Preferential clearance of apoB-48-containing lipoproteins after heparin-induced lipolysis is modulated by lipoprotein lipase activity[1]

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Abstract The acute effects of intravenous heparin administration (50 U/kg body weight) on apolipoprotein (apo)B-48 and apoB-100-containing lipoproteins in relation to postheparin lipase activities were studied in ten healthy normolipidemic volunteers. Five subjects returned to receive sham injections with saline. Lipoproteins were separated from plasma by density gradient ultracentrifugation at baseline, 3, and 20 min postheparin. ApoB-48 and apoB-100 in d $<$ 1.006 g/mL and 1.006 $<$ d $<$ 1.019 g/mL fractions were quantitatively measured after electrophoresis on 5% SDS polyacrylamide gels and Coomassie-blue staining. No significant changes were observed after saline injections. Heparin administration released lipoprotein lipase (LPL) and hepatic lipase (HL) activities after 20 min, and significantly reduced apoB-48 concentrations in d $<$ 1.006 g/mL fractions only. ApoB-100 concentrations showed a trend to decrease in d $<$ 1.006 g/mL fractions and to increase in 1.006 $<$ d $<$ 1.019 g/mL fractions. LPL activity was related to the percentual disappearance of apoB-48 ($r = 0.81$, $P = 0.004$) and apoB-100 ($r = 0.91$, $P < 0.001$) in d $<$ 1.006 g/mL fractions. When little LPL was released (LPL activity $<$ 120 mU/mL) by heparin, apoB-48 was preferentially eliminated over apoB-100. However, when abundant LPL was released (LPL activity $>$ 140 mU/mL), comparable percentual reductions for apoB-48 and apoB-100 were seen. Pharmacokinetic analysis revealed first-order kinetics for the clearance of apoB-48 in d $<$ 1.006 g/mL fractions, but zero-order kinetics for apoB-100 clearance. Under conditions of artificially enhanced lipolysis, the first catabolic step of apoB-48-containing lipoproteins and hepatic VLDL showed different pharmacokinetics. ApoB-48-containing lipoproteins were the preferred substrate for LPL, and only when abundant LPL was present, clearance of hepatic VLDL occurred.—**van Beek, A. P., H. H. J. J. van Barlingen, F. C. de Ruijter-Heijstek, H. Jansen, D. W. Erkelens, G. M. Dallinga-Thie, and T. W. A. de Bruin.** Preferential clearance of apoB-48-containing lipoproteins after heparin-induced lipolysis is modulated by lipoprotein lipase activity. *J. Lipid Res.* 1998. **39:** 322–332.

Supplementary key words chylomicron • apoB-100 • triglyceride-rich lipoproteins • hepatic lipase • chylomicron-remnant clearance • pharmacokinetics

Triglycerides (TG) in plasma are transported by two different types of apoB-containing triglyceride-rich lipoproteins (TRL), chylomicrons, and hepatic VLDL. Chylomicrons, with apoB-48 as their structural protein, are synthesized by the intestine and carry dietary triglycerides. Hepatic VLDL, with apoB-100 as major protein component, bring endogenous triglycerides to peripheral tissues. In the postprandial state, both apoB-48 and apoB-100 levels are increased (1–3), and both chylomicrons and hepatic VLDL compete for lipoprotein lipase in the "common lipolytic pathway" (4). The first catabolic step of TRL, hydrolysis of core TG, requires these particles to bind to the vascular endothelium, the site of lipase action. Several studies have contributed evidence that chylomicrons are preferentially lipolyzed over VLDL, thereby temporarily impeding normal lipolytic degradation of VLDL (5, 6).

It has been known for over 50 years that intravenous heparin administration to human subjects leads to enhanced hydrolysis of triglycerides (7), due to the release of two key enzymes in lipoprotein catabolism, lipoprotein lipase (LPL) and hepatic lipase (HL) (8). Heparin-induced lipolysis has been used to study the contribution of LPL and HL to apolipoprotein metabo-

Abbreviations: TRL, triglyceride-rich lipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase; d, density; apo, apolipoprotein.

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lism (9–11), the formation of lipoprotein subclasses (12–14), and reversed cholesterol transport (15). Berr, Eckel, and Kern (16) reported that plasma decay of chylomicron remnants was not affected by enhanced plasma lipolytic activity. Weintraub et al. (17) demonstrated that, in normolipidemic human subjects, continuous intravenous heparin administration resulted in a depletion of LPL and a subsequent reduced tolerance for an oral fat load. In addition to its pivotal role as triglyceride hydrolyzing enzyme, LPL mediates lipoprotein binding to cell surfaces (18).

The acute effects of heparin i.v. on apoB-48 and apoB-100 containing lipoproteins has not yet been studied in detail. Recently, quantitative measurements of apoB-48 and apoB-100 have been described (19, 20). It was the objective of the present study to examine the effects of acute heparin i.v. administration on lipoprotein particle release from endothelial surfaces. Further, to test whether fasting apoB-48-containing lipoproteins, in the state of excess availability of LPL, are still preferentially cleared relative to hepatic VLDL. We report that heparin-induced lipolysis in the fasting state resulted in preferential clearance of apoB-48 containing lipoproteins from $d < 1.019$ g/mL fractions relative to hepatic VLDL. The disappearance velocities of both lipoprotein species were pharmacokinetically different.

SUBJECTS AND METHODS

Subjects

Studies were performed in ten healthy Caucasian volunteers (eight women, two men), ranging in age from 18 to 25 years. All participants were normolipidemic and had no medical history. Their body mass indices (21.4 ± 2.8 (mean $\pm$ SD) kg/m^2) and waist–hip ratios (men: 0.81 ± 0.00; women 0.76 ± 0.03) were all within normal range. One woman used oral contraceptives; two participants were current smokers. None had the E2/E2 phenotype. Each volunteer received a heparin injection, as described below. Five subjects participated in control experiments, receiving a sham injection (NaCl 0.9%, 0.01 mL/kg body weight), in a single-blind cross-over design. This study was approved by the ethical committee of the Academic Hospital, Utrecht. Subjects were recruited by local advertisement. All subjects gave written informed consent prior to the study.

