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Effect of heparin-stimulated plasma lipolytic activity on VLDL APO B subclass metabolism in normal subjects

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Abstract

Heparin given intravenously enhances lipolysis, although fasting lipids are not markedly altered in long-term administration. In the present study we investigated heparin-induced acute perturbation of VLDL subclass metabolism. Eight men were examined during a control study and during an 8.5 h infusion of heparin. $^2H_3$-leucine was used as tracer and kinetic constants derived using a non-steady-state model. Heparin infusion increased both plasma lipoprotein and hepatic lipase activity and raised plasma FFAs two-fold ($P < 0.001$). The fractional catabolic rate (FCR) of VLDL1 apo B increased on heparin (25.7 ± 4.2 and 10.8 ± 1.7 pools/d, heparin vs. control, $P < 0.02$). The FCR of VLDL2 apo B increased to 12.6 ± 1.9 pools/d on heparin vs. 8.8 ± 1.1 pools/d during the control (NS). Total VLDL apo B production was not significantly changed (824 ± 45 and 692 ± 91 mg/d, heparin vs. control, NS). We conclude that during heparin infusion, the catabolism of especially large triglyceride-rich VLDL1 apo B is greatly increased. However, although the FFA levels were high during the heparin study, the production of total VLDL apo B did not rise. These findings are consistent with the known action of heparin on lipoprotein lipase but indicate that acute increase in plasma FFA levels does not lead to a rise in VLDL apo B production. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

Heparin, in addition to its anticoagulant properties, has a profound influence on lipid metabolism. Studies conducted 1943 by Hahn et al. showed that alimentary lipemia is abolished following an injection of heparin [1]. This clearance effect is due to enhanced activity of the lipolytic enzymes, lipoprotein lipase (LPL) and hepatic lipase (HL). LPL is synthesized in parenchymal cells and normally bound on endothelial surfaces in arteries and capillaries by a glycosaminoglycan, heparan-sulfate [2]. The main role of LPL is to hydrolyze triglycerides from chylomicrons and VLDL particles to produce free fatty acids for peripheral tissue energy needs. HL is synthesized in the liver and anchored on the sinusoidal surfaces of hepatic endothelial cells with a main role in the catabolism and remodelling of remnant particles generated by LPL. HL participates in the conversion of smaller and more dense VLDL2 particles to IDL and IDL to LDL. HL also hydrolyzes HDL triglycerides and phospholipids [2]. Only low levels of LPL and HL are found free in the circulation. An injection of heparin rapidly increases the level of LPL and HL in plasma over 100-fold and the consequent activation of lipolysis increases rapidly the FFAs in the circulation. This heparin effect has been widely used to study the activity of LPL and HL in different metabolical and pathological states.

The major subclasses of VLDL particles are large triglyceride-rich VLDL1 (Svedberg flotation rate, Sf 60–400) and smaller and more dense VLDL2 (Sf 40–60). Recent studies have shown that the metabolism of

Abbreviations: Apo B, apolipoprotein B; LPL, lipoprotein lipase; HL, hepatic lipase; FFA, free fatty acid; GC–MS, gas chromatography mass spectrometry.

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these subclasses is independently regulated [3,4]. In the fasting state large VLDL1 particles serve as liver's chylomicrons delivering energy for needs of peripheral tissues. In the fed state VLDL1 particles compete for same lipolytic pathways with chylomicrons [5]. Heparin-induced disturbances in the production and catabolism of VLDL subclasses may thus have marked effects on the post-prandial dyslipidemia. Weintraub et al. [6] showed that chronic heparin administration caused an accumulation of chylomicrons in the circulation in healthy men, probably as a result of a marked decrease in serum lipolytic activity after continuous heparin infusion for 4 days. This is of particular importance because postprandial dyslipidemia has been suggested to be an independent risk factor for coronary heart disease [7]. There is, however, at present no information on the effect of heparin on VLDL subclass metabolism.

