

## High Density Lipoprotein<sub>2</sub>

### 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<sub>2</sub> (HDL<sub>2</sub>), to hepatic lipase (HL) activity, and to HDL<sub>2</sub> levels. LPL activity correlated indirectly with lipemia, triglyceride content of HDL<sub>2</sub>, HL activity, and levels of HDL<sub>2</sub> but not of HDL<sub>3</sub>. HL activity correlated directly with lipemia and indirectly with HDL<sub>2</sub> levels.

Triglyceride content of HDL<sub>2</sub> correlated directly with lipemia and indirectly with HDL<sub>2</sub> levels. In HDL<sub>2</sub>, abundance of apolipoprotein (apo) A-II and the apoA-I/apoA-II ratio varied widely. The latter correlated positively with LPL activity and HDL<sub>2</sub> levels, and, inversely, with HL activity, lipemia, and triglyceride content of HDL<sub>2</sub>. 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<sub>2</sub>.

#### Introduction

The negative association between coronary heart disease (CHD)<sup>1</sup> 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<sub>2</sub>, but not of HDL<sub>3</sub>, 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<sub>2</sub> fraction.

The mechanism(s) underlying the negative association between CHD and HDL<sub>2</sub> is unknown. HDL<sub>2</sub> could directly interfere with the atherosclerotic process by playing a major role in the "reverse cholesterol transport" (10). Alternatively, HDL<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> that, in turn, affects the levels of HDL<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub>, and consequently the lower the plasma concentration of HDL<sub>2</sub>. 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<sup>2</sup> 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<sub>2</sub>.** Concentrations of HDL<sub>2</sub> and HDL<sub>3</sub> 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<sub>2</sub> (13). Cholesterol was quantified in the respective supernatants. In this way, HDL-cholesterol and HDL<sub>2</sub>-cholesterol were measured, and HDL<sub>2</sub>-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<sub>2</sub> and HDL<sub>3</sub> 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<sub>2</sub> and HDL<sub>3</sub> protein could be determined, from which the concentrations of the entire HDL<sub>2</sub> and HDL<sub>3</sub> particle mass were calculated (7). Cholesterol recovered from the two zonally isolated HDL fractions averaged 93.8% of HDL-cholesterol.

For determination of HDL<sub>2</sub>-cholesterol, zonal ultracentrifugation (7) and sequential precipitation (13) gave satisfactory agreement. By using the former method we obtained mean levels for HDL<sub>2</sub>-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<sub>2</sub> levels with other parameters of lipid transport were very similar for both methods of HDL<sub>2</sub> quantification. Total HDL-cholesterol, for example, correlated with HDL<sub>2</sub> 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.

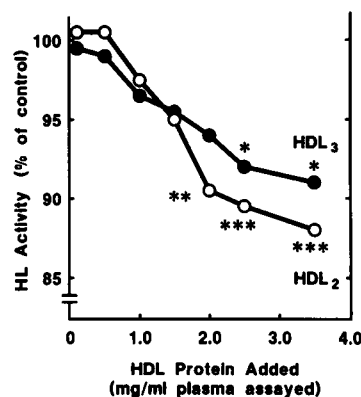
To release LPL and HL into the circulation, heparin sodium, U. S. Pharmacopeia from beef lung, was used (Upjohn Co., Kalamazoo, MI). A blood sample was drawn in the postabsorptive state (preheparin plasma), then 2,280 U/m<sup>2</sup> heparin was administered intravenously. A second blood sample (postheparin 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 [<sup>14</sup>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<sub>2</sub> and HDL<sub>3</sub> in postheparin plasma. HL is a single enzyme molecule displaying TG lipase, monoglyceride lipase, and phospholipase activities (24). TG and phospholipids of HDL<sub>2</sub> serve as substrates for HL (25), and HDL<sub>2</sub> compete with native TG-rich lipoproteins and artificial TG-rich emulsions as substrate for the enzyme (26). Endogenous HDL<sub>2</sub>, 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<sub>2</sub> and HDL<sub>3</sub> from the pre- and postheparin plasma of two individuals (7), one male and one female. Comparison of preheparin to postheparin plasma showed that HDL<sub>2</sub> levels rose by 2 and 6%, and HDL<sub>3</sub> 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 [<sup>14</sup>C]triolein substrate. As summarized in Fig. 1, addition of HDL<sub>2</sub> and HDL<sub>3</sub> indeed reduced the observed activity of HL. However, a 5% reduction of HL activity required the addition of HDL<sub>2</sub> in concentrations equivalent to ~ 150 mg HDL<sub>2</sub> protein/dl plasma. The levels of HDL<sub>2</sub> 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<sub>2</sub> and HDL<sub>3</sub> by < 10%. Also, HDL<sub>2</sub> or HDL<sub>3</sub> 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<sub>2</sub> 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<sub>2</sub>-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<sub>2</sub> concentrations in postabsorptive plasma and in the TG levels after ingestion of the fatty test meal.