Study design

The protocol for the heparin test has been described previously (21). Subjects had been fasting for 12 h and refrained from smoking and alcohol in the 2 days preceding the test. Heparin (Heparine Leo, Leo Pharmaceutical Products BV, Weesp, The Netherlands) was given as a bolus injection in a dose of 50 U/kg body weight via an indwelling catheter in an antecubital vein. Control experiments were performed on average 14 days before or after the heparin test. Blood was drawn from the contralateral antecubital vein in precooled tubes and these were put instantly into ice water. Plasma was prepared by centrifugation at 4°C (3000 RPM, 15 min) and maintained at this temperature. Average recoveries of triglyceride and cholesterol in all lipoprotein fractions compared to plasma were $84 \pm 8\%$ and $101 \pm 5\%$, respectively, throughout the preparation procedures. To study whether heparin administration resulted not only in the release of endothelium-bound lipases, but also in the (simultaneous) release of endothelium-bound lipoproteins, blood was collected after 3 min. This early time point was chosen to avoid the counteracting effects of enhanced plasma lipolysis at later time points. To evaluate the effects of lipolysis on apoB-48 and apoB-100 containing lipoproteins, blood was obtained 20 min postheparin. These blood samples were collected in EDTA-containing tubes (Venoject, 0.047 mmol EDTA/10 mL tube). No lipase inhibitor was used at any timepoint. To determine lipase activities and mass, blood was also drawn after 20 min in heparinized tubes (Vacutainer, lithium heparin 143 U/10 mL tube). To prevent scission of apoB, we administered aprotinin to a final concentration of 100 IU/mL.

Postheparin lipase assays

Lipoprotein lipase and hepatic lipase activities were measured by the method of Hüttünen et al. (22) using an anti-hepatic lipase antibody. Results are expressed as nmol of released free fatty acids min^{-1} (mU) per mL plasma. Lipoprotein lipase mass was measured in a commercially available one-step sandwich immuno assay using two distinct monoclonal antibodies (Markit-F LPL, Dainippon Pharmaceutical Co., Osaka) (23, 24).

Preparation of lipoproteins

Lipoprotein fractionation was done by density gradient ultracentrifugation according to Redgrave, Roberts, and West (25). Preparation of lipoprotein fractions was carried out on the test day.

Quantitation of apoB-48 and apoB-100

A modification of the method as described by Karpe and Hamsten (19) was used. Portions of 600 μl VLDL or IDL fractions were delipidated in a methanol–diethyl ether solvent system (19). The precipitates were dissolved in 60 μl sample buffer (2% SDS, 12.5% glycerol, 0.15 m sodium phosphate, 5% β-mercapto-ethanol, and 0.005% bromophenol blue) for 30 min, and

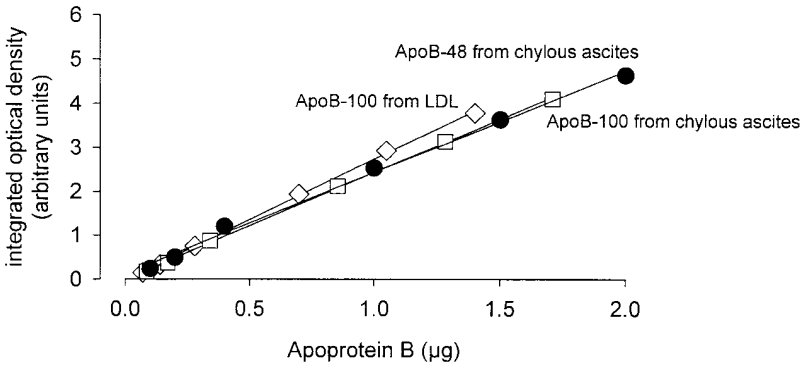

Fig. 1. Standard curves of human apoB-48 (●) and apoB-100 (□) containing chylous ascites isolated from d < 1.006 g/mL fractions and apoB-100 from human LDL (◇). Chylous apoB-48: y = 2.31* × −0.12 (r^2 = 0.996); chylous apoB-100: y = 2.44* × −0.01 (r^2 = 0.999); LDL apoB-100; y = 2.77* × −0.03 (r^2 = 0.999).

subsequently denatured for 10 min at 80°C. Portions of delipidated protein samples were used to quantify apoB-48; 10- or 20-fold diluted samples were used to determine apoB-100 concentrations. Aliquots for apoB determination were stored at −20°C and assayed within a week. Gel electrophoresis was carried out using 5% SDS-polyacrylamide gels with a 3% stacking in a Mini-Protean II (Bio-Rad Laboratories, Richmond, CA) vertical gel apparatus. Gels were pre-run at 40 V for 20 min. Twenty μl of dissolved protein/sample buffer mixture was applied to each lane. Electrophoresis was carried out at 40 V for 30 min followed by 80 V for approximately 90 min. Gels were fixed and stained overnight using a Coomassie G-250 staining kit (Novex, San Diego, CA). Destaining was achieved with at least four changes of distilled water. To assess the equality of chromogenicities of apoB-48 and apoB-100, standard curves of human chylous ascites and LDL, with known absolute amounts of protein (kindly determined by Dr. F. Karpe) were made (**Fig. 1**). In three experiments, the mean and SD for the ratio of apoB-48 slope/apoB-100 slope in chylous ascites was 0.95 ± 0.03, confirming the equality of chromogenicities of both apoB species found by others (19, 20, 26). In our assay, standard curves were straight lines up to 2.0 μg of apoB-48 or apoB-100 protein. For measurement of study samples, standard curves were made form LDL apoB-100, isolated by ultracentrifugation (1.030 < d < 1.045 g/mL). A standard curve ranging from 0.10 to 2.0 μg apoB-100 protein was applied to six lanes of the gel (**Fig. 2**). all apoB-100 was shown to enter the gel. Protein content of the LDL standard was measured by the assay of Lowry et al. (27) using bovine serum albumin as a standard. Determination of the purity of the LDL standard showed that <5% of the Coomassie staining was outside the apoB-100 band while no additional bands were

