Postprandial lipemia differentially influences high density lipoprotein subpopulations LpAI and LpAI,AII

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Abstract The behavior of apolipoprotein-defined subpopulations LpAI and LpAI,AII within high density lipoprotein (HDL) subclasses 2 and 3 was analyzed in the postprandial phase after a fat load. For the whole group of subjects, increases in plasma concentrations of HDL, principally due to the influx of lipoprotein surface components, were largely confined to the HDL_3 density range and involved LpAI,AII and LpAI. However, the degree of postprandial lipemia influenced the distribution of surface remnants between the subfractions. In subjects with a limited postprandial rise in triglycerides, increased HDL mass was predominantly associated with LpAI,AII, and equally distributed between HDL_2 and HDL_3. Conversely, subjects with exaggerated postprandial lipemia manifested increased mass primarily within the HDL_3 density range, implicating both LpAI,AII and LpAI. Stepwise regression analysis identified a two-variable model, involving LpAI,AII within HDL_2 and LpAI within HDL_3, as best defining the relationship between postprandial lipemia and the increase in HDL mass. Postprandial increases in triglyceride content were observed for all HDL subfractions, whilst modifications to the core lipid mass ratios were significant only for LpAI,AII. Stepwise regression analysis revealed a significant correlation between postprandial lipemia and the increase in triglyceride concentration only of LpAI,AII within HDL_3. The results suggest that postprandial lipemia differentially influences apolipoprotein-defined HDL subfractions. The extent of postprandial lipemia may determine the involvement of different HDL subfractions in postprandial lipoprotein metabolism.—**James, R. W., and D. Pometta.** Postprandial lipemia differentially influences high density lipoprotein subpopulations LpAI and LpAI,AII. *J. Lipid Res.* 1994. **35:** 1583–1591.

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High density lipoproteins (HDL) enjoy a reputation as anti-atherogenic particles, a conclusion supported by an impressive, multi-disciplinary array of evidence (1–8). Facilitated catabolism and, thus, rapid elimination of triglyceride-rich lipoproteins (chylomicrons, very low density lipoproteins (VLDL)) has been proposed as one mechanism by which HDL beneficially influence the atherogenic process (8–10). Amongst other aspects, it involves the efficient management of the large quantities of lipid and protein material released during lipolysis. The principal elements are phospholipids and apolipoproteins (11–13) which derive from the lipoprotein surface layer rendered redundant consequent to shrinkage of the triglyceride-rich core. The task is assumed by HDL particles residing principally within the HDL_3 density spectrum, where absorption of surface remnants modifies their density and composition towards that of HDL_2 (11–13). During the post-absorptive phase, disposal of acquired material, probably mediated by hepatic lipase (14, 15), completes a hypothetical, recycling process (16) by regenerating HDL_3 particles. There is, however, a surprising lack of detailed information about how this fundamental process functions, although ultracentrifugal separation into HDL subclasses 2 and 3 has been instrumental in providing a measure of understanding (11–13, 15, 16).

In recent years, several groups have exploited apolipoprotein composition to effectuate subfractionation of HDL (17–21). In terms of their quantitatively major apolipoproteins (A-I and A-II), HDL can be separated into particles containing apoA-I and A-II (LpAI,AII or Lp(AI with AII)) and those containing apoA-I but no apoA-II (LpAI or Lp(AI without AII)). Both types of particle are found within subclasses HDL_2 and HDL_3 (19, 22, 23). The physiological relevance of these subpopulations is presently under investigation. Available evidence suggests that LpAI may be primarily involved in the reverse transport of cholesterol (24, 25) although it remains a point of contention (26). There is no clear indication as to the role of LpAI,AII; speculation has focused on links with the metabolism of triglyceride-rich lipoproteins (16).

Abbreviations: VLDL, very low density lipoproteins; HDL, high density lipoproteins; PBS, phosphate-buffered saline; apo, apolipoprotein; LCAT, lecithin:cholesterol acyltransferase.

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A recent study from our laboratory provided compositional data consistent with such speculation (23).

The objective of the present study was to follow the behavior of apolipoprotein-defined subpopulations within HDL_2 and HDL_3 during a fat challenge. Their plasma concentrations and compositions were examined as indicators of potential roles in the postprandial phase of lipoprotein metabolism. The results provide the first direct evidence of the divergent effects of postprandial lipemia on LpAI and LpAI,AII.