**Figure 1.** Effect of HDL<sub>2</sub> and HDL<sub>3</sub> on observed activity of HL. HDL<sub>2</sub> and HDL<sub>3</sub> were isolated from one female (31 mg/dl HDL<sub>2</sub>-cholesterol, 32 mg/dl HDL<sub>3</sub>-cholesterol) and one male (9 mg/dl HDL<sub>2</sub>-cholesterol, 35 mg/dl HDL<sub>3</sub>-cholesterol) before and 15 min after injection of heparin. Increasing amounts of each preparation (two

preheparin and two postheparin) of HDL<sub>2</sub> ( $n = 4$ ) and HDL<sub>3</sub> ( $n = 4$ ) were added to assay mixtures for HL activity. Each mixture contained the same postheparin plasma from a male individual with low HDL<sub>2</sub> plasma levels (HDL<sub>2</sub> protein was 0.15 mg/ml; HDL<sub>3</sub> protein, 1.0 mg/ml plasma; and HDL-cholesterol, 51 mg/dl). As can be seen, ~ 1.5 mg HDL<sub>2</sub> protein/ml plasma (equivalent to 150 mg HDL<sub>2</sub> protein/dl) was required to reduce apparent activity of HL by 5%. HDL<sub>2</sub> 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<sub>2</sub> and HDL<sub>3</sub> 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 <sub>2</sub> -Ch	HDL <sub>3</sub> -Ch	ApoA-I	ApoA-II	ApoB	Triglyceridemic response*	Activity in postheparin plasma <sup>‡</sup>	
	mg/dl <sup>§</sup>	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl	mg/dl		LPL	HL
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<sub>2</sub>-Ch, or HDL<sub>3</sub>-Ch, cholesterol associated with total HDL, HDL<sub>2</sub>, or HDL<sub>3</sub>, respectively. \* Triglyceridemic response expressed as milligrams per deciliter times 8-h TG area (8). <sup>‡</sup> Activity expressed as micromoles of free fatty acids released per milliliter plasma per hour at 28°C. <sup>§</sup> Milligrams per deciliter of postabsorptive plasma.

The magnitude of postprandial lipemia and the levels of HDL<sub>2</sub> in postabsorptive plasma showed a significant negative association (Table II). The association was virtually identical using either the entire HDL<sub>2</sub> lipoprotein values obtained by zonal ultracentrifugation or the HDL<sub>2</sub>-cholesterol values, by the precipitation procedure. Also, this negative association persisted when HDL<sub>2</sub>-cholesterol was replaced by other HDL<sub>2</sub> components such as HDL<sub>2</sub>-protein, HDL<sub>2</sub>-phospholipid, or HDL<sub>2</sub>-TG. HDL<sub>2</sub>-TG, however, displayed a much weaker association than did the other HDL<sub>2</sub> components. No correlation existed between the magnitude of postprandial lipemia and the plasma levels of HDL<sub>3</sub> or any of its components.