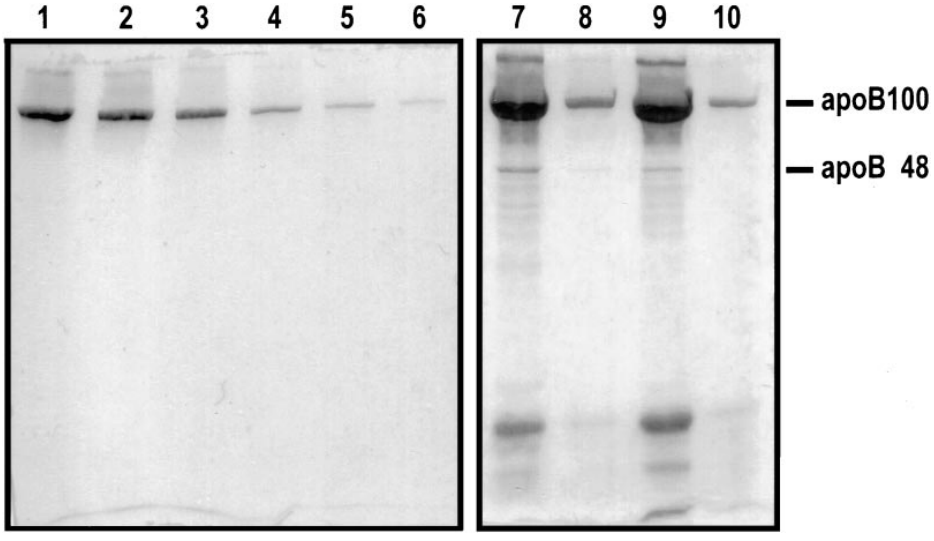

Fig. 2. Analytical SDS-PAGE on 5% homogeneous slab gel after Coomassie brilliant blue staining as described in Methods. Left panel (lanes 1–6): a standard curve of human LDL (1.030 < d < 1.045 g/mL) ranging from 2.0 to 0.1 μg apoB-100 protein. Right panel: typical bands for apoB-48 (lanes 7 and 9) and apoB-100 (lanes 8 and 10) determination.

seen. The albumin contamination of the LDL standard was estimated to be 2.5% (determined by F. Karpe). Gels were scanned on the IBAS image analysis system (Zeiss/Kontron, Echting Germany), using a Panasonic CCD camera (WV-CD 50 Eching, Germany) with an orange filter for contrast enhancement. After digitalization of the gels, each individual band was delineated and the original image was corrected for background color in each separate lane. Output was measured as integrated optical density (IOD) in arbitrary units (U) and was a function of the size and the absorbance of the apoB-48 or apoB-100 band. To construct standard curves, the chromogenicities of LDL standard with known amounts of protein were evaluated by simple linear regression analysis. All standard curves were straight lines, passed through the origin and had r^2 values exceeding 0.99. The interassay coefficient of variation (CV) of the SDS-PAGE step was 8% for quantification of both apoB-48 and apoB-100. The combined CVs of ultracentrifugation, delipidation and SDS-PAGE in $d < 1.006$ g/mL or $1.006 < d < 1.019$ g/mL fractions were less than 15% for both apoB-48 and apoB-100. The plasma lower limits of detection for apoB-48 and apoB-100 were 0.2 mg/L. Ratios of apoB-48 and apoB-100 are expressed on a molar basis (28).

Other methods

Plasma and lipoprotein fractions were assayed for cholesterol and triglycerides with commercial enzymatic reagents. In $d < 1.063$ g/mL fractions, protein quantitation was done by the method of Lowry et al. (27) and phospholipid analysis by means of a commercial assay (Phospholipids B, Wako Chemicals, GmbH, Germany). Plasma apoB and apoA-I were determined by immunonephelometry (Behringwerke AG, Marburg, Germany) and standardized according to the International Federation for Clinical Chemistry. ApoE genotype was determined as described (29).

Statistical and pharmacokinetic analyses

Heparin-induced changes are expressed as absolute differences between two concentrations or as percentual changes from baseline concentrations. Data were subjected to a repeated measures ANOVA to determine significant changes in time. Paired *t*-tests were used to compare the significance of mean differences. Multiple and simple linear regression analysis was used to calculate the strength of the associations. It was assumed that heparin injection resulted in the instantaneous release of LPL or HL and in their immediate and homogeneous distribution throughout the body.

Disappearance velocities were calculated as the decrease in substrate concentrations per min. Apparent first-order constants (k_e) and half-life times ($\ln 2/k_e$) were calculated according to Berr et al. (16), based on baseline and 20-min postheparin concentrations. Statistical analyses were done with SPSS 6.1 for Windows.

RESULTS

Postheparin release of LPL and HL

Heparin injection in 10 participants released 113.4 ± 36.4 mU/mL lipoprotein lipase activity (normal range: 57–217) and 280.3 ± 100.8 mU/mL hepatic lipase activity (normal range: 116–536) after 20 min. Preheparin lipase activity was very low (<3 mU/mL). Similar LPL and HL activities were found after saline injection (1.6 ± 1.5 and 2.8 ± 0.8 mU/mL, respectively). Pre- and postheparin LPL masses were determined in a subgroup of six participants. At baseline, 58.3 ± 43.5 ng/mL LPL mass was detected, and after 20 min 323.0 ± 90.6 ng/mL LPL mass. There was a significant correlation between released LPL mass and activity in postheparin plasma ($r = 0.88$, $P = 0.020$). The specific activity of the released LPL was 0.49 ± 0.08 mU/ng).

