteryl esters (651 mol wt). * Weight-percentage contributions.

positively with LPL and HDL_2 levels, and, negatively with alimentary lipemia, TG content of HDL_2, and HL activity.

Discussion

The fasting plasma levels of HDL-cholesterol and HDL_2 are major discriminators for atherosclerosis. Although it is generally used for assessment of lipid risk factors, the fasting plasma represents an equilibrated state of the lipid transport system in the circulation. This system, however, has individually variable buffering capacity for maintaining homeostasis of lipid transport. When the plasma lipid transport system of normolipidemics is subjected to a physiological challenge such as the intake of a fatty meal, the magnitude of the ensuing postprandial lipemia varies among individuals (8). In fasting plasma, the HDL_2 class shows interindividual variations of the same magnitude ranging from 5 to 150 mg/dl plasma (8, 31). Among all parameters of lipid transport in fasting plasma, HDL_2 levels exhibit by far the strongest association with postprandial lipemia (8). This phenomenon prompted us to study why postprandial lipemia and HDL_2 levels differ so widely among normolipidemic individuals and why they are associated so closely.

In a recent study we observed that, 2–4 h after ingestion of a fatty meal, plasma TG levels peak and that HDL_2 become enriched with TG somewhat later, ~ 4–8 h after fat intake (31). The proportion of TG accumulating in the core of HDL_2 varies with the magnitude of preceding postprandial lipemia (31). The TG content of HDL_2 appears to be very important for the metabolic fate of HDL_2 because TG-enriched HDL_2 can be converted to HDL_3 by HL in vitro (31). The physiological relevance of this finding is supported by the following observation.

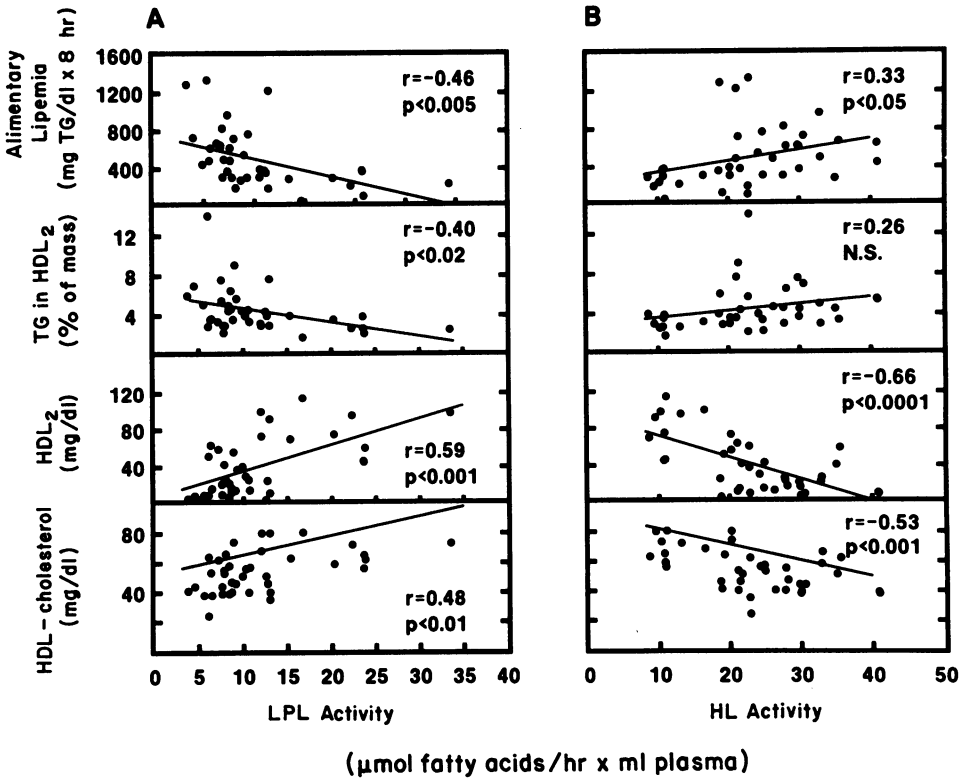

Figure 2. Linear regression analyses comparing the magnitude of alimentary lipemia, TG content of HDL_2, the plasma levels of HDL_2, and HDL-cholesterol (*ordinates*) with the activity of LPL (*A*) and HL (*B*) in postheparin plasma (*abscissae*).

