Postprandial Lipemia

A Key for the Conversion of High Density Lipoprotein2 into High Density Lipoprotein3 by Hepatic Lipase

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Abstract. In this study, we have investigated the effects of alimentary lipemia in 15 normotriglyceridemic individuals on high density lipoproteins2 (HDL2) with respect to structure, composition, and substrate efficacy for hepatic lipase in vitro. In the study subjects, HDL2 levels ranged widely from 4.7 to 151.7 mg/dl plasma. HDL2 were isolated in the postabsorptive (pa) state and in the postprandial (pp) state, i.e., 7 h after ingestion of a standard fatty meal. In going from the pa state to the pp state, HDL2 exhibited higher flotation rates and lower densities due to a decreased proportion of protein (38.7 → 36.2%) and a higher abundance in phospholipid (32.5 → 34.9%). There was a variable increase in triglyceride at the expense of cholesteryl esters; this increase was correlated positively with the magnitude of pp lipemia ($r = 0.69$, $P < 0.01$) and inversely with HDL2 levels ($r = -0.72$, $P < 0.01$). HDL2 fractions were incubated with human hepatic lipase in vitro. Product lipoproteins formed from lipolysis of pa-HDL2 and triglyceride-poorer pp-HDL2 were reduced in phospholipid content (by 25 and 50%, respectively) but remained in the size and density range of native HDL2. By contrast, a major fraction of triglyceride-richer pp-HDL2 was converted to particles with density, size, and apoprotein composition of native HDL3. Changes consistent with these findings in vitro were observed in vivo also, where 15 h postprandially, individuals with high-level lipemia showed a decrease in HDL2 and rise in HDL3, while those with lower-level lipemia did not. This study indicates that the magnitude of postprandial lipemia determines the proportion of triglyceride in pp-HDL2, which in turn determines whether or not HDL2 are converted to HDL3 by hepatic lipase action.

Introduction

High density lipoproteins2 (HDL2) are one of the two major HDL subclasses (1). In normotriglyceridemic subjects, HDL2 levels correlate with HDL cholesterol (2) which has a powerful inverse correlation with the incidence of coronary heart disease (3, 4). Therefore, it appears very important to identify the mechanism(s) that control HDL2 levels in the plasma. We provide evidence that ingestion of a fatty meal can raise the triglyceride content of HDL2, which varies directly with the magnitude of postprandial (pp)[1] lipemia and indirectly with HDL2 levels. We also show that the extent of pp enrichment of HDL2 with triglycerides determines whether or not HDL2 can be converted into HDL3 by hepatic lipase in vitro.

Methods

Study subjects were healthy males and females ranging in age from 29 to 37 yr. No participant took medication. Fasting plasma triglyceride levels were <160 mg/dl and HDL-cholesterol ranged from 27 to 87 mg/dl. Concentrations of HDL2 particles in fasting plasma of the 15 volunteers ranged from 4.7 to 151.7 mg/dl. Postabsorptive (pa)-HDL2

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1. *Abbreviations used in this paper:* apo, apolipoprotein; $F^\circ_{1.21}$, flotation rate in aqueous NaBr solution of density 1.21 g/ml at 20°C expressed in Svedbergs (10^{-13} s); pa-HDL2 and pp-HDL2, HDL2 isolated from plasma in the pa state and the pp state, respectively; pa, postabsorptive, after a 12–14-h overnight fast; pp, postprandial, 7 h after oral ingestion of a fatty meal.

were isolated from plasma after a 14-h overnight fast. Thereafter, a standard liquid fatty meal, whose composition has been described in detail previously (2), was administered orally. Each subject ingested 65 g of fat/m² of body surface. 7 h after ingestion of the meal, a second blood sample was obtained for isolation of pp-HDL₂. Additional minor blood sampling was performed at 2, 4, 6, and 8 h to determine pp triglyceride levels. The magnitude of pp lipemia was quantified as reported previously (2) as the area under the time dependent pp triglyceride level curve, and is expressed as milligrams per deciliter times 8 h triglyceride area. In some subjects, blood samples were obtained also at 15 h to analyze plasma for changes in HDL₂ and HDL₃ levels at this time. Fresh plasma was subjected to zonal ultracentrifugation (5) and the volume fraction containing HDL₂ was subjected to gel filtration chromatography using Bio-gel A-5M in 2.5 cm × 95-cm columns (Bio-Rad Laboratories, Richmond, CA). This isolation strategy gave highly purified pa- and pp-HDL₂, even from subjects with extremely low levels of HDL₂ in the presence of vast amounts of triglyceride-rich lipoproteins.