Fasting and apoB concentrations and postheparin lipase activities

In the fasting state, 5.3 ± 2.3% of the lipoprotein particles present in the $d < 1.006$ g/mL fractions were apoB-48-containing lipoproteins (chylomicrons). In $1.006 < d < 1.019$ g/mL fractions this was similar: 4.3 ± 2.8% (chylomicron-remnants). ApoB-48 and apoB-100 concentrations in $d < 1.006$ g/mL showed a strong relationship ($r = 0.76$, $P < 0.001$), but not in $1.006 < d < 1.019$ g/mL fractions. Very strong associations existed in the $1.006 < d < 1.019$ g/mL fractions between apoB-100 and cholesterol ($r = 0.96$, $P < 0.001$). Individual fasting levels of apoB-48 and apoB-100 in the two density fractions were not related to postheparin lipase activities. In contrast, hepatic lipase activity was positively associated with the ratio of apoB-100 concentrations of $d < 1.006$ g/mL to $1.006 < d < 1.019$ g/mL fractions ($r = 0.69$, $P < 0.027$) and the ratio of apoB-48 to apoB-100 in $d < 1.019$ g/mL fractions ($r = 0.66$, $P = 0.038$).

Postheparin release of lipoprotein particles

No conclusive pattern was observed in plasma apoB or apoA-I (data not shown) nor in any plasma lipid or lipoprotein constituent (**Table 1**), indicative of lipoprotein particle release. Lipoprotein constituents in $d < 1.019$ g/mL fractions invariably showed a decrease in 3- and 20-min postheparin samples.

Postheparin responses in d < 1.006 and 1.006 < d < 1.019 g/mL fractions

Table 1 shows concentrations of d < 1.006 and 1.006 < d < 1.019 g/mL constituents during the heparin and the saline tests. Responses to sham injections were not significantly different from baseline in any fraction. In response to heparin administration, plasma TG levels decreased from 1.02 to 0.78 mmol/L after 20 min ($P < 0.001$). This decrease was fully accounted for by a TG decrease in d < 1.006 g/mL fractions from 0.50 to 0.24 mmol/L ($P < 0.001$) and a simultaneous TG increase in 1.006 < d < 1.019 g/mL fractions from 0.08 to 0.09 mmol/L ($P = 0.019$). As shown in **Fig. 3**, apoB-48 in d < 1.006 g/mL fractions decreased significantly 20 min postheparin to 38.0 ± 16.4% of the initial concentration ($P < 0.001$). Unexpectedly, apoB-48 concentrations in 1.006 < d < 1.019 mg/L fractions remained constant and did not show any increments. ApoB-100 concentrations showed a trend to decrease in d < 1.006 g/mL fractions and to increase in 1.006 < d < 1.019 g/mL fractions. Heparin administration, therefore, resulted in preferential disappearance of apoB-48 compared to apoB-100. The amount of apoB-48-containing lipoproteins (i.e., molecules apoB-48 in d < 1.019 g/mL fractions) expressed as percentage of hepatic VLDL particles (molecules apoB-100 in d < 1.019 g/mL fractions) fell from 5.1% at baseline to 4.9% after 3 min and 3.0% after 20 min ($P < 0.001$).

Compositional changes after heparin injection

The effect of heparin-induced lipolysis on the composition of the lipoprotein particles in d < 1.006 g/mL fractions was measured as the amount of lipoprotein constituents per apoB (apoB-48 + apoB-100). We observed (**Fig. 4**) that heparin administration caused a depletion of triglycerides ($P < 0.001$) and phospholipids ($P < 0.007$) per lipoprotein particle. No significant change was seen in the cholesterol content per particle in d < 1.006 g/mL fractions.

Elimination of apoB-48 and apoB-100

To obtain more insight into the process of preferential elimination of apoB-48 over apoB-100, the percent changes in both apoB species were compared. LPL activities were markedly associated to the individual percentual disappearance of both apoB-48 ($r = 0.81$, $P < 0.004$) and apoB-100 ($r = 0.91$, $P < 0.001$) in d < 1.006 g/mL fractions after 20 min (**Fig. 5**). The effects of sham injection reflect the background changes associated with the i.v. procedure (triangular symbols). To induce a 50% reduction in apoB-48 from baseline concentrations, an estimated 80 mU/mL (Range 70–110 mU/mL) released LPL activity was required. In contrast, the heparin-induced percentual disappearance curve of apoB-100 was shifted to the right and had a higher slope. Approximately 130 mU/mL LPL activity was needed to induce a 50% reduction in fasting apoB-100 concentrations at 20 min. Therefore, when little LPL was released (LPL activity < 120 mU/mL), apoB-48 was preferentially eliminated over apoB-100. However, when abundant LPL was released (LPL activity > 140 mU/mL) by heparin injection, comparable reductions in apoB-48 and apoB-100 were observed at 20 min. It should be noted that two subjects showed an increase in apoB-100 concen-

TABLE 1. Composition of d < 1.006 g/mL and 1.006 < d < 1.019 g/mL density gradient fractions before and after heparin or saline administration

	Heparin (n = 10) d < 1.006 g/mL			Saline (n = 5) d < 1.006 g/mL		
	baseline	3 min	20 min	baseline	3 min	20 min
Triglycerides (mmol/L)	0.50 ± 0.26	0.41 ± 0.23[ac]	0.22 ± 0.19[ac/bc]	0.32 ± 0.20	0.33 ± 0.21	0.33 ± 0.23
Cholesterol (mmol/L)	0.17 ± 0.09	0.15 ± 0.09[ad]	0.12 ± 0.08[ad/be]	0.12 ± 0.07	0.12 ± 0.08	0.12 ± 0.07
Phospholipids (mmol/L)	0.12 ± 0.10	0.10 ± 0.09[ad]	0.06 ± 0.07[ae/be]	0.10 ± 0.07	0.11 ± 0.08	0.10 ± 0.07
Total protein (mg/L)	100 ± 50	90 ± 50[ad]	50 ± 60[ad/be]	80 ± 40	80 ± 30	70 ± 30
ApoB-48 (mg/L)	1.16 ± 0.66	1.04 ± 0.82	0.47 ± 0.34[ac/be]	0.57 ± 0.31	0.48 ± 0.19	0.43 ± 0.24
ApoB-100 (mg/L)	40.7 ± 21.2	37.2 ± 22.0	30.5 ± 23.4	31.8 ± 11.8	33.3 ± 14.5	27.5 ± 9.9
	1.006 < d < 1.019 g/mL			1.006 < d < 1.019 g/mL		
	baseline	3 min	20 min	baseline	3 min	20 min
Triglycerides (mmol/L)	0.08 ± 0.05	0.08 ± 0.05	0.09 ± 0.05[ae/be]	0.07 ± 0.02	0.07 ± 0.03	0.08 ± 0.03
Cholesterol (mmol/L)	0.16 ± 0.11	0.16 ± 0.11	0.19 ± 0.13[be]	0.15 ± 0.09	0.14 ± 0.06	0.13 ± 0.05
Phospholipids (mmol/L)	0.02 ± 0.03	0.02 ± 0.03	0.03 ± 0.04[ae/bd]	0.06 ± 0.03	0.06 ± 0.02	0.06 ± 0.02
Total protein (mg/L)	70 ± 50	60 ± 50	70 ± 50	60 ± 40	70 ± 30	70 ± 20
ApoB-48 (mg/L)	0.54 ± 0.15	0.55 ± 0.22	0.52 ± 0.33	0.66 ± 0.41	0.59 ± 0.29	0.62 ± 0.32
ApoB-100 (mg/L)	41.0 ± 39.0	40.0 ± 40.4	46.4 ± 36.2	37.2 ± 27.3	30.5 ± 21.0	39.6 ± 18.5