This study was designed to test the hypothesis that heparin-induced enhanced lipolysis increases the catabolism of VLDL1 or VLDL2 apo B, or both. We also tested whether heparin has an effect on the production of VLDL apo B subclasses. In earlier studies the production of VLDL apo B and triglycerides was increased when heparin was infused together with Intralipid infusion [8]. The authors suggested that the elevation of plasma FFA acutely stimulates VLDL apo B and triglyceride production in vivo in healthy men. The effect of FFA has also been widely studied in in vitro studies, which suggest that increased FFA availability promotes the production of VLDL triglycerides [9–11], the results on apo B being more controversial [10,12–14]. In this study we tested whether heparin-induced increase in plasma FFA has an effect on the production of VLDL subclasses without simultaneous Intralipid infusion. Eight healthy men were studied during an 8.5 h heparin infusion and during a control study. VLDL apo B subclass metabolism was traced with a bolus-injection of stable isotope $^{2}H_{3}$-leucine.

2. Research design and methods

2.1. Subjects

Eight healthy men participated in the study. None of the subjects used any medication. All subjects underwent a history and physical examination and laboratory tests for exclusion of hepatic, renal, thyroid and haematological abnormalities. All subjects had a normal oral glucose tolerance test according to WHO criteria [15]. Apo E2 allele carriers were excluded from the study. Physical and biochemical characteristics of the subjects are shown in Table 1.

The purpose, nature and potential risks of the study was explained to the subjects before their written informed consent was obtained. The study protocol was approved by the Ethical Committee of the Helsinki University Hospital.

2.2. Study design

Subjects participated in random order in two apo B turnover studies (control study and heparin study) with a 1–2-month interval between the studies. The subjects were instructed to ingest an isocaloric, weight-maintaining diet for 1 month before the studies and during the entire study period. They were asked to record their dietary intake for 24 h before the first phase and to replicate this diet before the second. The clinical studies and laboratory measurements were done in Helsinki, while gas chromatography mass spectrometry (GC–MS) analyses were performed in Glasgow.

2.3. Apo B turnover protocol

All subjects were fasted overnight (12 h) and admitted to the metabolic ward at the Helsinki University Central Hospital before each turnover study. An indwelling cannula was inserted in an antecubital vein for infusions. A second cannula was inserted retrogradely

Table 1
Physical and biochemical characteristics of the subjects[a]

Subject	Apo E	Age (years)	Weight (kg)	BMI (kg/m^2)	Body fat (%)	Triglycerides (mmol/l)	Total chol (mmol/l)	HDL chol (mmol/l)
1	3/3	25	76.8	22.9	15.5	1.25	5.03	1.07
2	4/3	31	89.5	25.1	19.7	1.11	5.17	1.03
3	4/4	32	93.9	27.5	27.3	1.31	4.65	0.99
4	4/4	54	89.1	27.2	24.6	1.39	5.62	1.31
5	3/3	35	98.9	28.6	28.1	1.58	5.40	1.14
6	4/4	47	77.5	24.1	20.6	1.16	5.19	1.38
7	3/3	43	77.8	22.3	18.0	1.33	4.48	1.40
8	4/3	52	90.7	28.0	27.9	1.28	4.38	1.25
Mean ± SE		40 ± 4	86.8 ± 3.2	25.7 ± 0.9	22.7 ± 1.8	1.30 ± 0.11	4.99 ± 0.20	1.20 ± 0.06

[a] Figures represent the mean of the fasting measurements

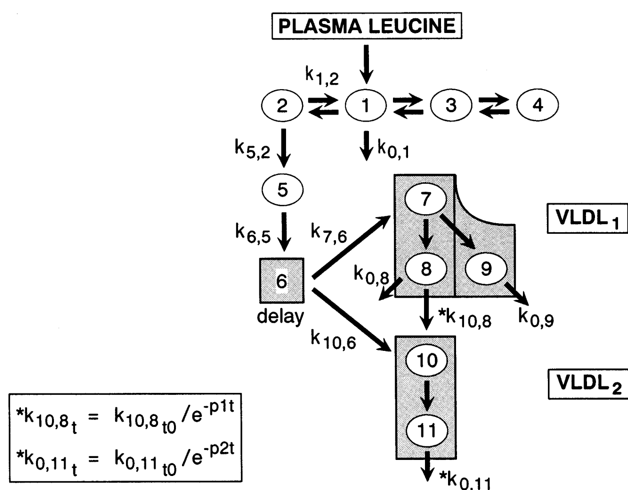

Fig. 1. Multicompartmental model of VLDL1 and VLDL2 apo B metabolism. The equations permitting the increase in $k_{10,8}$ and $k_{0,11}$ with time are given. Exponential functions were used with constant p_1 and p_2 representing the fractional decrease in input per unit time (h). t denotes the value at time t, t_0 the value at zero time.