All incubations were performed at 25°C in a final volume of 15 ml. Incubation mixtures contained HDL₂ (between 5.0 and 5.5 mg phospholipid) in 20 mM Tris-HCl buffer, pH 8.2 and fatty acid-free bovine serum albumin (Sigma Chemical Co., St. Louis, MO) at a concentration of 4 g/dl. To prepare hepatic lipase, human postheparin plasma was chromatographed on heparin-Sepharose (6). Fractions containing the peak of hepatic lipase activity and separated from lipoprotein lipase were pooled. This pool was incubated with antilipoprotein lipase immunoglobulins to remove any traces of lipoprotein lipase (7) and was rechromatographed on N-desulfated, acetylated heparin-Sepharose (6). A typical hepatic lipase preparation used contained 0.05 mg protein/ml of 0.9 M NaCl, 10 mM Tris, pH 7.4, 20% glycerol. At completion of preparation, the specific activity with trioleylglycerol as substrate (6) at pH 8.5, 25°C was ~200 μmol fatty acid released per minute per mg protein. For every comparative experiment such as those shown in Fig. 1 and Fig. 4, aliquots of one enzyme pool were used. In control experiments, 0.15 M NaCl was substituted for hepatic lipase. Incubation time was typically 15 h and ranged from 3 to 30 h. After incubation, the reaction was stopped by chilling the mixture to 4°C and adjusting the density with NaBr to 1.4 g/ml for zonal ultracentrifugation to analyze and isolate HDL (5). Recoveries of HDL-protein and HDL-total cholesterol averaged 89.3 and 91.8%, respectively. Phospholipid, triglyceride, cholesterol, cholesteryl esters, protein, and phospholipid classes were quantified by standard procedures used and described (1, 2, 5). For analysis of particle size, HDL fractions were subjected to polyacrylamide gradient gel electrophoresis (8). Apolipoproteins were separated by polyacrylamide gel electrophoresis in 0.1% SDS (1) and apolipoprotein (apo) A-I and A-II were quantified by respective double antibody radioimmunoassay procedures used previously (2). Analytical ultracentrifugation was performed at 52,000 rpm, 20°C, and at d = 1.21 g NaBr/ml. Flotation rates are expressed as Svedbergs (10^{-13} s) and are designated $F^{\circ}_{1.21}$ (1).

Results

The compositions of pa-HDL₂ and pp-HDL₂ from 15 subjects are contrasted in Table I. Pp-HDL₂ had a lower proportion of protein and were enriched in phospholipids. Thus, they were less dense and had higher $F^{\circ}_{1.21}$ values in agreement with observations reported by Tall et al. (9). They also contained a lower proportion of cholesteryl esters and a higher one of triglycerides. The percentage of core components, as computed from the sum of triglycerides and cholesteryl esters, was not significantly different. As can be seen from the last column of Table I, triglyceride contributed an average of 18% to the mass of the pa-HDL₂ core; this contribution increased to an average of 30% in pp-HDL₂ at the expense of cholesteryl esters. However, there were great individual differences in the postprandial triglyceride increase in HDL₂ as can be appreciated from the large standard deviations (13.25%) and the wide range (8.6–52.7%). This increase correlated directly with the magnitude of postprandial lipemia (r = 0.69, P < 0.01), and inversely with the HDL₂ levels in plasma (r = −0.72, P < 0.01).