Data are means ± standard deviation.
[a]Significantly different from baseline, or 3-min concentrations[b], [c]$P < 0.001$, and [d]$P < 0.005$, and [e]$P < 0.05$.

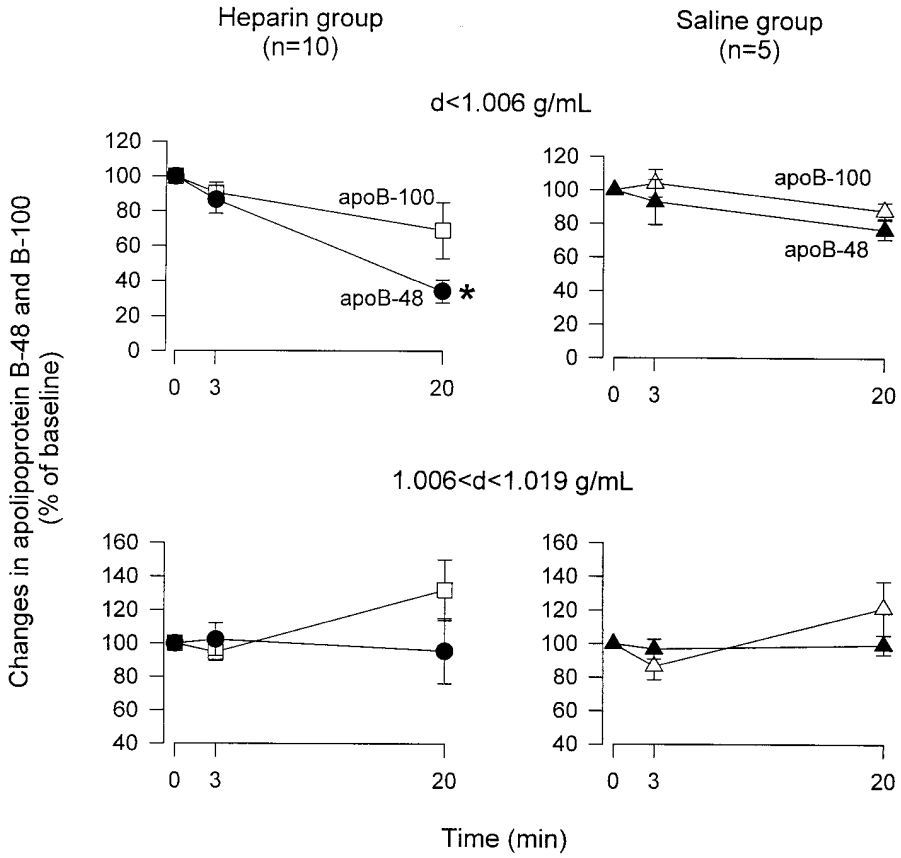

Fig. 3. Percentual changes from baseline of apoB-48 (●/▲) and apoB-100 (□/△) in response to heparin (left panels) and saline injection (right panels) in d < 1.006 g/mL fractions (upper panels), and in 1.006 < d < 1.019 g/mL fractions (lower panels). Data are mean ± SEM. Triangular symbols are controls. *, significantly different from baseline.

trations in d < 1.006 g/mL to approximately 135% of baseline values.

Pharmacokinetic analysis

Disappearance velocities of both apoB-48 and apoB-100 in d < 1.006 g/mL were calculated for each individual. ApoB-100 disappearance velocities ($V_{apoB\text{-}100}$) in d < 1.006 g/mL fractions were associated with LPL activity ($r = 0.80$, $P = 0.005$) (**Fig. 6**), but not with fasting apoB-100 concentrations. Multiple regression analysis revealed that, in addition to LPL activity, the presence of triglyceride-depleted apoB particles (corresponding to small, dense VLDL) at baseline contributed significantly to the disappearance velocity of apoB-100 in d < 1.006 g/mL fractions (**Table 2**). In contrast, apoB-48 disappearance velocities ($V_{apoB\text{-}48}$) in d < 1.006 g/mL were highly related to fasting apoB-48 concentrations ($r = 0.91$, $P < 0.001$) (Fig. 6). In multiple regression analysis (Table 2), LPL activity was only of borderline significance, in addition to fasting apoB-48 concentrations, to account for the apoB-48 disappearance velocities from d < 1.006 g/mL fractions. The apparent first-order rate constants (and therefore half-life times) of apoB-48 ($K_{e,\ apoB\text{-}48}$) were related to LPL activities ($r = 0.82$, $P = 0.004$) (**Fig. 7**). Heparin-induced half-life times of apoB-48 in d < 1.006 g/mL fractions were 15.5 ± 6.0 min. Half-life times of apoB-48 in d < 1.019 g/mL fractions were 26.4 ± 12.7 min. Triglycerides in d < 1.006 g/mL fractions followed essentially the same pattern as apoB-48. Disappearance velocities (V_{TG}) were related to fasting triglyceride concentrations ($r = 0.69$, $P = 0.027$) and the apparent first-order rate constants ($k_{e,TG}$) were associated with LPL activity ($r = 0.81$, $P = 0.005$).