MATERIALS AND METHODS

Healthy volunteers who were normotriglyceridemic in a pre-study control (triglycerides < 200 mg/dl; 2.3 mmol/l) were recruited from the personnel and students of the University Hospital in Geneva (n = 17, 10 males, 7 females). Informed consent was obtained from all volunteers entering the study which was reviewed by and performed according to the guidelines of the Human Studies Ethical Commission of the University Hospital, Geneva. After an overnight fast, a standard fat-rich meal was provided as a drinkable liquid and consumed within 10 min. It provided 65 g fat/m^2 of body surface and was composed of 460 ml of whipped cream containing 2.2% protein, 12% carbohydrate, 85.8% fat (with a polyunsaturated/saturated fat ratio of 0.06) and 480 mg cholesterol. No other food was allowed during the next 8 h. Blood samples were taken prior to (t0) and 2 h, 4 h, 6 h, and 8 h after ingestion of the meal. Lipid and apolipoprotein measurements were carried out on all blood samples, and postprandial triglyceridemia was calculated as the function of triglyceride levels during the 8-h postprandial phase by the trapezoidal rule, as described previously (9). In addition, HDL were subfractionated from the 0, 2 h, and 6 h samples as described below.

Plasma samples were sequentially fractionated into HDL subclasses 2 and 3 then subfractions LpAI and LpAI,AII as described in detail previously (27). Briefly, HDL subclasses were isolated by density gradient ultracentrifugation (13) from 2 × 2.0 ml plasma. Peaks corresponding to HDL_2 and HDL_3 were identified either by measuring total cholesterol, or by monitoring the absorbance at 280 nm, pooled and dialyzed against phosphate-buffered saline (PBS; NaCl 0.14 M, KCl 2.7 mM, phosphate buffer 10 mM, pH 7.4, EDTA 1.0 mM). An aliquot of each subclass (0.5 mg protein) was incubated with an excess of immunoabsorbent anti-apoA-II gel by gentle end-over-end rotation (2 h, 4°C) in stoppered, plastic tubes. After low speed centrifugation (5 min) to remove gel bound HDL, the supernatant was recovered. It corresponded to HDL containing A-I but no A-II, i.e., HDL-LpAI. Parallel incubations were performed using HDL (0.1 mg) and an anti-apoA-I immunoabsorber to control for the presence of non-HDL material (virtually all HDL particles contain apoA-I (19, 22)). As indicated previously (27), such material did not exceed 2% of total protein or lipids. Additional control incubations showed negligible binding of HDL to immunoabsorbents prepared using non-immune mouse IgG.

This highly reproducible procedure (27) allows quantification of four HDL subfractions: HDL_2-(LpAI) and HDL_3-(LpAI) whose lipid and total protein components were assayed in the unbound fraction after incubation of the relevant HDL subclass with anti-apoA-II immunoabsorbent; and HDL_2-LpAI,AII and HDL_3-LpAI,AII which were computed by subtraction of LpAI values from nonfractionated HDL_2 and HDL_3, respectively. For brevity, these fractions are herein referred to as $Lp(AI)_2$, $Lp(AI,AII)_2$, $Lp(AI)_3$, and $Lp(AI,AII)_3$. (As described previously (27), inter-assay coefficients of variation for the immunofractionation step of 3.4, 1.1, 2.2, and 0.5 for $Lp(AI)_2$, $Lp(AI,AII)_2$, $Lp(AI)_3$, and $Lp(AI,AII)_3$, respectively, were observed.) For clarity, the term 'subclasses' is reserved for HDL_2 and HDL_3 as isolated by ultracentrifugation. Recovery of HDL-cholesterol by ultracentrifugation (as compared to HDL-cholesterol measured in whole plasma after phosphotungstate precipitation) was 92.0 ± 10.1% (n = 51).

Lipids (phospholipids, triglycerides, free and esterified cholesterol) were measured by standardized enzymatic procedures (28). ApoA-I and apoB were quantified by electroimmunoassay (29) and apoA-II by enzyme-linked immunoassay (23). Total protein was assayed by the procedure of Lowry et al. (30).

Statistical analyses were performed using the Statworks statistical package (Abacus concepts). Comparisons between samples were made with the two-tailed Student's *t*-test. The association between postprandial lipemia and various HDL parameters was analyzed by means of the Pearson product moment correlation. The least squares method was used for regression analyses that were subsequently subjected to analysis of variance in order to obtain the significance of F ratios.

RESULTS

Plasma lipid and apolipoprotein levels measured in fasting and postprandial blood samples are given in **Table 1.** Highly significant increases in plasma triglycerides were observed up to the 6-h postprandial sample; these had virtually returned to fasting levels by t8. Less dramatic changes in other lipid levels were noted (Table 1). Plasma cholesterol rose slightly, whereas apoB levels fell gradually, attaining significance (at $P \leq 0.05$) for t6 and t8. Minor variations in HDL-cholesterol were observed; conversely, apoA-I and A-II tended to rise throughout the postprandial phase.