Table I. Weight Percentage Chemical Composition of pa- and pp-HDL₂ from 15 Subjects*‡

		$F^{\circ}_{1.21}$	Protein	PL	UC	CE	TG	TG + CE	$\frac{TG}{TG + CE} \times 100$
pa-HDL₂	Mean	5.3	38.7	32.5	5.0	19.5	4.3	23.8	18.0
	±SD	0.29	2.36	1.16	0.96	2.61	1.47	2.21	6.53
	Range	4.7–5.5	36.1–43.6	30.2–34.3	2.7–6.5	13.6–22.6	2.1–6.7	18.6–26.2	8.8–26.9
pp-HDL₂	Mean	6.2	36.2	34.9	5.0	16.8	7.1	24.0	30.1
	±SD	0.18	1.59	1.79	0.97	3.71	2.96	2.07	13.25
	Range	5.7–6.3	33.7–39.0	32.2–38.9	3.2–6.7	9.7–21.5	2.0–11.8	19.7–26.7	8.6–52.7
	P§	<0.001	<0.001	<0.001	NS	<0.001	<0.001	NS	<0.001

* Abbreviations used are: PL, phospholipid; UC, unesterified cholesterol (387 mol wt); CE, cholesteryl esters (651 mol wt); TG, triglyceride. ‡ The HDL₂ levels in the 15 subjects averaged 48 mg/dl (mean)±43 (SD) and ranged from 5 to 152 mg/dl fasting plasma. The magnitude of postprandial lipemia as quantified by the trapezoidal rule (2) averaged 665.3±420.8 ranging from 101 to 1,344 mg/dl·8 h TG area. The difference of percentage TG of core (Δ (TG)/(TG + CE) × 100) in going from pa-HDL₂ to pp-HDL₂ averaged 12.1±10.70 ranging from −1.5 to 27.9 and correlated positively with magnitude of lipemia (r = 0.687, P < 0.01) and inversely with HDL₂ levels (r = −0.724, P < 0.01). § Statistics obtained by paired t test.

For in vitro incubation studies with hepatic lipase, we first performed a series of pilot experiments to establish the optimal conditions for comparing various HDL_2 preparations and their respective product lipoproteins. Two pools of pp-HDL_2, each obtained from two individuals, and their respective pa-HDL_2 were incubated for increasing lengths of time. With three additions of enzyme aliquots of 200–300 μl each at 0, 3, and 12 h, phospholipid hydrolysis in both pa-HDL_2 and pp-HDL_2 had approached a plateau at 15 h. At this time, ~25% of phospholipid (40% of phosphatidylcholine) was hydrolyzed from pa-HDL_2, and 49–55% (or 60–70% of phosphatidylcholine) from pp-HDL_2. In both types of HDL_2, triglyceride hydrolysis averaged 85% at 12 h and 93% at 15 h. Because we aimed at comparing product lipoproteins formed from HDL_2 at end-point conditions, the selected incubation conditions unless stated otherwise, were: 25°C for 15 h and additions of enzyme aliquots at 0, 3, and 12 h.

For comparative studies with hepatic lipase in vitro, we selected from 8 of the 15 study subjects pa-HDL_2 and respective pp-HDL_2 whose triglyceride content differed widely; three preparations of pp-HDL_2 had a very low proportion of triglyceride (< 15% of core), two pp-HDL_2 had medium triglyceride content (29 and 30% of core), and three other pp-HDL_2 had a very high triglyceride content (36–53% of core). Each HDL_2 preparation from every individual was tested in separate experiments, performed in duplicate. Figure 1 shows representa-
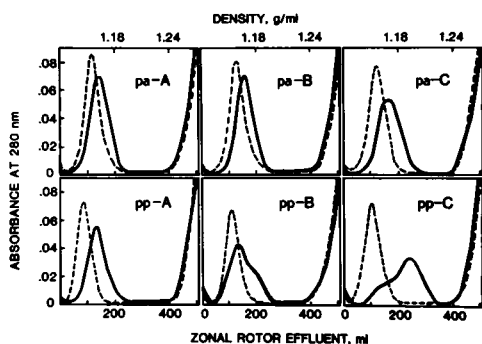
tative zonal ultracentrifugal analyses of these incubation experiments. A, B, and C refer to individuals whose pp-HDL_2 contained low, medium, and high amounts of triglycerides, respectively. Upper and lower panels show pa-HDL_2 and their respective pp-HDL_2. pp-HDL_2 (dashed lines in Fig. 1, bottom panels) banded at earlier rotor volume fractions than their respective pa-HDL_2 (dashed lines in Fig. 1, top panels), in agreement with higher $F°_{1.21}$ values and lower protein content (Table I).