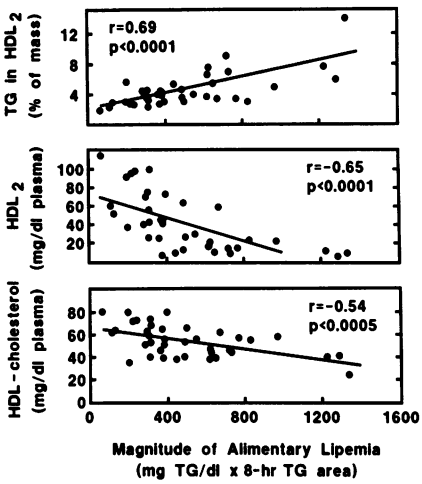

Figure 3. Linear regression analyses comparing TG content of HDL_2, HDL_2 levels, and HDL-cholesterol (*ordinates*) with the magnitude of alimentary lipemia. TG content of HDL_2 was inversely associated with HDL_2 levels ($r = -0.56$, $P < 0.005$). TG content of HDL_3, albeit weaker than that of HDL_2, showed also a positive association with the magnitude of lipemia ($r = 0.56$, $P < 0.0005$). No association existed, however, between lipemia and plasma levels of HDL_3 nor with TG content of HDL_3 and HDL_3 levels.

In individuals with pronounced postprandial lipemia, whose HDL_2 become TG-rich postprandially, fat intake causes, after 12–15 h, a drop in the levels of HDL_2 and a rise in those of HDL_3 (31). The data in the study presented here support this notion; the larger postprandial lipemia, the higher is the TG content of HDL_2 and the lower are HDL_2 levels in plasma (Fig. 3). Based on these findings we propose the relationship schematically illustrated in Fig. 4. Ingestion of fat is followed by the appearance of chylomicrons in plasma whose accumulation in plasma is limited by LPL. Accordingly, its activity is associated inversely with the magnitude of postprandial lipemia (Fig. 2 *A*). With low LPL activity, a pronounced lipemia provides TG for transfer from chylomicrons into HDL_2 through the action of lipid transfer proteins (32). By this mechanism, the activity of LPL is associated inversely with TG in HDL_2 (Fig. 2 *A*). TG-enriched HDL_2 are converted to HDL_3 by HL (31). The result is a low steady state level of HDL_2. In this metabolic scenario, LPL would be the origin of a chain of events whose interplay determines HDL_2 levels. This view is supported by an in vivo experiment in which LPL was selectively blocked. Specific blockage of LPL in the chicken causes massive accumulation of TG-rich lipoproteins, the enrichment of HDL with TG at the expense of cholesteryl esters, and, ultimately, the replacement of the larger HDL_2 by smaller, denser HDL_3 (33). These direct in vivo findings underscore the importance of LPL in determining the composition and plasma levels of HDL_2. Two important factors in this sequence of reactions appear to be lipid transfer protein(s) and HL.

In this study, we have not quantified the activities of lipid transfer protein in plasma. We hypothesize, however, that interindividual differences in lipid transfer activity are not critical for differences in TG content of HDL_2 for the following reasons. Postprandial enrichment of HDL_2 correlates with the magnitude of postprandial lipemia (31), and TG abundance in HDL_2 correlates directly with magnitude of lipemia (Fig. 3). The positive association between plasma TG levels and TG content of HDL in normolipidemic and hypertriglyceridemic individuals has been reported by others (34). Thus, enrichment of HDL_2 with TG in man appears to be governed primarily by the relative abundance of donor and acceptor lipoproteins (35).

TG content of HDL_3 showed also a positive association with lipemia and paralleled that in HDL_2 ($r = 0.81$). TG-enriched HDL_3 serve also as substrate for HL and are converted to particularly small, dense HDL_3 (Patsch, J. R., and G. Bengtsson-Olivecrona, unpublished observations). In some of our study subjects with extremely low HDL_2 levels, below 10 mg/dl, HDL_3 levels were also low, and the HDL_3 showed higher densities typical for HDL_{3D} (36). This observation is consistent with the view that in individuals with very pronounced lipemia, little HDL_3 is formed because of low HDL_2 levels. The TG-enriched HDL_3 are converted to smaller, denser HDL_3, which leads to a predominance of this HDL subspecies as has been observed in hypertriglyceridemic subjects (36, 37). Because of this heterogeneity of HDL_3, the postulated precursor-product relationship between HDL_2 and HDL_3 (27, 31) does not always have to be reflected in a simple reciprocal association of high HDL_2 levels with low HDL_3 levels or vice versa.