TABLE 1. Lipid and apolipoprotein parameters in fasting and postprandial blood samples

Parameter	t0	t2	t4	t6	t8
Triglycerides	1.07 ± 0.57	1.93 ± 0.96[a]	2.14 ± 1.08[a]	1.88 ± 1.06[a]	1.19 ± 0.62
Cholesterol	4.72 ± 0.93	4.86 ± 0.94[d]	4.81 ± 0.84	4.81 ± 0.79	4.81 ± 0.80
HDL-cholesterol	1.33 ± 0.26	1.36 ± 0.32	1.32 ± 0.28	1.29 ± 0.24	1.36 ± 0.28
ApoA-I	131.6 ± 25.0	133.7 ± 25.0	133.7 ± 24.8	138.6 ± 25.5[c]	139.9 ± 25.6[b]
ApoB	96.6 ± 13.4	96.4 ± 15.0	95.3 ± 14.0	93.0 ± 13.2[d]	92.5 ± 11.3[d]
ApoA-II	45.8 ± 14.0	46.3 ± 12.5	46.6 ± 14.7	47.3 ± 13.0[d]	47.2 ± 12.8[d]

Blood lipid and apolipoprotein levels (± SD; n = 17) measured in fasting blood (t0) and 2 h, 4 h, 6 h, and 8 h after ingestion of a fatty meal. Lipid values are in mmol/l, and apolipoproteins in mg/dl. Postprandial samples were compared to the fasting values using the Student's paired *t*-test.

[a] $P < 0.001$.
[b] $P < 0.005$.
[c] $P < 0.01$.
[d] $P < 0.05$.

Concentrations of HDL_2 and HDL_3, calculated as the sum of their individual components, are given in **Table 2A.** HDL_3 mass rose in postprandial plasma, the increase, already evident at t2, becoming highly significant 6 h postprandially. Increases in HDL_2 were minor in the 2-h sample, but significant ($P < 0.025$) at t6. Accompanying these changes was a tendency for both HDL_2 and HDL_3 to float at lower densities. Postprandial lipemia (measured as the 8-h triglyceride area) was negatively correlated with the mass of HDL_2 ($r = -0.45$; $P < 0.05$) in fasting plasma, but showed no correlation with HDL_3 ($r = -0.05$).

In terms of individual components, these rises particularly reflected increments in phospholipid and free cholesterol concentrations of both subclasses, although it was more marked for HDL_3 (Table 2A); increases in the total protein and triglyceride components were also noted. In contrast, there were no significant changes in the absolute concentrations of esterified cholesterol. Most of the modifications were significant for the 6-h postprandial sample. Translated into composition (weight percent, Table 2B), there was a highly significant enrichment of HDL_3 in phospholipids and free cholesterol and, to a lesser extent, triglycerides. Conversely, the relative esterified cholesterol content was significantly reduced. Compositional modifications of HDL_2 were less marked, apart from a highly significant reduction in esterified cholesterol (Table 2).

Plasma concentrations of the subfractions obtained by immunoaffinity absorption of ultracentrifugally isolated HDL subfractions are given in **Table 3A.** As compared to the fasting blood sample, levels of $Lp(AI)_3$ and

TABLE 2. Composition of HDL_2 and HDL_3 in fasting and postprandial plasma samples

Subclass	Protein	Triglycerides	Phospholipids	Free Cholesterol	Esterified Cholesterol	Total Mass
A) Absolute concentrations (mg/dl) of HDL subclass components						
HDL_2						
t0	33.5 ± 20.8	2.0 ± 1.9	23.3 ± 16.5	5.0 ± 3.4	19.4 ± 12.2	83.4 ± 53.9
t2	35.1 ± 20.8	2.4 ± 2.1	23.3 ± 15.3	5.3 ± 3.5	19.5 ± 12.0	85.5 ± 52.7
t6	36.0 ± 19.8	2.7 ± 2.6[a]	26.5 ± 16.9[c]	5.8 ± 3.7[d]	19.7 ± 11.7	90.8 ± 53.3[c]
HDL_3						
t0	120.2 ± 21.0	2.7 ± 1.4	48.2 ± 10.5	6.3 ± 1.3	40.6 ± 7.5	218.0 ± 38.0
t2	126.9 ± 22.3	2.9 ± 1.4	50.5 ± 12.3	6.7 ± 1.3	41.8 ± 7.8	228.7 ± 39.9
t6	134.5 ± 19.0[b]	3.7 ± 2.0[c]	60.9 ± 17.9[d]	8.3 ± 1.5[d]	40.8 ± 10.3	248.3 ± 41.4[c]
B) Relative concentrations (g/100 g) of HDL subclass components						
HDL_2						
t0	40.5 ± 8.8	2.3 ± 1.1	27.5 ± 3.6	6.1 ± 0.9	23.5 ± 2.9	
t2	41.6 ± 3.9	2.8 ± 1.8	26.5 ± 3.7	6.1 ± 1.1	23.0 ± 2.8	
t6	40.1 ± 2.9	2.9 ± 1.6	28.6 ± 4.8	6.4 ± 1.0	21.6 ± 2.4[d]	
HDL_3						
t0	55.1 ± 2.8	1.2 ± 0.5	22.1 ± 2.4	2.9 ± 0.3	18.6 ± 1.4	
t2	55.6 ± 3.4	1.2 ± 0.5	21.9 ± 2.7	2.9 ± 0.4	18.3 ± 2.1	
t6	54.6 ± 5.1	1.5 ± 0.7[b]	24.3 ± 3.7[d]	3.3 ± 0.4[d]	16.3 ± 3.0[c]	