into a heated hand vein to obtain arterialized venous blood for blood sampling. At 0 min, infusions of saline (100–150 ml/h) (control and heparin study) and heparin (heparin study) were started. Heparin (Heparin, Löwens, Denmark) was started with a 100 IU bolus followed by a continuous infusion of 1000 IU/h. All timings for the turnovers are related to the start (0 min was at 08:30 h) of the infusions. In each turnover, a bolus injection of $^2H_3$-leucine (C/D/N Isotopes Inc., Vaudreuil, Quebec, 7 mg/kg of body weight) dissolved in saline was given at 30 min. Blood samples for the determination of plasma $^2H_3$-leucine concentrations were taken immediately before the bolus injection, and at 32, 34, 36, 38, 40, 42, 45, 50, 60, 75 min and 1.5, 2.5, 3.5, 4.5, 6.5, and 8.5 h. To measure VLDL1 and VLDL2 apo B $^2H_3$-leucine enrichment, blood samples were taken before the tracer bolus administration and at 45, 60, 75, 90, 105, 120, 150 and 180 min and 3.5, 4.5, 5.5, 6.5, 7.5 and 8.5 h. The composition and apo B mass concentration of plasma VLDL1 and VLDL2 fractions were determined three times during the study period at 30 min, 4.5 and 8.5 h. The subjects were fasting until 17:00 h when the last blood sample was withdrawn.

2.4. Isolation of lipoproteins and analysis of leucine in apo B

VLDL1 and VLDL2 were isolated from plasma by cumulative flotation gradient ultracentrifugation using a six-step discontinuous salt gradient as previously described [4,16,17]. Pool sizes for apo B were calculated from the product of plasma volume (assumed to be 4.5% of body weight) times the plasma concentration of apo B in VLDL1 and VLDL2. The leucine content of the apo B pool was calculated from the amino acid composition of apo B [18]. Apo B in isolated lipoprotein fractions was precipitated by isopropanol [4,19]. To determine plasma leucine enrichments, proteins were precipitated using trichloroacetic acid [4].

2.5. Determination of leucine enrichment by GC–MS

Amino acids were converted into tert-butyl-dimethyl-silyl-(TBDMS-) derivatives and enrichments were determined immediately by gas chromatography mass spectrometry (GC–MS) using a quadrupole GC–MS instrument (MD 800, Fisons, Manchester, UK) as described [4]. The method employed for the analysis of $^2H_3$-leucine enrichment in protein hydrosylates and plasma amino acids has been described in detail elsewhere [20].