The second major factor to be considered in this metabolic scenario is HL. According to the scheme outlined in Fig. 4, the TG content of HDL_2 rather than the activity of the enzyme would determine whether or not HDL_2 is converted to HDL_3

Table IV. Proportion of ApoA-I and ApoA-II in HDL_2 and HDL_3

	Percent of protein		Molar ratio
	ApoA-I	ApoA-II	ApoA-I/ApoA-II
HDL_2			
Mean	80.1	12.0	4.6
±SD	6.4	5.0	1.5
Range	68.2–97.8	4.5–29.5	1.4–7.3
HDL_3			
Mean	65.7	25.7	1.6
±SD	4.3	2.6	0.2
Range	57.0–73.7	21.0–31.8	1.2–2.1

Table V. Pearson Correlation Coefficients between the Relative Proportion of ApoA-I and ApoA-II in HDL_2 and Other Parameters of Lipid Transport*

	Percentage of the two major apoproteins in HDL_2		Molar ratio in HDL_2
	ApoA-I	ApoA-II	ApoA-I/ApoA-II
LPL activity	−0.26 (NS)	−0.21 (NS)	0.36 ($P < 0.05$)
Alimentary lipemia	−0.20 (NS)	0.22 (NS)	−0.36 ($P < 0.05$)
Percent TG in HDL_2	0.06 (NS)	0.38 ($P < 0.04$)	−0.50 ($P < 0.002$)
HL activity	−0.36 (NS)	0.41 ($P < 0.02$)	−0.54 ($P < 0.002$)
HDL_2 levels	0.35 (NS)	−0.47 ($P < 0.007$)	0.61 ($P < 0.0001$)

* No correlations existed between apoA-I and apoA-II associated with HDL_3 and any of the parameters listed.

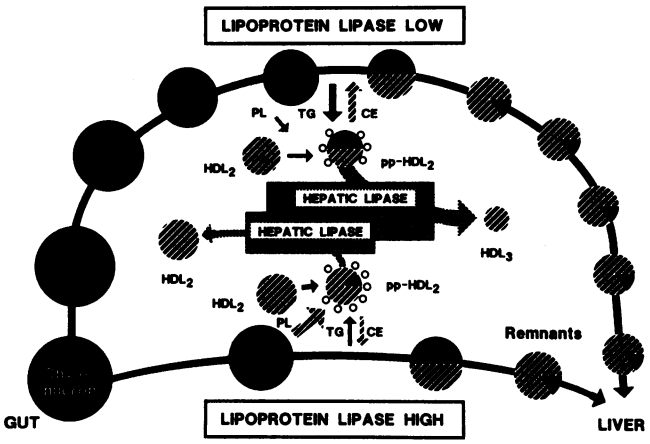

Figure 4. Model of interrelationship of LPL, postprandial lipemia, TG content of HDL_2, and HL on HDL_2 levels. In individuals with high LPL activity, there is little, if any, accumulation of chylomicrons. Chylomicron-derived phospholipids (*open circles*) are transferred to HDL_2. HL now removes the excess phospholipid from these postprandial HDL_2 particles ($pp\text{-}HDL_2$) so that the phospholipid-depleted HDL_2 can continue to serve as phospholipid acceptors during renewed chylomicron catabolism. Under these conditions HDL_2 steady state levels are not reduced by fat intake. By contrast, when LPL activity is low, chylomicrons accumulate in the blood. Chylomicron TG (*black areas*), through the action of lipid transfer proteins, are transferred to HDL_2 in exchange for cholesteryl esters (*shaded*). HL whose activity is elevated now removes not only the phospholipids from the surface, but also the TG from the core of HDL_2 so that smaller HDL_3 particles are formed. In this situation, HL reduces the steady state levels of HDL_2 by converting some of them to HDL_3.

by HL. Indeed, the TG content of HDL_2 varied inversely with HDL_2 levels ($r = -0.56$, $P < 0.0005$). However, HL activity differed also from subject to subject and correlated inversely with HDL_2 levels (Fig. 2 *B*). At this point we have no explanation why the activity of HL is increased in individuals with low LPL activities and with low HDL_2 levels.

Regarding the composition of HDL_2, not only the TG content, but also the apoA-I/apoA-II molar ratio was highly variable among individuals because of differing amounts of apoA-II in HDL_2 (Table IV). The higher the apoA-II content in HDL_2, the higher are the proportion of TG in HDL_2 and HL activity, and the lower are HDL_2 levels in plasma (Table V). In a previous study, we have observed that only those HDL_2 particles that contain apoA-II are converted to HDL_3 by HL in vitro (31). Taken together, the in vitro data (31) and the data from this study suggest a possible function of apoA-II in this process: apoA-II could be important for TG content of HDL_2 by being involved in neutral lipid exchange, or the apoprotein could be an effector for HL. HDL containing apoA-II (in addition to apoA-I) are present both in HDL_2 and HDL_3 (38, 39) and could represent the postulated shuttle vector in going from HDL_2 to HDL_3 (31) via lipid exchange and HL (Fig. 4) and, from HDL_3 to HDL_2 through assimilation by HDL_3 of surface components of lipolyzed TG-rich lipoproteins (27). The study presented here, further strengthens the view that HDL_2 and, thus, HDL-cholesterol should not be considered, neither biochemically nor epidemiologically, as an independent lipid transport mechanism nor as an independent risk factor for atherosclerosis, but rather as being a dependent variable to TG transport as exemplified in the postprandial phase.

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