Incubation of pa-HDL_2 with hepatic lipase produced particles with slightly higher density (solid lines in Fig. 1, upper panels). This was the case with all pa-HDL_2 tested, and in no case were lipoprotein populations with HDL_3 density observed. When pp-HDL_2 with low triglyceride content were incubated with hepatic lipase, the product particles banded as a single peak at somewhat higher densities in the zonal rotor (Fig. 1, pp-A). The peak position of this single band was between 120 and 170 ml in six individual experiments (using pp-HDL_2 from three different donors), which is within the elution volume of native pa-HDL_2 in the zonal rotor (1). The HDL formed in vitro from panels pa-A ($n = 3$), pa-B ($n = 2$), pa-C ($n = 3$), and pp-A ($n = 3$) showed very similar percentage composition ($n = 11$): 44.5±2.0% protein, 23.5±1.7% phospholipid, 4.8±1.7% cholesterol, 25.3±0.9% cholesteryl ester, and 1.7±1.4% triglyceride.

The two pp-HDL_2 samples with medium triglyceride content both yielded denser peaks within the HDL_2 density range and a trailing shoulder (Fig. 1, pp-B). Material from panel pp-B was not analyzed for composition because of poor resolution of HDL-peaks. All triglyceride-rich pp-HDL_2 (Fig. 1, pp-C), when incubated with hepatic lipase, gave rise to a minor peak within HDL_2 density and a main peak banding at a volume between 200 and 250 ml, which is in the range of native HDL_3 (1). The HDL_3, formed in these experiments exhibited mean peak $F°_{1.21}$ rates of 2.9 (Table II) typical of the most abundant HDL_3 class, also termed HDL_{3L} (1). Quantitative compositional analysis of HDL from Fig. 1, pp-C is illustrated in Table II.

In Fig. 1, only the first 500 ml of the entire rotor volume (665 ml) is shown. Material eluting past 400 ml contained the albumin added to the incubation mixture. In absence of hepatic lipase, <0.2 mg phospholipid was recovered from this fraction. With hepatic lipase present, between 0.6 and 0.8 mg phospholipid was recovered from albumin in pa-A, pa-B, and pa-C, and between 1.6 and 2.1 mg phospholipid in panels pp-A, pp-B, and pp-C. More than 85% of this phospholipid was lysophosphatidylcholine.

The size distributions as determined by gradient gel electrophoresis are given in Fig. 2. Pa- and pp-HDL_2 from all subjects were indistinguishable by size (Fig. 2, lanes 3, 5, 7, and 9). When incubated with hepatic lipase, pa-HDL_2 were reduced in size only slightly and none of them were converted into particles within the size range of HDL_3 (Fig. 2, lanes 4 and 6). Similarly, triglyceride-poor pp-HDL_2 were reduced in size only slightly to particles that were not in the size range of native HDL_3 (Fig. 2, lane 8). By contrast, triglyceride-rich pp-

Figure 1. Representative zonal ultracentrifugal analyses of incubation mixtures in the absence (- - -) and presence (———) of hepatic lipase. Upper panels, pa-HDL_2; lower panels, respective pp-HDL_2 of three volunteers, A, B, and C. HDL_2 levels were 152, 41, and 11 mg/dl in individuals A, B, and C, respectively. The percentage triglyceride of pa vs. respective pp-HDL_2 core was in A 13.8 vs. 15.2%, in B 16.5 vs. 28.1%, and in C, 11.2 vs. 36.1%. In the other two subjects represented in panel C, HDL_2 levels were 8 and 5 mg/dl, and triglyceride in pp-HDL_2 core was 40.7 and 52.7%. Each mixture contained 5.5 mg HDL_2 phospholipid and was incubated at 25°C for 15 h. For conciseness, only the first 500 ml of the entire rotor volume (665 ml) is shown. All volume fractions under HDL peaks were pooled to one respective sample except for those under solid line in panel pp-C where HDL_2-like material ("HDL_2") was collected from 50 to 180 ml, and HDL_3-like material ("HDL_3") from 200 to 320 ml.