2.6. Kinetic analysis of VLDL1 and VLDL2 apo B

Tracer/tracee ratios and apo B masses were used to derive kinetic rate constants describing the production and catabolism of VLDL1 and VLDL2 apo B. The data were analyzed with the SAAM II program [21] using multicompartmental model shown in Fig. 1 (Table 2). The model was based on the multicompartmental model for the kinetics of VLDL1 and VLDL2 apo B described in detail in our previous articles [4,22,23]. The model had the following specific features. First, plasma leucine kinetics were described by a four compartment subsystem. The present experimental design did not allow determination of all the rate constants for plasma leucine. $k_{3,4}$, $k_{4,3}$, $k_{3,1}$, $k_{1,3}$ and $k_{2,1}$ were fixed at population mean values of 0.0275, 0.181, 2.528, 0.0469 and 3.012 respectively based on values derived from previous studies on long term (14-day) data in a large group of subjects [20]. Others ($k_{0,1}$, $k_{1,2}$, $k_{5,2}$) were allowed to vary to adjust the plasma leucine curve between individuals. $k_{5,2}$ denoted transfer of tracer from plasma to the apo B synthetic compartment. Compartment 5 was added to the model to provide input of tracer mass. $k_{6,5}$ was fixed to 100. Time zero in the model was 50 h before the actual experiment was started to permit masses to achieve steady state. Input of leucine into VLDL1 and VLDL2 apo B occurred from compartment 2 via a delay component (compartment 6). The delay was set at 0.5 h initially but this value was adjusted if required by the data. VLDL1 apo B was modelled as a short delipidation chain and a remnant compartment was included in line with previously published systems [20]. Input of apo B occurred at compartment 7 in VLDL1 and at compartment 10 in VLDL2. VLDL2 apo B was modelled as a short delipidation chain with a single output. To reduce the number of unknowns, a number of dependent constants were defined: $k_{8,7} = k_{10,8}$; $k_{0,9} = k_{9,7}$; $k_{11,10} = k_{0,11}$.

To test the hypothesis whether increased lipolysis could explain the decrease in VLDL apo B concentration we allowed the FCR of VLDL1 and VLDL2 apo B to increase with time. This was done using the following equations:

$$k_{(10,8)_t} = \frac{k_{(10,8)_{t_0}}}{\exp(-p_1 t)}$$

$$k_{(0,11)_t} = \frac{k_{(0,11)_{t_0}}}{\exp(-p_2 t)}$$

with constants p_1 and p_2 representing the fractional increase in rate constants per unit time (h). t denotes the value at time t, t_0 the value at zero time. Using this approach an excellent fit was obtained for both the tracer data and the change in VLDL1 mass. On heparin administration lipolysis in some patients may have been so rapid that it is possible that the VLDL1 released from the liver was converted to VLDL2 before sampling could take place from a peripheral vein. The proportion of this rapidly lipolyzed VLDL1 was not possible to detect using the current methodology. The same model was used for both control and heparin phases.

We also tested whether decreased synthesis could explain the decrease in VLDL concentration. In a previous study [23], in which we studied the effect of insulin and acipimox on VLDL apo B metabolism, both decreased the pool size of VLDL 1 apo B secondary to a fall in VLDL1 apo B production. Five subjects of the present study participated in insulin study of that previous experiment (subjects 3, 5, 6, 7 and 8). Mean tracer masses for $^2H_3$-leucine in VLDL1 and VLDL2 apo B for these subjects during the control, heparin and insulin studies are shown in Fig. 2. On heparin the VLDL1 apo B tracer mass rose almost to the same level as control but the decay was more rapid. On insulin the entire curve was lowered. Thus permitting VLDL1 or VLDL2 apo B production to decrease with time, i.e. application of the model in [4,23] did not result in a satisfactory fit to the observed data in present study with heparin. Although the production rate was not allowed to vary by time it was allowed to vary freely between the turnovers. Because we had to use separate models for the separate experiments (insulin infusion and heparin infusion) we have generated two comparable but not identical sets of rate constants for subjects in the basal state (control study). This is a limitation of the modelling process since we cannot adjust production and delipidation in a single model—it would yield poorly defined constants.

The experimental tracer/tracee ratios were weighted within CONSAM by applying a standard deviation of approximately 4×10^{-5} to the data. This represented a coefficient of variation of about 1% for peak ratios in VLDL1 and VLDL2 apo B $^2H_3$-leucine. A coefficient of variation of 5% was applied to the apo B pool sizes.