HL activity was associated with apoB-100 changes in the 1.006 < d < 1.019 g/mL fractions, illustrated by the strong relations of HL activity with the appearance velocities ($r = 0.85$, $P = 0.002$) and the percentual increase ($r = 0.80$, $P = 0.005$) of apoB-100 in 1.006 < d < 1.019 g/mL fractions.

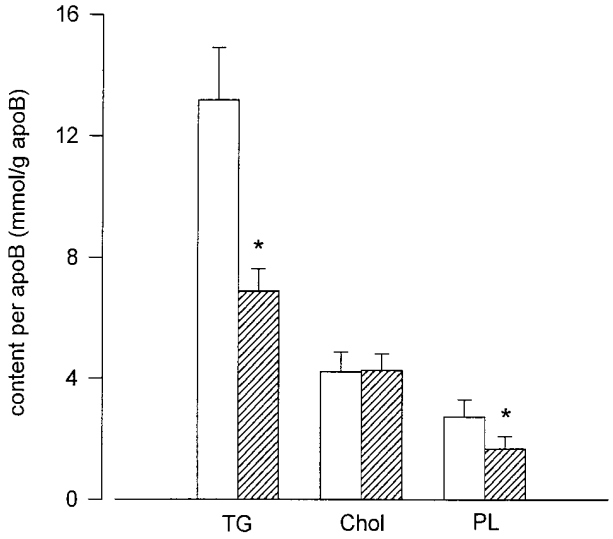

Fig. 4. Composition of lipoprotein particles in d < 1.006 g/mL fractions at baseline (open bars) and 20 min after heparin injection (hatched bars). Triglycerides (TG), cholesterol (Chol), and phospholipids (PL) are expressed in mmol/g apoB. Data are mean ± SEM. *, significantly different from baseline.

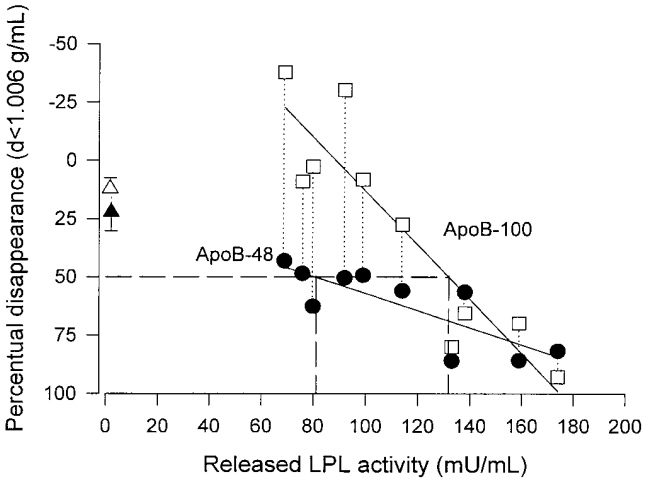

Fig. 5. Percentual disappearance curves after heparin i.v. and saline administration for apoB-48 (●) and apoB-100 (□) in d < 1.006 g/mL fractions. Triangular symbols show results obtained with saline injection (▲, apoB-48; △, apoB-100). The dotted lines join apoB-48 and apoB-100 percentual reductions from one individual. Note that approximately 80 (range 70–110) mU/mL LPL activity results in a 50% reduction of apoB-48, whereas approximately 130 mU/mL LPL activity is necessary to induce a quantitatively similar effect in apoB-100.

DISCUSSION

We undertook this investigation to study the 'common lipolytic pathway.' It is widely accepted that chylomicrons and hepatic VLDL compete for the same enzyme, lipoprotein lipase, but it remains unclear how these particles compete with each other and which factors influence this first catabolic step (30). The common lipolytic pathway, proposed by Brunzell and coworkers (4), is clearly manifested in the postprandial state, when there is competition between chylomicrons and hepatic VLDL. In this situation, it is likely that accessibility of lipoprotein particles to lipase action is a limiting factor for TRL catabolism (30). In the present experiments we created an opposite situation, in which lipase activity was available in excess and where competition between particles was at a minimum. This allowed us to study a state in which lipoprotein access to lipase action was not rate-limiting. The experiments were short-term in order to rule out any confounding effects of enhanced VLDL secretion induced by the experimental design. Direct quantitative measurement of apoB-48 and apoB-100 as indicator for whole particle catabolism provides reliable information without the disadvantages of other external labels (31, 32).

TABLE 2. Multiple regression analysis of contributing factors to disappearance velocities of apoB-48, apoB-100 and triglyceride in d < 1.006 g/mL fractions

	Disappearance Velocities			
	Variable	R^2 (%)[a]	β[b]	*P* Value[c]
ApoB-48	apoB-48 conc	0.83	0.95	<0.001
	LPL activity	0.90	0.27	0.06
ApoB-100	LPL activity	0.65	0.69	0.005
	TG/apoB conc	0.91	−0.53	0.003
Triglycerides	TG conc	0.48	0.88	0.003
	LPL activity	0.77	0.57	0.021

Abbreviations: conc, concentration; TG, triglycerides; LPL, lipoprotein lipase.
[a]Cumulative R^2.
[b]Standardized partial regression coefficient.
[c]*P* value of variable.