Table II. Incubation of Triglyceride-Rich pp-HDL₂* with Hepatic Lipase In Vitro

	Hepatic lipase absent	Hepatic lipase present	
	pp-HDL₂	"HDL₂"	"HDL₃"
Weight percent			
Protein	38.4±1.62	44.7±0.58	51.5±1.15
PL	34.1±1.25	28.6±1.46	28.0±1.46
UC	5.1±0.45	6.4±1.03	4.9±0.15
CE	12.1±2.59	16.5±0.36	15.4±0.42
TG	10.4±1.18	3.8±0.29	0.3±0.10
Peak $F°_{1.21}$	6.0±0.19	4.9±0.65	2.9±0.19
Apo A-I:Apo A-II‡	3.0±0.75	4.0±1.39	1.7±0.49

* HDL fractions are those defined in Fig. 1, pp-C. Numbers are mean±SD from six (*n* = 6) experiments using pp-HDL₂ from three subjects in duplicate experiments. Hydrolysis of phospholipid and triglyceride from HDL₂ ranged from 50.8 to 55.9% and from 90.4 to 95.9%, respectively. The ratio of HDL₂ to HDL₃ formed from pp-HDL₂ from the three subjects was 0.17, 0.39, and 0.73, respectively. Abbreviations are those used in Table I.
‡ Molar ratio.

HDL₂ (Fig. 2, lane 9) were transformed into two types of particles, i.e., one with HDL₂ size (Fig. 2, lane 10) and one (Fig. 2, lane 11) with size of native HDL₃ (Fig. 2, lane 2).

Particles formed from pa-HDL₂ and from triglyceride-poor pp-HDL₂ had the same apoprotein composition as the particles prior to treatment with hepatic lipase (data not shown). By contrast, the two particle populations formed from all triglyceride-rich pp-HDL₂ tested (Fig. 3 *A*) differed considerably in their apoprotein composition. The HDL₂-like particles had a low abundance of apo A-II, (Fig. 3 *B*), equivalent to an apo A-I/apo A-II molar ratio of 4.0 (Table II). The HDL₃-like particles, however, had a much higher abundance of apo A-II
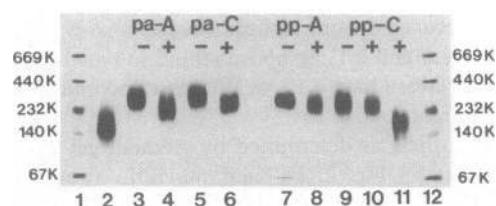
Figure 2. Behavior of HDL fractions on gradient gel electrophoresis. Lanes 3–11 show HDL fractions from Fig. 1; –, without hepatic lipase; +, with hepatic lipase; lanes 10 and 11, "HDL₂" and "HDL₃" from Fig. 1, pp-C. Lanes 1 and 12, calibration proteins (high molecular weight, Pharmacia, Piscataway, NJ) ranging from 669,000 to 67,000 mol wt; lane 2, native HDL₃.
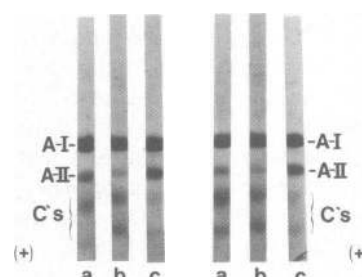
Figure 3. Electrophoretic behavior of apoproteins of HDL from two different donors represented in Fig. 1, pp-C. a, without hepatic lipase; b, "HDL₂"; c, "HDL₃". The same result was obtained with triglyceride-rich pp-HDL₂ from the third donor (for apo A-I/apo A-II molar ratios of all three subjects, see Table II).

(Fig. 3 *C*), and hence an apo A-I/apo A-II molar ratio of 1.7 (Table II).