2.7. Analytical methods

Plasma glucose concentrations were measured in duplicate using the glucose oxidation method (Beckman Glucose Analyzer II, Beckman Instruments, Fullerton,

Table 2
Computed rate and function constants[a]

Subject	$k_{5,2}$	$k_{7,6}$	$k_{9,7}$	$k_{10,8}$	$k_{0,11}$	p_1	p_2
Control							
1	0.228	0.521	0.103	1.089	0.449	0.000	0.010
2	0.608	0.392	0.001	0.573	0.475	0.021	0.000
3	2.100	0.855	0.005	0.539	1.195	0.000	0.000
4	0.543	0.774	0.000	0.827	0.890	0.000	0.000
5	0.299	0.809	0.000	0.733	0.559	0.000	0.000
6	0.228	0.611	0.048	1.084	0.530	0.044	0.120
7	0.389	0.693	0.496	1.773	0.807	0.000	0.000
8	0.291	0.745	0.000	0.825	0.769	0.000	0.003
Heparin							
1	0.484	0.442	0.586	1.091	0.652	0.062	0.180
2	1.663	0.287	0.052	0.471	0.423	0.101	0.295
3	8.930	0.661	0.268	1.138	0.722	0.000	0.027
4	0.447	0.507	0.439	1.558	0.833	0.007	0.096
5	0.884	0.570	0.000	0.689	0.465	0.123	0.182
6	0.300	0.517	0.571	1.450	0.609	0.101	0.143
7	0.490	0.583	0.490	0.838	0.679	0.000	0.111
8	0.411	0.415	0.000	1.019	0.715	0.066	0.000

[a] p_1 and p_2 are the constants used in exponential functions in Fig. 1. Rate constants $k_{i,j}$ indicate transfer from compartment j to compartment i as pools/d. The indices i,j refer to the numbering of subcompartments as shown in Fig. 1. $k_{8,7} = k_{10,8}$; $k_{0,9} = k_{9,7}$; $k_{0,11} = k_{11,10}$; $k_{10,6} = 1 - k_{7,6}$.

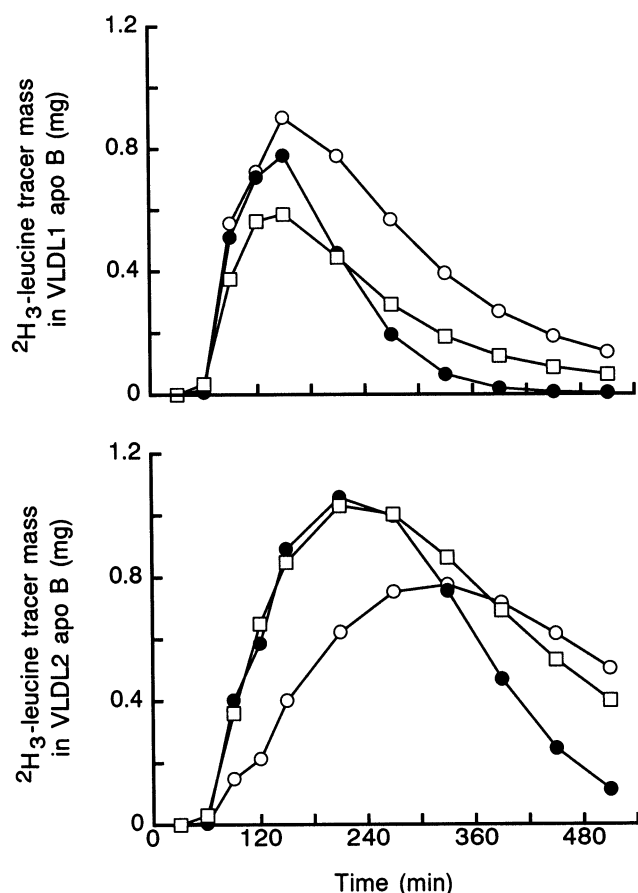

Fig. 2. Mean $^2H_3$-leucine tracer mass (mg) in VLDL1 (upper panel) and VLDL2 (lower panel) apo B during the control study (○), the heparin study (●) and the insulin study (□) for five subjects. Insulin data is from reference [23].

CA) [24]. Serum free insulin concentrations were determined by double antibody radioimmunoassay (Pharmacia Insulin RIA Kit, Pharmacia, Uppsala, Sweden) after precipitation with polyethylene glycol. Concentrations of cholesterol, triglyceride (Hoffman–La Roche kits Nos. 0736805 and 0736642 respectively), phospholipids (Wako Chemicals, Neuss, Germany) and free (non-esterified) cholesterol (Boehringer, Mannheim, Germany) were measured enzymatically in an automated Cobas Mira analyzer (Basel, Switzerland). The concentration of cholesteryl ester was calculated as the difference between total and free cholesterol. Serum FFA were quantified by the fluorometric method of Miles et al. [25]. Serum glycerol was determined by an enzymatic spectrophotometric method [26]. The concentration of apo B and total proteins in isolated lipoprotein fractions was determined according to Kashyap et al. [27]. Plasma LPL and HL were analyzed as previously described [28,29].