Fasting apoB-48-containing particles with a buoyant density < 1.006 g/mL are generally found in fasting plasma (1–3). Cohn and coworkers (2) found that approximately 25% of the fasting plasma triglycerides was contained in apoB-48 TRL. Fasting apoB-48-containing particles in d < 1.006 g/mL are not full blown chylomicrons resulting from fat feeding, but represent intestinally derived particles loaded with absorbed biliary lipid and lipid derived from sloughed enterocytes (33). We observed after heparin i.v. administration that apoB-48-containing lipoproteins preferentially disappeared from d < 1.006 g/mL fractions relative to hepatic VLDL. This provided direct evidence that intestinally derived lipoprotein particles are the preferred substrate of LPL, even in the fasting state. In postprandial studies, several investigators (1, 3) have reached

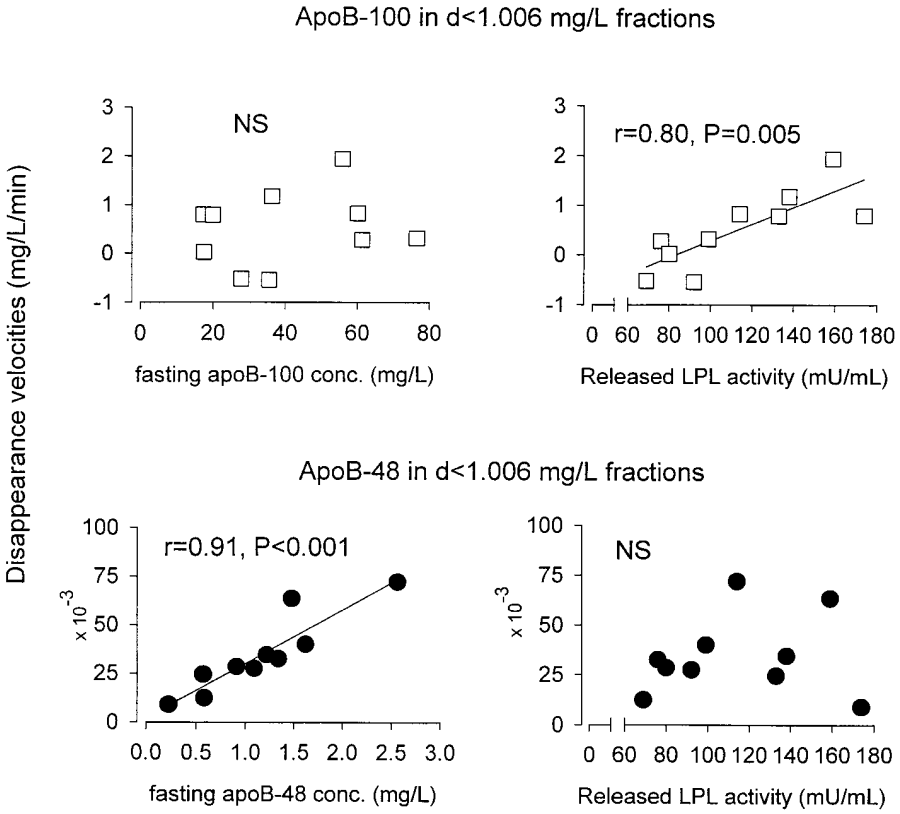

Fig. 6. Scatterplots of disappearance velocity rates from d < 1.006 g/mL fractions for apoB-100 (□) (upper panels) and apoB-48 (●) (lower panels) versus fasting apoB-48/100 concentrations (left panels) and LPL activity (right panels). NS, non-significant.

similar conclusions because of temporal relationships between apoB-48 and apoB-100 or on the basis of disturbed precursor–product relationships of apoB-100. Thus, in the fasting state as well as in the postprandial state, apoB-48-containing lipoproteins effectively eliminate hepatic VLDL. ApoB-48 disappearance in d < 1.006 g/mL fractions was not accompanied by a simultaneous increase in 1.006 < d < 1.019 g/mL fractions, as was the case with apoB-100. This is remarkable as lipolysis after heparin injection shifted from the endothelium to the plasma compartment and lipolytic products with higher densities might have been detected. This lack of precursor–product relationship has also been observed in another study (32). These combined findings suggest immediate uptake of LPL–chylomicron complexes. ApoB-100, in contrast to apoB-48, showed an entirely different response to heparin administration. We observed a trend to decrease in d < 1.006 g/mL fractions and to increase in 1.006 < d < 1.019 g/mL fractions, resulting in virtually unchanged concentrations in the combined density fractions. These processes were related to LPL and HL activity, respectively. The control group had lower baseline concentrations in the d < 1.006 g/mL fractions. This is most likely due to biological variation, because the study was performed in a single-blind cross-over design.

We found that apoB-48-containing lipoproteins disappeared from d < 1.006 g/mL fractions in a concentration-dependent way and its first-order elimination constant (k_e) was a function of LPL activity. Similar findings have been reported by Berr and Kern (34), using autologous reinfusion of retinyl palmitate-labeled

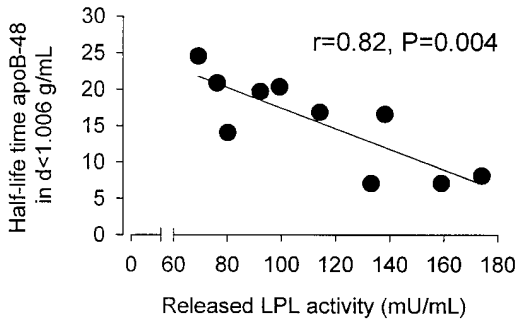

Fig. 7. Scatterplot of LPL activity versus half-life times ($T_{1/2}$, in min) of apoB-48 (●) in d < 1.006 g/mL fractions.