Because we used zonal ultracentrifugation, in addition to the precipitation technique, we were able not only to quantitate HDL<sub>2</sub> and HDL<sub>3</sub>, 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<sub>2</sub> was much larger than that of HDL<sub>3</sub>, 170–312 mg/dl. In HDL<sub>2</sub>, 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<sub>2</sub>, which varied as much as 7.8-fold among individuals. Quite similar to HDL<sub>2</sub>, the TG content of HDL<sub>3</sub> varied also greatly among individuals. The TG content of HDL<sub>2</sub> and HDL<sub>3</sub> changed in a parallel fashion ( $r = 0.81$ ,  $P < 0.0001$ ).

Table II. Pearson Correlation Coefficients between the Magnitude of Postprandial Lipemia\* and HDL<sub>2</sub> Component Levels<sup>‡</sup> in Postabsorptive Plasma

	<i>r</i>	<i>P</i>
Total HDL <sub>2</sub>	-0.65	0.0001
HDL <sub>2</sub> -cholesterol <sup>§</sup>	-0.63	0.0001
HDL <sub>2</sub> -cholesterol	-0.61	0.0001
HDL <sub>2</sub> -phospholipid	-0.56	0.0008
HDL <sub>2</sub> -protein	-0.53	0.0018
HDL <sub>2</sub> -triglyceride	-0.39	0.0278

\* Milligrams per deciliter times 8-h TG area (8).

<sup>‡</sup> Milligrams per deciliter plasma.

<sup>§</sup> 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<sub>2</sub>, plasma levels of HDL<sub>2</sub>, and total HDL-cholesterol are illustrated in Fig. 2 A. LPL activity was inversely correlated with alimentary lipemia and TG content of HDL<sub>2</sub>, but positively correlated with plasma concentrations of HDL<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>. 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<sub>2</sub>, plasma levels of HDL<sub>2</sub>, and HDL-cholesterol. Lipemia displayed a strong positive association with TG content of HDL<sub>2</sub>, and an equally strong negative association with plasma levels of HDL<sub>2</sub> ( $r = 0.69$  vs.  $r = -0.65$ ). TG content of HDL<sub>2</sub>, as the percent TG of HDL<sub>2</sub> mass, was inversely associated with HDL<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> and HDL<sub>3</sub> is summarized in Table IV. In HDL<sub>2</sub>, apoA-I represented ~ 80% of the HDL<sub>2</sub> protein. ApoA-II averaged 12% of the protein, but varied widely, ranging from 4.5 to 29.5% of the HDL<sub>2</sub> protein. This resulted in an apoA-I/apoA-II molar ratio of  $4.6 \pm 1.5$ , ranging from 1.4 to 7.3. In HDL<sub>3</sub>, the amount of apoA-II was more than that in HDL<sub>2</sub> and exhibited a much lesser degree of variation, which resulted in an apoA-I/apoA-II molar ratio of  $1.6 \pm 0.2$ , ranging from 1.2 to 2.1.

Table V compares abundance of apoA-I and apoA-II in HDL<sub>2</sub> with activity of LPL, magnitude of lipemia, TG content of HDL<sub>2</sub>, HL activity, and plasma levels of HDL<sub>2</sub>. Abundance of apoA-I did not correlate with any of the listed parameters. However, apoA-II in HDL<sub>2</sub> correlated positively with TG content of HDL<sub>2</sub> and with HL activity, and, negatively with HDL<sub>2</sub> levels in plasma. The apoA-I/apoA-II molar ratio in HDL<sub>2</sub> correlated

Table III. Plasma Levels and Weight-Percentage Composition of HDL<sub>2</sub> and HDL<sub>3</sub> from 38 Subjects

	Total lipoprotein	Protein	PL	UC	CE	TG
	mg/dl plasma	%*	%*	%*	%*	%*
<b>HDL<sub>2</sub></b>						
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
<b>HDL<sub>3</sub></b>						
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<sub>2</sub> levels, and, negatively with alimentary lipemia, TG content of HDL<sub>2</sub>, and HL activity.