Absolute (A) and relative (B) concentrations (± SD, n = 17) of HDL subclasses separated by density gradient ultracentrifugation from fasting (t0) and postprandial (t2, 2 h; t6, 6 h) plasma. Postprandial samples were compared to t0 using Student's paired *t*-test; [a] $P \leq 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.005$; [d] $P \leq 0.001$.

TABLE 3. Composition of apolipoprotein-defined HDL subfractions in fasting and postprandial plasma samples

Subfraction	Protein	Triglycerides	Phospholipids	Free Cholesterol	Esterified Cholesterol	Total Mass
A) Absolute concentrations (mg/dl)						
$Lp(AI)_2$						
t0	11.3 ± 9.4	1.4 ± 1.6	8.8 ± 8.1	2.1 ± 1.6	5.5 ± 4.2	29.2 ± 24.2
t2	11.5 ± 9.1	1.4 ± 1.6	8.6 ± 8.4	2.1 ± 1.7	5.4 ± 4.6	29.1 ± 24.1
t6	12.1 ± 9.1	1.6 ± 1.9	9.7 ± 8.9[a]	2.5 ± 1.8[b]	5.7 ± 4.9	31.7 ± 25.9
$Lp(AI,AII)_2$						
t0	21.8 ± 10.5	0.8 ± 1.1	14.6 ± 8.9	3.0 ± 2.1	13.3 ± 7.0	53.5 ± 28.6
t2	23.5 ± 12.1	1.0 ± 0.8	14.6 ± 7.7	3.1 ± 1.9	13.8 ± 7.8	56.1 ± 28.9
t6	24.0 ± 11.0	1.1 ± 1.0	16.8 ± 8.8[b]	3.5 ± 1.8[b]	13.9 ± 7.1	59.1 ± 28.4[a]
$Lp(AI)_3$						
t0	29.5 ± 11.2	1.6 ± 0.8	8.9 ± 4.2	1.7 ± 0.6	5.8 ± 2.6	47.5 ± 16.5
t2	33.1 ± 13.3	1.7 ± 0.7	9.3 ± 3.9	1.7 ± 0.7	6.4 ± 2.8	52.2 ± 17.4
t6	33.1 ± 9.0	2.0 ± 1.0[a]	12.2 ± 5.3[d]	2.3 ± 0.8[d]	7.6 ± 4.4[a]	57.2 ± 16.6[b]
$Lp(AI,AII)_3$						
t0	90.5 ± 16.2	1.0 ± 0.9	39.3 ± 7.8	4.5 ± 0.9	34.7 ± 6.2	170.2 ± 28.5
t2	93.7 ± 17.2	1.2 ± 0.9	41.2 ± 9.8	4.9 ± 0.9	35.3 ± 6.5	176.4 ± 31.2
t6	100.8 ± 17.2[c]	1.7 ± 1.3[c]	48.7 ± 14.6[c]	6.0 ± 1.1[d]	33.1 ± 9.3	190.4 ± 34.6[d]
B) Relative concentrations (g/100 g)						
$Lp(AI)_2$						
t0	38.4 ± 5.8	4.9 ± 2.6	28.6 ± 5.7	7.8 ± 2.4	20.2 ± 5.3	
t2	40.4 ± 8.7	4.6 ± 2.3	27.2 ± 5.2	7.6 ± 2.6	18.6 ± 5.4	
t6	39.9 ± 6.8	4.9 ± 2.7	29.8 ± 6.3	8.4 ± 2.1	17.0 ± 5.5[a]	
$Lp(AI,AII)_2$						
t0	41.4 ± 4.7	1.3 ± 0.9	27.0 ± 4.0	5.4 ± 1.4	24.9 ± 2.9	
t2	42.3 ± 5.0	1.9 ± 1.8	25.8 ± 4.5	5.5 ± 0.9	24.4 ± 2.7	
t6	40.9 ± 3.3	1.9 ± 1.3[c]	27.9 ± 4.8	5.6 ± 0.9	23.5 ± 2.3[a]	
$Lp(AI)_3$						
t0	61.9 ± 7.6	3.6 ± 1.8	18.6 ± 4.8	3.7 ± 1.1	12.1 ± 3.2	
t2	62.1 ± 10.6	3.4 ± 1.5	18.7 ± 7.8	3.5 ± 1.1	12.2 ± 3.1	
t6	58.3 ± 7.5[a]	3.6 ± 1.9	21.0 ± 4.9[b]	4.2 ± 1.4	12.7 ± 4.8	
$Lp(AI,AII)_3$						
t0	53.1 ± 2.8	0.6 ± 0.4	23.1 ± 2.3	2.7 ± 0.4	20.4 ± 1.8	
t2	53.2 ± 3.4	0.6 ± 0.4	23.2 ± 2.9	2.8 ± 0.3	20.1 ± 2.3	
t6	53.3 ± 5.7	0.9 ± 0.6[b]	25.4 ± 4.3[b]	3.2 ± 0.5[d]	17.2 ± 3.5[d]	