chylomicrons. In contrast, hepatic VLDL disappearance from d < 1.006 g/mL fractions was not a concentration-dependent process, but rather a zero-order process limited by LPL activity. The preferential clearance of apoB-48-containing lipoproteins and the Janus-faced properties of LPL (first-order vs. zero-order kinetics) are likely to be brought about by different Michaelis-Menten constants (K_m) for chylomicrons and hepatic VLDL. It seems likely that LPL has different affinities for both lipoprotein species. Vilella and colleagues (35) showed that LPL activity and mass was associated with LDL and HDL. Electron microscopy of immunostained fractions revealed reaction for LPL and apoB or apoA-I on the same particles. Based on these findings, they argued that there was a frequency of 1 LPL homodimer per 800 LDL particles in postheparin plasma (assuming half of the LPL molecules were on LDL). Zabon and coworkers (36) showed that dimeric postheparin lipoprotein lipase was bound to triglyceride-rich particles and that differences with previous studies resulted from the use of a lipase inhibitor. It was found that the postheparin LPL-dimer/apoB molar ratio in the d < 1.006 g/mL was 1 on 100 to 500 VLDL particles. Based on the latter finding, that dimeric LPL is only bound to TRL in d < 1.006, we extended these calculations under two extreme conditions, namely that the affinity of postheparin dimeric LPL is far greater for apoB-48 TRL than for apoB-100 TRL or that there is an equal affinity of LPL for apoB-48 and apoB-100 TRL. In the first situation, ($K_{m,\text{apoB-48}} >> K_{m,\text{apoB-100}}$), one LPL-dimer is available for every 3 (range: 0.3–5) chylomicrons (apoB-48 in d < 1.006 g/mL). In the second situation ($K_{m,\text{apoB-48}} = K_{m,\text{apoB-100}}$), approximately 50 (range: 10–110) triglyceride-rich lipoproteins (apoB-48 and apoB-100 in d < 1.006 g/mL) compete for one released LPL-dimer. Differences in molar ratio estimations may result from differences in identified lipoprotein species and differences between groups with conceivably different LPL mass and VLDL particle concentrations. It should be noted that calculated ranges of Zambon et al. (36) and ours overlap.

The fact that LPL activity was related to the percentual disappearance of both apoB-48 and apoB-100 in d < 1.006 g/mL fractions was of considerable interest. At low levels of LPL activity (but within the normal range), only apoB-48 disappeared. At higher levels of LPL activity an equal removal was observed in apoB-48 and apoB-100. It seems plausible that the presence of apoB-48, by binding most of the LPL, hampers apoB-100 catabolism. However, this impediment can be overcome when abundant LPL is present. Alternatively, apoB-48 clearance may be relatively insensitive to LPL levels. Furthermore, the observed differences between apoB-48 and apoB-100 may reflect differences in particle size, rather than unique attributes of apoB-48 versus apoB-100. It is likely that fasting apoB-48-containing particles are larger than apoB-100-containing particles. The data imply that heterozygous LPL deficiency carriers and other patient groups with low LPL activity (37), are expected to show abnormalities in apoB-100 removal, but to a much lesser extent in apoB-48 removal. These abnormalities can be further aggravated in hypersecretion states, such as FCH and obesity. Homozygous LPL-deficiency is known to completely block catabolism of both apoB-48 and apoB-100 (38).

Multiple regression analysis revealed that disappearance velocities of apoB-100 in d < 1.006 g/mL fractions were not only related to LPL activity but also to a low triglyceride content per lipoprotein particle (small, dense VLDL). This confirms the importance of particle composition. In contrast to the in vitro studies of Connelly et al. (39), we did not find any evidence that particle number was of primary importance for triglyceride elimination velocities.

We found that heparin-induced half-life times of apoB-48-containing lipoproteins in d < 1.006 g/mL fractions averaged 15.5 ± 6.0 min. Berr et al. (16) demonstrated that plasma chylomicron half-life times were not affected by enhanced plasma lipolytic activity. Estimated half-life times in these experiments were nearly 12 min.

Ex vivo lipolysis was counteracted by immediate cooling of all blood samples and by preparation of lipoprotein fractions on the test day. LPL binding to lipoproteins is known to be disrupted by high salt concentrations and centrifugal forces (40). Recently, Zambon et al. (36) showed that ex vivo lipolysis in postheparin whole plasma was extensive when no LPL inhibitor was used. Thus, it is possible that part of the observed phenomena could be due to ex vivo changes. However, we feel that the ex vivo changes are limited, because by 3 min postheparin more than 75% of the lipase activity is already present in the plasma (unpublished results, A. P. Van Beek, F. C. De Ruijter-Heijstek, H. Jansen, D. W. Erkelens, and T. W. A. De Bruin), but the lipase-induced changes in triglyceride and apoB-48 are at that moment only 25% of those observed 20 min postheparin. Did we observe apoB-48 clearance from blood or disappearance from the d < 1.006 fractions to other density fractions? Due to the currently used methodology, it is not possible to measure apoB-48 concentrations in whole plasma or in density fractions other than d < 1.019 g/mL. Literature supports the idea of direct removal of chylomicrons, without the transition into denser fractions (32). Recently, it was shown that not only is the formation of large chylomicrons into chylomicron remnants limited, but also that large chylomicrons/chylomicron remnants are more avidly bound to

the vascular endothelium than smaller lipoprotein particles (41).

The apoB-48 and apoB-100 concentrations in d $<$ 1.019 g/mL fractions in our study group were 1.70 $\pm$ 0.74 and 81.7 $\pm$ 56.1 mg/L, respectively. These values are in agreement with fasting levels found by Schneeman et al. (3), Kotite, Bergeron, and Havel (20), and Karpe et al. (32). ApoB-48 and apoB-100 were strongly associated (r = 0.76, P = 0.001) in d $<$ 1.006 g/mL, most probably reflecting competition, even in the fasting state. In contrast, apoB-48 and apoB-100 in 1.006 $<$ d $<$ 1.019 g/mL were not related, indicating that beyond the level of the common lipolytic pathway, no competition existed in the fasting state.

Postheparin hepatic lipase activity was associated with baseline distributions of apoB-48 and apoB-100 in the d $<$ 1.019 g/mL fractions. This implies an important metabolic role for HL ont only postprandially, but also in the fasting state and is in accord with several studies, showing that HL is important for remodeling and uptake of both chylomicrons and β-VLDL (42, 43).

Our results show that the common lipolytic pathway has a markedly varying capacity to cope with TRL, depending on the LPL activity. Therefore, it is likely that small changes in LPL activity have a major impact on both fasting and postprandial lipid metabolism. Studies in the postprandial state are necessary to gain further insight into the relationship of LPL, chylomicrons and hepatic VLDL.

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