To determine whether formation of HDL₃-like particles from triglyceride-enriched pp-HDL₂ could be observed with shorter incubation times and with smaller amounts of the enzyme preparation, we reduced incubation time to 6 h with two enzyme additions (at 0 and 3 h), and to 3 h with addition of one aliquot of enzyme at 0 h. The results are illustrated in Fig. 4. Reduction of incubation time and amount of enzyme resulted in progressive reduction of phospholipid hydrolysis that was paralleled by reduced density of the product lipoproteins. With triglyceride-poor pp-HDL₂, no HDL₃-like particles were formed (Fig. 4, upper panel). With triglyceride-rich pp-HDL₂, HDL₃-like particles were formed after 15 h of incubation. However, the existence of two lipoprotein populations was evident already at 3 h of incubation using one aliquot of hepatic lipase (Fig. 4, lower panel). This experiment demonstrates that it is the composition of HDL₂ rather than the experimental conditions that allow HDL₃ formation.

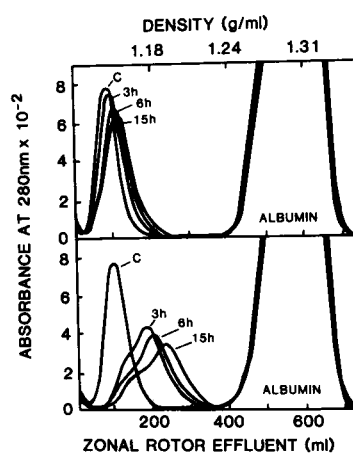After establishing that triglyceride-enriched pp-HDL₂ can

Figure 4. Effect of incubation time and enzyme additions on formation of HDL₃ in vitro. Upper panel, triglyceride-poor pp-HDL₂ (15.5% triglyceride of core). Lower panel, triglyceride-rich pp-HDL₂ (40.7% of core). C, control incubation for 15 h in absence of hepatic lipase. With 3 h incubation time, one 300-μl aliquot of enzyme was added at start of incubation. With 6 h incubation time, two 300-μl aliquots were added at 0 and 3 h. With 15 h incubation, three aliquots of enzyme were added at 0, 3, and 12 h, respectively. Other conditions were those described in Fig. 1. Extent of phospholipid hydrolysis in both panels averaged 50% at 15 h, 35% at 6 h, and 20% at 3 h.

be converted to HDL_3 by hepatic lipase in vitro, we tested these observations for their in vivo relevance. We administered to some of the study subjects whose HDL_2 were used for the experiment shown in Fig. 1 the standardized oral fat load and analyzed their plasma for HDL subclass distribution at 0 h (pa state), 7 h (pp state), and 15 h postprandially. Fig. 5 illustrates the results with two individuals with high HDL_2 levels. In these subjects, concentrations of HDL_2 in plasma did not decrease throughout the observation period. Different results were observed with individuals with medium-low HDL_2 levels (Fig. 6). At 15 h, HDL_2 concentrations in the plasma of these subjects were decreased by 23 and 13%, respectively. Concomitantly, there was an increase of HDL_3 by 6 and 7%, respectively.

As can be seen from Fig. 6, at 7 h the HDL_2 from both subjects banded at lower densities than at 0 h (in agreement with the higher $F°_{1.21}$ rates observed for pp-HDL_2 when compared with pa-HDL_2, Table I). At 15 h, however, the HDL_2 banded at higher densities than at 0 h. Therefore, the flotation rates ($F°_{1.21}$) of these HDL_2 were measured. $F°_{1.21}$ from subject A (B) were at 0 h 5.1 (5.3), at 7 h 5.8 (6.1), and at 15 h 4.8 (4.9). Protein content (weight percent) was at 0 h 39 (40)% and at 15 h 43 (44)%. At 15 h, the HDL_2 of these subjects thus showed higher abundance of protein, higher densities, and lower flotation rates than at 0 h and hence were very similar to the "HDL_2" found in vitro after action of hepatic lipase on triglyceride-rich pp-HDL_2 (Fig. 1, pp C and Table II).