Absolute (A) and relative (B) concentrations (± SD, n = 17) of HDL subfractions separated from fasting (t0) and postprandial (t2, 2 h; t6, 6 h) plasma. Postprandial samples were compared to fasting values using Student's paired *t*-test; [a] $P \leq 0.05$; [b] $P \leq 0.01$; [c] $P \leq 0.005$; [d] $P \leq 0.001$. All other comparisons were not significant at $P = 0.05$.

$Lp(AI,AII)_3$ rose in both 2-h ($0.05 > P < 0.07$) and 6-h postprandial plasma, the increases being highly significant in the latter sample. Similarly, $Lp(AI,AII)_2$ mass was significantly increased in the 6-h sample whilst no significant changes in $Lp(AI)_2$ were recorded. The principal contributors to the increases in the masses of the subfractions were phospholipids and free cholesterol (Table 3A); they also occasioned substantial changes in the composition of $Lp(AI,AII)_3$ (Table 3B).

Postprandial lipemia is thought to alter the core and surface entities of HDL by distinct mechanisms (11–13, 31 and Discussion). The consequences of such modifications at the LpAI and LpAI,AII level were examined by analyzing particle composition.

In both HDL subclasses and apolipoprotein-defined subfractions, the total mass of components of the lipoprotein outer layer (phospholipids, free cholesterol, and protein) rose gradually in the postprandial phase. Increases were already evident at t2 and became significant at t6. They were particularly marked for HDL_3-derived subfractions ($Lp(AI,AII)_3$, 155.5 ± 27.0 v 134.4 ± 22.9 mg/dl, $P < 0.001$; $Lp(AI)_3$, 47.6 ± 13.1 v 40.1 ± 14.1 mg/dl, $P < 0.05$) compared to fractions within the HDL_2 density range ($Lp(AI,AII)_2$, 44.1 ± 21.1 v 39.3 ± 20.9 mg/dl, $P < 0.01$; $Lp(AI)_2$, 24.3 ± 19.6 v 22.3 ± 18.9 mg/dl, $P < 0.05$). In contrast, there was no increase at t6 in the core lipid mass (triglycerides + esterified cholesterol) for either subclass or for immunoaffinity isolated subfraction, with the exception of $Lp(AI)_3$ (9.6 ± 4.7 v 7.5 ± 3.1 mg/dl, $P < 0.05$). There were, however, changes in the mass ratios of the two lipids in the apoA-II-containing subfractions in the postprandial phase (t0 v t6: $Lp(AI,AII)_3$, 35.0 ± 18.3 v 23.4 ± 12.8, $P < 0.05$; $Lp(AI,AII)_2$, 24.6 ± 14.2 v 16.8 ± 11.8, $P < 0.025$); LpAI subfractions showed no significant changes in the relative mass ratios of the core lipids.