### Discussion

The fasting plasma levels of HDL-cholesterol and HDL<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> levels exhibit by far the strongest association with postprandial lipemia (8). This phenomenon prompted us to study why postprandial lipemia and HDL<sub>2</sub> 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<sub>2</sub> become enriched with TG somewhat later, ~ 4–8 h after fat intake (31). The proportion of TG accumulating in the core of HDL<sub>2</sub> varies with the magnitude of preceding postprandial lipemia (31). The TG content of HDL<sub>2</sub> appears to be very important for the metabolic fate of HDL<sub>2</sub> because TG-enriched HDL<sub>2</sub> can be converted to HDL<sub>3</sub> by HL in vitro (31). The physiological relevance of this finding is supported by the following observation.

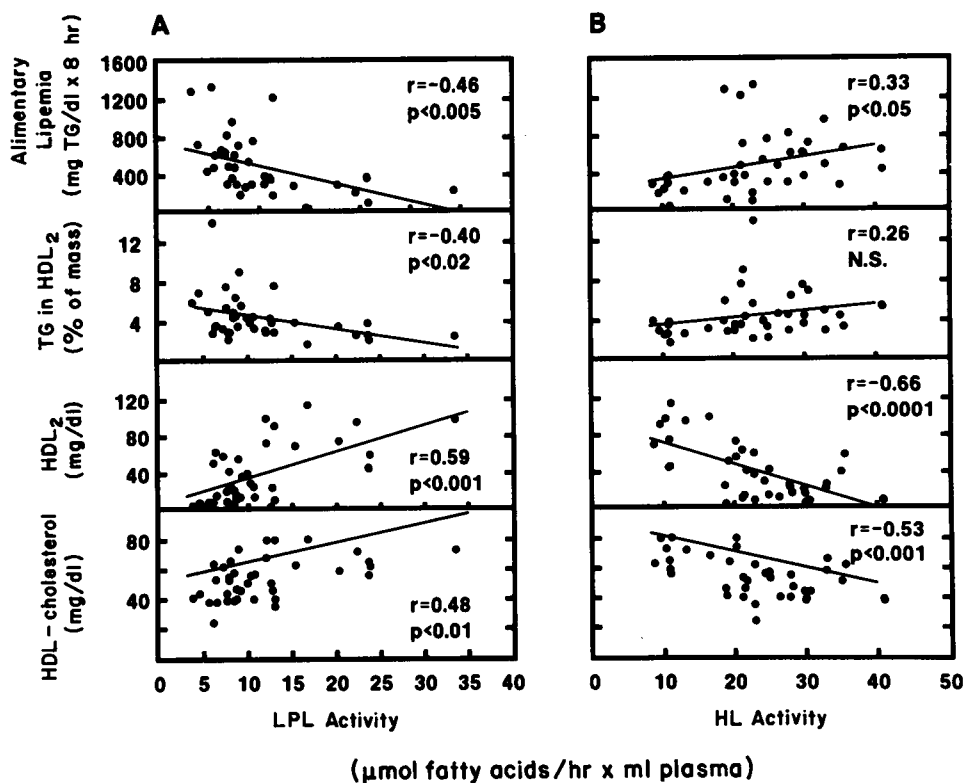
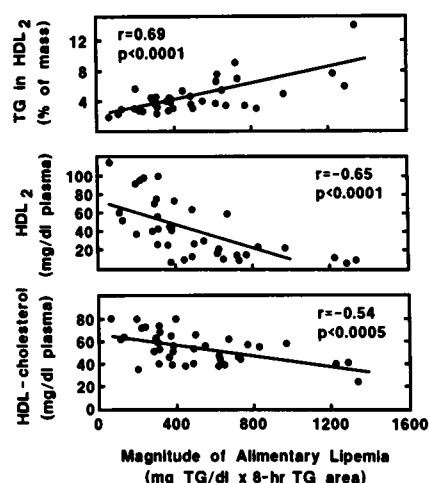


Figure 2. Linear regression analyses comparing the magnitude of alimentary lipemia, TG content of HDL<sub>2</sub>, the plasma levels of HDL<sub>2</sub>, and HDL-cholesterol (ordinates) with the activity of LPL (A) and HL (B) in postheparin plasma (abscissae).