Discussion

The rationale for this study was provided by our discovery that, in normolipidemic subjects, ingestion of a standard fatty meal altered HDL_2 that had large individual variations with
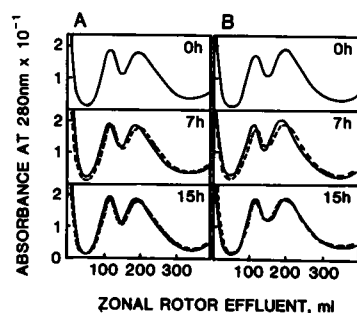
Figure 5. Zonal ultracentrifugal analysis for HDL subfractions in two of the study subjects whose HDL_2 were used in the experiments represented in Fig. 1 A. Plasma was obtained in the pa state (0 h) and 7 h and 15 h, respectively, after ingestion of the standard fatty meal. 0 h effluent pattern is included in 7- and 15-h panels in dashed line (- - -) to facilitate comparison. Magnitude of pp lipemia was 125 and 58 mg/dl · 8 h TG area in subject A (male) and subject B (female), respectively. In subject A (respective values for subject B in parentheses), HDL_2 levels at 0, 7, and 15 h were 130 (115), 145 (124), and 143 (122) mg/dl plasma. Triglyceride abundance in HDL_2 core was at 0 h 12.6 (12.3)%, at 7 h 12.5 (12.1)%, and at 15 h 9.9 (8.8)%. HDL_3 levels were 242 (280), 251 (285), and 244 (279) mg/dl at 0, 7, and 15 h, respectively.
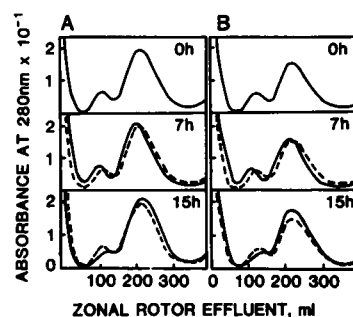
Figure 6. Zonal ultracentrifugal analyses for postprandial changes of HDL subclass distribution in two study subjects, A (male) and B (female). 0 h effluent patterns (- - -) are superimposed on 7-h and 15-h panels to facilitate comparison. Magnitude of pp-lipemia was 727 and 626 mg/dl · 8 h TG area in subjects A and B, respectively. HDL_2 levels in subject A (B) were 47 (45) mg/dl at 0 h, 49 (50) mg/dl at 7 h, and 36 (39) mg/dl at 15 h. Triglyceride abundance in HDL_2 core was at 0 h 14.4 (12.9)%, at 7 h 34.1 (29.3)%, and at 15 h 11.4 (10.8)%. HDL_3 levels were 259 (241) at 0 h, 261 (235) at 7 h, and 275 (257) mg/dl at 15 h.

respect to triglyceride content (Table I). In subjects with very high HDL_2 levels, little or no change in core components occurs. In individuals with extremely low HDL_2 levels and pronounced pp lipemia, the HDL_2 particles become enriched with triglycerides at the expense of cholesteryl esters. This is most likely caused by protein-mediated (10, 11) net exchange of triglycerides and cholesteryl esters between chylomicrons and HDL_2 and appears to be governed by the relative abundance of donor and acceptor particles (12).

The compositional variance among pp-HDL_2 suggested they may yield different product lipoproteins upon interaction with hepatic lipase since the enzyme hydrolyzes both phospholipids and triglycerides in HDL_2 (13). From clinical studies, evidence has accumulated that hepatic lipase plays a role in the catabolism of HDL_2 (for review, see reference 14). In familial deficiency of hepatic lipase, the levels of HDL_2 are elevated (15) and in healthy humans the activity of hepatic lipase in postheparin plasma is correlated negatively with HDL_2 levels (16). However, it has been demonstrated that HDL_2 from fasting normolipidemic plasma are not converted into HDL_3 by hepatic lipase in vitro (13) and our experiments with pa-HDL_2 confirm this finding (Fig. 1, upper panels). As could be expected from our experiments, a higher proportion of phospholipids was hydrolyzed from the phospholipid-rich pp-HDL_2 than from pa-HDL_2. One primary physiologic function of hepatic lipase could be to remove, from HDL_2, the postprandially derived phospholipids so that HDL_2 can continue to serve as phospholipid acceptors during renewed chylomicron catabolism. With triglyceride-poor pp-HDL_2, hepatic lipase would maintain the structural integrity of HDL_2 essentially without reducing HDL_2 levels. With triglyceride-enriched pp-HDL_2, removal of triglycerides from the core of HDL_2 would form smaller HDL_3 particles. In this situation, hepatic lipase would reduce HDL_2 levels by converting them into HDL_3. Between these two extremes, there appears to be a continuous transition (Fig. 1, pp-B). A similar mechanism involving lipoprotein lipase instead of hepatic lipase has been postulated for the formation of small LDL (17).