A wide range of postprandial responses (0.62–14.2 mmol/l triglyceride area at 8 h) to the fat challenge was observed in the subjects. To further examine this aspect, participants were divided into two groups representing

TABLE 4. HDL subfraction concentrations in fasting and postprandial samples from subjects with a high and low metabolic response to the fat challenge

	Group A		Group B	
Subfraction	t0	t6	t0	t6
HDL_2	103.0 ± 66.5	112.3 ± 68.0	64.8 ± 28.6	71.8 ± 27.0[a]
HDL_3	224.4 ± 25.3	232.1 ± 21.6	211.8 ± 47.4	261.4 ± 51.3[b]
$Lp(AI)_2$	39.1 ± 31.7	41.4 ± 35.1	20.5 ± 10.6	23.0 ± 9.1
$Lp(AI,AII)_2$	63.9 ± 35.3	70.8 ± 33.4	44.3 ± 18.7	48.8 ± 19.4
$Lp(AI)_3$	48.5 ± 19.0	50.8 ± 18.8	46.7 ± 15.1	63.0 ± 12.6[b]
$Lp(AI,AII)_3$	175.8 ± 12.7	181.4 ± 19.9	165.2 ± 35.8	198.4 ± 43.5[b]

HDL subfraction concentrations (mg/dl ± SD) were computed as the sum of protein and lipid components. Statistical comparisons (t6 v t0 within each subgroup) were made with the Student's paired *t*-test; [a] $P < 0.02$; [b] $P < 0.005$.

subjects with a high (group A) or low (group B) metabolic response to the fat load. Partition of the subjects was based on a triglyceride value of 200 mg/dl (2.3 mmol/l) which is the threshold for definition of hypertriglyceridemia promoted by American and European guidelines (32, 33); subjects with no triglyceride measurement above this value during the postprandial phase were assigned to group A whilst those with at least one value superior to 2.3 mmol/l were allocated to group B. Group A (n = 8; 3 males and 5 females) had a significantly lower ($P < 0.0001$) postprandial response (2.72 ± 1.71 mmol/l triglyceride area at 8 h) than group B (8.35 ± 3.2 mmol/l triglyceride area at 8 h; n = 9; 7 males, 2 females). Fasting HDL-cholesterol levels were 1.42 ± 0.32 and 1.28 ± 0.20 mmol/l (nonsignificant) in the high and low metabolic responders, respectively. Average fasting HDL_2 mass was substantially higher in group A (104.3 ± 69.4 v 64.9 ± 28.2 mg/dl, although nonsignificant due to large inter-individual variations: HDL_3 levels were similar in both groups (224.9 ± 25.0 and 212.0 ± 47.4 mg/dl). Plasma concentrations of the apolipoprotein-defined subfractions are shown in **Table 4** for fasting and 6-h samples. Several points merit attention. First, the increase in postprandial HDL mass was less marked in group A than group B. Second, the increased HDL mass in group A was largely confined to apoA-II-containing subfractions and was equally distributed between $Lp(AI,AII)_3$ and $Lp(AI,AII)_2$. Third, higher HDL mass concentrations in group B were mainly associated with HDL_3-derived subfractions, $Lp(AI)_3$ making a substantial contribution to

TABLE 5. Stepwise regression analysis of the relationship between postprandial lipemia and modifications to HDL subfraction surface and core components

	Coefficient	SEM	Partial Coefficient	*P*
Surface components				
All subjects				
$Lp(AI,AII)_2$	− 0.036	0.01	− 0.61	< 0.01
$Lp(AI)_3$	0.028	0.006	0.76	< 0.01
	$r = 0.81$ $r^2 = 0.66$		Adjusted $r^2 = 0.61$	< 0.01
Group A				
$Lp(AI)_2$	− 0.024	0.1	− 0.38	< 0.05
$Lp(AI,AII)_2$	− 0.32	0.007	− 0.72	< 0.01
$Lp(AI)_3$	0.015	0.005	0.42	< 0.05
	$r = 0.97$ $r^2 = 0.95$		Adjusted $r^2 = 0.89$	< 0.01
Triglyceride content				
All subjects				
$Lp(AI,AII)_3$	0.269	0.106	0.56	< 0.05
	$r = 0.56$ $r^2 = 0.31$		Adjusted $r^2 = 0.27$	< 0.05
Group B				
$Lp(AI,AII)_2$	0.213	0.074	0.71	< 0.05
$Lp(AI)_3$	− 0.129	0.064	− 0.49	< 0.05
	$r = 0.81$ $r^2 = 0.65$		Adjusted $r^2 = 0.59$	< 0.05

Stepwise regression analyses were applied to the whole group and to the subgroups. The independent variable was log postprandial lipemia (triglyceride area at 8 h) and the dependent variables were the increases (t6 − t0) in the mass of surface components or triglycerides of the four apolipoprotein-defined subfractions. Only analyses that gave significant models are shown.

increased levels. Increases in the concentrations of the surface components essentially accounted for the postprandial rise in HDL mass (results not shown).