**Figure 3.** Linear regression analyses comparing TG content of HDL<sub>2</sub>, HDL<sub>2</sub> levels, and HDL-cholesterol (*ordinates*) with the magnitude of alimentary lipemia. TG content of HDL<sub>2</sub> was inversely associated with HDL<sub>2</sub> levels ( $r = -0.56$ ,  $P < 0.005$ ). TG content of HDL<sub>3</sub>, albeit weaker than that of HDL<sub>2</sub>, 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<sub>3</sub> nor with TG content of HDL<sub>3</sub> and HDL<sub>3</sub> levels.

In individuals with pronounced postprandial lipemia, whose HDL<sub>2</sub> become TG-rich postprandially, fat intake causes, after 12–15 h, a drop in the levels of HDL<sub>2</sub> and a rise in those of HDL<sub>3</sub> (31). The data in the study presented here support this notion; the larger postprandial lipemia, the higher is the TG content of HDL<sub>2</sub> and the lower are HDL<sub>2</sub> 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<sub>2</sub> through the action of lipid transfer proteins (32). By this mechanism, the activity of LPL is associated inversely with TG in HDL<sub>2</sub> (Fig. 2 A). TG-enriched HDL<sub>2</sub> are converted to HDL<sub>3</sub> by HL (31). The result is a low steady state level of HDL<sub>2</sub>. In this metabolic scenario, LPL would be the origin of a chain of events whose interplay determines HDL<sub>2</sub> levels. This view is supported

**Table IV.** Proportion of ApoA-I and ApoA-II in HDL<sub>2</sub> and HDL<sub>3</sub>

	Percent of protein		Molar ratio
	ApoA-I	ApoA-II	ApoA-I/ApoA-II
<b>HDL<sub>2</sub></b>			
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
<b>HDL<sub>3</sub></b>			
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

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<sub>2</sub> by smaller, denser HDL<sub>3</sub> (33). These direct *in vivo* findings underscore the importance of LPL in determining the composition and plasma levels of HDL<sub>2</sub>. 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<sub>2</sub> for the following reasons. Postprandial enrichment of HDL<sub>2</sub> correlates with the magnitude of postprandial lipemia (31), and TG abundance in HDL<sub>2</sub> 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<sub>2</sub> with TG in man appears to be governed primarily by the relative abundance of donor and acceptor lipoproteins (35).