Three aspects from this study support the physiologic relevance of our observations. The first is that triglyceride-rich pp-HDL_2, upon treatment with hepatic lipase, consistently yielded two lipoprotein populations. One population has the size and flotation characteristics of HDL_2. A second population has the characteristics of HDL_3. When we gradually reduced incubation times from 15 to 6 to 3 h, the existence of two particle populations was always apparent (Fig. 4, lower panel). Thus, formation of HDL_3 was initiated early in lipolysis as a quantized change and was not the result of conversion of "HDL_2" to HDL_3 due to prolonged incubation. With triglyceride-poor pp-HDL_2, no HDL_3-like particles were formed even after 15 h of incubation (Fig. 4, upper panel). Therefore, it is the intrinsic property of pp-HDL_2 that determines whether HDL_3 is formed. Infusion of heparin into individuals with lipoprotein lipase deficiency causes a decrease in HDL_2 and an increase in HDL_3 caused by hepatic lipase (18). Since the HDL_2 of these severely hypertriglyceridemic subjects must be enriched with triglycerides, the study by Rao et al. (18) suggests that hepatic lipase can degrade such triglyceride-rich HDL_2 in vivo at significant rates.

The second consideration related to the physiological role of this process is the finding that the HDL_2-like particles that remained in vitro contained less apo A-II than the starting pp-HDL_2 (Table II and Fig. 3). The HDL_3-like particles contained large amounts of apo A-II with an apo A-I/apo A-II molar ratio of 1.7, which is that of native HDL_3 (1, 19). The product lipoproteins formed from triglyceride-rich pp-HDL_2 thus not only had the density and size but also the apoprotein composition of native HDL_3.

The third finding supporting the physiologic relevance of our in vitro observations are the kinetics of HDL_2 and HDL_3 distribution in the plasma of our study subjects between 0 and 15 h postprandially. In the subjects with high HDL_2 levels and low postprandial lipemia, HDL_2 were not enriched with triglyceride at 7 h postprandially (Fig. 5, legend) and were not converted to HDL_3 by hepatic lipase in vitro (Fig. 1, pp-A). In these subjects, ingestion of the standard fatty meal failed to cause a decrease in HDL_2 plasma levels throughout the observation period (Fig. 5).

In the individuals with lower HDL_2 levels and pronounced lipemia, HDL_2 were enriched with triglyceride at 7 h postprandially (Fig. 6) and were converted partly to HDL_3 by hepatic lipase in vitro (Fig. 1, pp-B, pp-C). In these subjects ingestion of the standard fatty meal caused a decrease in HDL_2 concentration in plasma (Fig. 6). Composition and density of these HDL_2 at 15 h resemble that of "HDL_2" found after hepatic lipase action in vitro on triglyceride-rich pp-HDL_2. Observations consistent with these findings have been reported by Kashyap et al. (20) who monitored in six subjects the changes in relative HDL subfraction distribution using gradient gel electrophoresis from 0 to 12 h postprandially. In the two individuals with low HDL_2 levels and pronounced lipemia, HDL_2 levels decreased 12 h postprandially. The subjects with high HDL_2 levels showed no decrease in HDL_2.

Our findings suggest that the following sequence of events could occur in vivo: Dietary ingestion of fat is followed by postprandial chylomicronemia which allows transfer of triglycerides from chylomicrons into HDL_2 in exchange for cholesteryl esters. The resulting triglyceride-rich pp-HDL_2 are converted by hepatic lipase to HDL_3. Postprandial lipemia would be a prerequisite for the initiation of the above sequence of events and the magnitude of postprandial lipemia would determine the degree of enrichment with triglycerides in HDL_2. Thus, with increasing magnitude of postprandial lipemia, the proportion of HDL_2 that is converted into HDL_3 would increase and, ultimately, decrease the steady-state levels of HDL_2 in plasma. This hypothesis provides an explanation for the great differences in HDL_2 levels among normotriglyceridemic individuals and for their negative association with the magnitude of postprandial lipemia (2).

Acknowledgments

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