Modifications to the apolipoprotein-defined subfractions were also examined as a function of postprandial lipemia. Linear regression analyses revealed that postprandial hypertriglyceridemia was positively correlated with the increase (t6 − t0) in the mass of surface components of $Lp(AI)_3$ ($r = 0.57$, $P = 0.02$) and $Lp(AI,AII)_3$ ($r = 0.35$, $P = 0.18$) and negatively correlated with $Lp(AI)_2$ ($r = -0.40$, $P = 0.12$) and $Lp(AI,AII)_2$ ($r = -0.37$, $P = 0.13$). Stepwise regression analysis identified a two-variable model that best explained the relationship between HDL subfractions and postprandial lipemia (adjusted $r^2 = 0.61$, $P < 0.01$). This involved $Lp(AI,AII)_2$ and $Lp(AI)_3$ (**Table 5**). Stepwise regression analysis within the subgroups revealed a three-variable model explaining 89% of the relationship between subfractions and postprandial lipemia in group A (Table 5); no significant model could be identified in group B.

A final series of analyses examined the triglyceride content of the subfractions. Linear regression analysis indicated positive correlations between postprandial lipemia and the increase in the triglyceride content (t6 − t0) of each subfraction, notably $Lp(AI,AII)_3$ ($r = 0.56$, $P = 0.02$) and $Lp(AI,AII)_2$ ($r = 0.45$, $P = 0.08$). When subjected to stepwise regression analysis only $Lp(AI,AII)_3$ emerged as a significant factor (adjusted $r^2 = 0.31$, $P < 0.05$; Table 5) for the whole group of subjects. Subgroup analyses identified a two-variable model with $Lp(AI,AII)_2$ and $Lp(AI)_3$ for the low metabolic response group (Table 5).

DISCUSSION

The concentration and composition of HDL are particularly vulnerable to fluctuations in the metabolism of triglyceride-rich lipoproteins. It is reflected in the negative correlations between plasma triglycerides and HDL-cholesterol (10, 34, 35) and, to an even greater extent, in the inverse relationship between postprandial hypertriglyceridemia and HDL mass (notably subclass 2) (9, 10, 16). Conversely, efficient removal of chylomicrons/VLDL is particularly beneficial as illustrated by the positive correlation between lipoprotein lipase activity and HDL (10, 16). Two processes are thought to underlie these metabolic ties. One, the assimilation of lipolytic surface remnants, undoubtedly represents an important physiological role of HDL. In contradistinction, the second phenomenon, an excessive influx of triglycerides consequent to hypertriglyceridemia, establishes a situation where pathological changes in HDL composition and distribution could ensue (31, 35). Postprandial lipemia thus provides the scenario within which both mechanisms would be active. We have exploited this approach to further examine the involvement of defined HDL subpopulations in the processes. Our results suggest differential involvement of LpAI and LpAI,AII in the two processes where the degree of postprandial lipemia may be an important factor.

Postprandial increases in the mass of the HDL subfractions (which were largely confined to lipoprotein surface components: phospholipids, free cholesterol, and protein) revealed an interesting disparity between LpAI,AII and LpAI. That is, postprandial lipemia was positively correlated with $Lp(AI)_3$ and negatively correlated with $Lp(AI,AII)_2$. Moreover, stepwise regression analysis indicated that a model including these two variables best defined the relationship between HDL subfractions and the metabolism of triglyceride-rich lipoproteins. This is further underlined by the subgroup analyses. In subjects who were able to limit the postprandial rise in triglycerides, increases in HDL mass were particularly marked for apoA-II-containing fractions, notably $Lp(AI,AII)_2$. This did not reflect adsorption of lipolytic surface remnants proportional to the relative concentrations of the subfractions as, for example, 30.0% and 10.1% of the increase in surface components of HDL at t6 were associated with $Lp(AI,AII)_2$ and $Lp(AI)_3$, respectively, whereas they accounted for 19.5% and 14.8% of total HDL mass. In subjects where there was a larger degree of postprandial lipemia (group B), increases in HDL mass were most marked in HDL_3-derived fractions, both $Lp(AI)_3$ and $Lp(AI,AII)_3$. Again, this did not reflect adsorption according to the relative concentrations of the subfractions, e.g., $Lp(AI,AII)_2$ accounted for 7.9% of adsorbed surface components whereas it represented 16% of HDL mass whilst corresponding figures for $Lp(AI,AII)_3$ were 24.3% and 16.8%. The results suggest that both LpAI and LpAI,AII contribute to adsorption of surface remnants during postprandial lipoprotein metabolism. However, with efficient catabolism of triglyceride-rich lipoproteins, LpAI,AII particles within both the HDL_2 and HDL_3 density ranges play the predominant role in the adsorptive process. In contrast, adsorption of lipolytic remnants is limited to the HDL_3 density range and LpAI plays a more important role when the postprandial lipemic response is exaggerated. The extent of postprandial lipemia would thus appear to be an important determinant of the contribution of each subfraction.