2.8. Other measurements

The percent body fat was determined using a single frequency bioelectrical impedance device (Bio-Electrical Impedance Analyzer System, Model # BIA-101A, Mt. Clemens, MI.) [30].

2.9. Statistical analysis

All data are expressed as the mean ± SEM. Statistical comparisons between the study occasions were made with the non-parametric Wilcoxon signed rank test. Two-way analysis of variance (ANOVA) for repeated measures was used to evaluate changes within and between the two turnover studies. Non-normally distributed variables (plasma and VLDL triglycerides) were logarithmically transformed before statistical comparisons. P-values less than 0.05 were considered to be statistically significant. Data analysis was performed using the SYSTAT statistical package (SYSTAT Inc., Evanston, IL).

3. Results

3.1. Insulin and glucose concentration

Serum free insulin averaged during the heparin study from 28 ± 4 to 22 ± 4 pmol/l (NS) and during the control study from 34 ± 5 to 20 ± 4 pmol/l ($P < 0.001$). Plasma glucose concentrations averaged 5.4 ± 0.1 mmol/l during the heparin study and 5.2 ± 0.1 mmol/l during the control study.

3.2. Plasma lipids and lipases

Plasma triglyceride concentrations (Fig. 3) decreased by 27% ($P < 0.01$) over the period of the heparin study ($P < 0.05$ compared with the control study). Plasma cholesterol concentration decreased slightly during the heparin (8%, $P < 0.01$) and control (5%, $P < 0.05$) studies. Plasma FFA (Fig. 3) increased rapidly after the initiation of heparin infusion and averaged 1073 ± 81 μmol/l during the heparin infusion ($P < 0.0001$) compared with the control study where plasma FFA averaged 526 ± 75 μmol/l. Plasma glycerol (Fig. 3) averaged 167 ± 25 μmol/l during the heparin study and 87 ± 10 μmol/l during the control study ($P < 0.05$ heparin vs. control study).

Plasma lipoprotein lipase (LPL) activity increased from 2.2 ± 0.3 to 21.4 ± 2.7 mU/ml ($P < 0.0001$) during the heparin study and from 1.5 ± 0.4 to 2.0 ± 0.1 mU/ml (NS) during the control study. Plasma hepatic lipase (HL) increased from 2.3 ± 0.4 to 123.5 ± 14.4 mU/ml ($P < 0.0001$) during the heparin study and from 1.2 ± 0.4 to 1.9 ± 0.2 mU/ml (NS) during the control study.

3.3. VLDL1 and VLDL2 composition

VLDL1 apo B pool size (Fig. 4) decreased during the heparin study ($P<0.05$) and was 63% lower at the end of the study than in the control study ($P<0.05$). VLDL2 apo B pool size (Fig. 4) was 25% higher after 30 min heparin infusion compared to control study, thereafter the pool sizes were comparable during the heparin and control studies.

Concentration of VLDL1 triglyceride, free cholesterol and phospholipid decreased significantly during the heparin study compared to control study ($P<0.05$) (Table 3). Concentration of components of VLDL2 particle did not change significantly during the studies (Table 3). The percentages of components in VLDL1 and VLDL 2 particles (triglyceride, free cholesterol, cholesteryl ester, phospholipid and protein) remained unchanged during the heparin and control studies (data not shown).