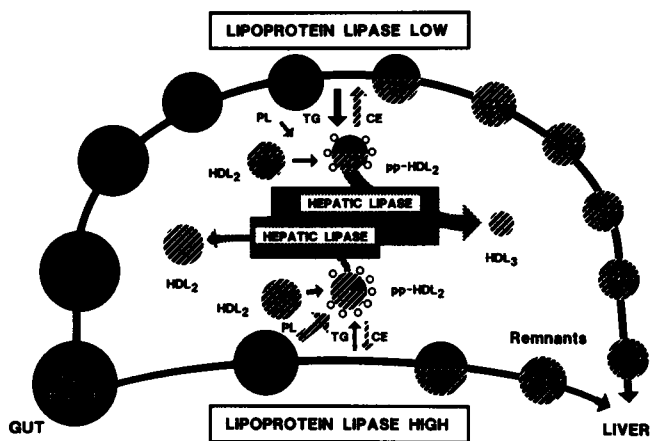
TG content of HDL<sub>3</sub> showed also a positive association with lipemia and paralleled that in HDL<sub>2</sub> ( $r = 0.81$ ). TG-enriched HDL<sub>3</sub> serve also as substrate for HL and are converted to particularly small, dense HDL<sub>3</sub> (Patsch, J. R., and G. Bengtsson-Olivecrona, unpublished observations). In some of our study subjects with extremely low HDL<sub>2</sub> levels, below 10 mg/dl, HDL<sub>3</sub> levels were also low, and the HDL<sub>3</sub> showed higher densities typical for HDL<sub>3D</sub> (36). This observation is consistent with the view that in individuals with very pronounced lipemia, little HDL<sub>3</sub> is formed because of low HDL<sub>2</sub> levels. The TG-enriched HDL<sub>3</sub> are converted to smaller, denser HDL<sub>3</sub>, which leads to a predominance of this HDL subspecies as has been observed in hypertriglyceridemic subjects (36, 37). Because of this heterogeneity of HDL<sub>3</sub>, the postulated precursor-product relationship between HDL<sub>2</sub> and HDL<sub>3</sub> (27, 31) does not always have to be reflected in a simple reciprocal association of high HDL<sub>2</sub> levels with low HDL<sub>3</sub> 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<sub>2</sub> rather than the activity of the enzyme would determine whether or not HDL<sub>2</sub> is converted to HDL<sub>3</sub>

**Table V.** Pearson Correlation Coefficients between the Relative Proportion of ApoA-I and ApoA-II in HDL<sub>2</sub>\* and Other Parameters of Lipid Transport

	Percentage of the two major apoproteins in HDL <sub>2</sub>		Molar ratio in HDL <sub>2</sub>
	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 <sub>2</sub>	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 <sub>2</sub> 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<sub>3</sub> and any of the parameters listed.



**Figure 4.** Model of interrelationship of LPL, postprandial lipemia, TG content of HDL<sub>2</sub>, and HL on HDL<sub>2</sub> levels. In individuals with high LPL activity, there is little, if any, accumulation of chylomicrons. Chylomicron-derived phospholipids (open circles) are transferred to HDL<sub>2</sub>. HL now removes the excess phospholipid from these postprandial HDL<sub>2</sub> particles (pp-HDL<sub>2</sub>) so that the phospholipid-depleted HDL<sub>2</sub> can continue to serve as phospholipid acceptors during renewed chylomicron catabolism. Under these conditions HDL<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> so that smaller HDL<sub>3</sub> particles are formed. In this situation, HL reduces the steady state levels of HDL<sub>2</sub> by converting some of them to HDL<sub>3</sub>.

by HL. Indeed, the TG content of HDL<sub>2</sub> varied inversely with HDL<sub>2</sub> levels ( $r = -0.56$ ,  $P < 0.0005$ ). However, HL activity differed also from subject to subject and correlated inversely with HDL<sub>2</sub> 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<sub>2</sub> levels.

Regarding the composition of HDL<sub>2</sub>, 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<sub>2</sub> (Table IV). The higher the apoA-II content in HDL<sub>2</sub>, the higher are the proportion of TG in HDL<sub>2</sub> and HL activity, and the lower are HDL<sub>2</sub> levels in plasma (Table V). In a previous study, we have observed that only those HDL<sub>2</sub> particles that contain apoA-II are converted to HDL<sub>3</sub> 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<sub>2</sub> 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<sub>2</sub> and HDL<sub>3</sub> (38, 39) and could represent the postulated shuttle vector in going from HDL<sub>2</sub> to HDL<sub>3</sub> (31) via lipid exchange and HL (Fig. 4) and, from HDL<sub>3</sub> to HDL<sub>2</sub> through assimilation by HDL<sub>3</sub> of surface components of lipolyzed TG-rich lipoproteins (27). The study presented here, further strengthens the view that HDL<sub>2</sub> 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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