There is little doubt that postprandial events occasion a decrease in the density of HDL particles (11, 12, 15, 36, 37). Under in vitro conditions, the influx of material is apparently sufficient to move HDL_3 into the HDL_2 density range (11). The situation in vivo is less clear, however, as there has been no convincing demonstration of such an extensive change in HDL flotation rate (12, 13). This may reflect the highly complex nature of competing activities at the HDL level, including lipid transfer, LCAT, and particularly hepatic lipase (8, 14–16, 36); the latter can

generate denser HDL particles, countering the tendency of postprandial HDL to manifest a higher flotation rate. Patsch et al. (16) have developed a model whereby HDL cycles between the subclass 3 and 2 density ranges during the postprandial phase and have suggested that LpAI,AII particles could be responsible for the shuttle mechanism (16). Our results are consistent with this model as regards subjects with a high metabolic response in that the $Lp(AI,AII)_2$ subfraction was particularly active. However, we are unable to determine whether this reflects adsorption by $Lp(AI,AII)_3$ and subsequent conversion to $Lp(AI,AII)_2$ as suggested by Patsch et al. (16) or adsorption by pre-existing subfractions within the HDL_2 and HDL_3 density ranges (13). There was a tendency for the $Lp(AI,AII)_3/Lp(AI,AII)_2$ mass ratio to fall in postprandial samples (3.56, 3.19, and 3.14 for t0, t2, and t6, respectively) but the changes were not significant and thus do not provide evidence for a substantial shift in the density of the LpAI,AII fraction.

Results from the subjects with a low metabolic response to the fat load were less consistent with the model as there was a greater involvement of LpAI particles. This could, however, represent one pathophysiological consequence of exaggerated postprandial lipemia. There are several indications that adsorption of lipolytic surface remnants by apoA-II-containing particles may be physiological desirable. First, due to its stronger lipid binding properties (38, 39) apoA-II may more effectively stabilize particles undergoing substantial compositional changes. Second, recent studies by Mowri et al. (40) demonstrated that in vitro hepatic lipase preferentially hydrolyzes lipids of apoA-II-containing lipoproteins. Thus they appear better equipped for elimination of adsorbed lipids and regeneration of acceptor particles. Third, it has long been known that particles within the HDL_3 spectrum are the most avid acceptors of cellular cholesterol (41), recent studies implicating LpAI particles (24, 25). This function could be affected by postprandial fluctuations in LpAI composition. Moreover, such particles may less efficiently shed acquired lipids due to the absence of apoA-II, thus prolonging any undesirable effects. In this context, one potential, pathological consequence of exaggerated postprandial lipemia could be interference with the functioning of LpAI, endangering the major pathway for elimination of cholesterol.

The second phenomenon associated with increased plasma triglyceride levels is an accelerated transfer of the lipid to HDL, resulting from the activity of lipid transfer proteins (8, 31, 42). This subsequently sets the stage for transformation of lipoproteins into smaller denser particles, a process in which hepatic lipase is implicated (14, 16, 43, 44). Although lipid transfer protein appears to be associated with LpAI (45), efficient transfer of lipids into both LpAI and LpAI,AII has been observed in vitro (40, 46). Our results showing significant changes to the core lipid mass ratios only in LpAI,AII subfractions could reflect preferential involvement of apoA-II-containing particles. The data should, however, be interpreted with caution. Generation of esterified cholesterol by LCAT could have masked modifications to the ratio in LpAI. Conversely, hepatic lipase-mediated hydrolysis of triglycerides (40) would limit apparent increases of the lipid in LpAI,AII. In this context, it should be noted that both absolute and relative concentrations of triglycerides were consistently lower in LpAI,AII as compared to LpAI particles (Tables 3A, B). Nevertheless, stepwise regression analysis identified triglyceride enrichment of $Lp(AI,AII)_3$ as being most strongly linked to postprandial hypertriglyceridemia and may reflect the fact that the bulk of esterified cholesterol resides within this subfraction. If this can be confirmed, it raises the question as to whether ongoing core lipid modifications could influence the capacity of LpAI,AII to act as acceptors of lipoprotein surface components.

In conclusion, our results demonstrate that postprandial lipemia differentially influences LpAI and LpAI,AII particles. ApoA-II-containing particles would appear to be principally involved in adsorption of surface remnants when the postprandial rise in triglyceride-rich lipoproteins is limited. Conversely, LpAI particles are implicated to a greater extent with excessive hypertriglyceridemia, with potential pathophysiological consequences. Triglyceride enrichment of all HDL subfractions is observed in the postprandial phase, although the greatest impact would appear to be on $Lp(AI,AII)_3$. The results reinforce suggestions that LpAI and LpAI,AII are distinct metabolic entities that should be fractionated if their physiological roles are to be fully appreciated.

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