3.4. VLDL apo B production

The production of VLDL1 apo B (Fig. 5) averaged 413 ± 38 mg/d during the heparin study and 505 ± 86 mg/d during the control study (NS). The production of VLDL2 apo B (Fig. 5) increased during the heparin study (412 ± 35 mg/d), compared with the control study (187 ± 20 mg/d, $P<0.02$). We speculate that during the heparin study a proportion of producted VLDL1 particles were rapidly lipolyzed before sampling and thus increased the calculated production rate of VLDL 2 apo B and decreased the calculated production rate of VLDL1 apo B. This is comparable with the observation that the VLDL1 triglyceride content was already reduced at 0.5 h on heparin compared to the control phase (Table 3). The study design did not allow determination of the proportion of rapid lipolysis. However, total VLDL apo B production (Fig. 5) did not change during the heparin study (824 ± 45 mg/d) compared with the control study (692 ± 91 mg/d, NS).

The amount of VLDL2 apo B derived from VLDL1 apo B increased during the heparin study (from 331 ± 35, at 0.5 h to 400 ± 37 mg/d at 8.5 h, $P<0.02$), but not during the control study (489 ± 89–492 ± 88 mg/d, NS).

3.5. VLDL apo B catabolism

The fractional catabolic rate (FCR) of VLDL1 apo B (Fig. 6) increased markedly during the period of the heparin study (from 11.5 ± 1.4, at 0.5 h to 25.7 ± 4.2 pools/d, at 8.5 h, $P<0.02$) but not during the control study (from 9.6 ± 1.3 to 10.8 ± 1.7 pools/d, NS). At 8.5 h the FCR of VLDL1 apo B on heparin was significantly faster than during the control study ($P<0.02$). The FCR of VLDL2 apo B (Fig. 6) increased during the heparin study (from 7.6 ± 0.6, at 0.5 h to 12.6 ± 1.9 pools/d, at 8.5 h, $P<0.05$), but did not change during the control study (from 8.6 ± 1.1 to 8.8 ± 1.1 pools/d, NS; heparin vs. control phases at 8.5 h, NS).

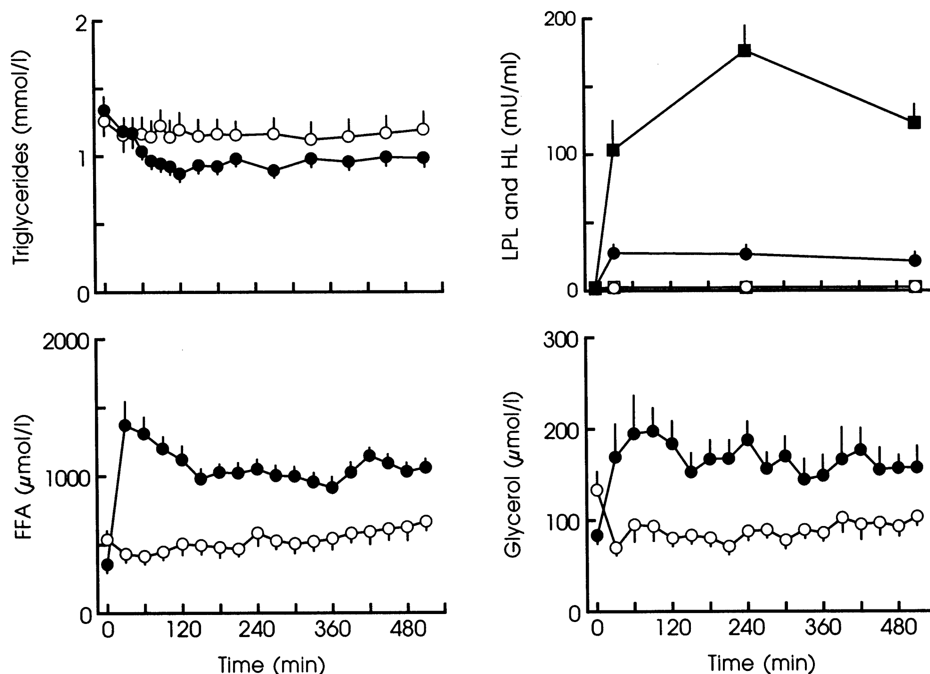

Fig. 3. Plasma concentration of triglycerides, FFA, glycerol and LPL during the control study (○) and the heparin study (●). HL is presented as open squares (the control study) and as closed squares (the heparin study). Bars represent SEM.

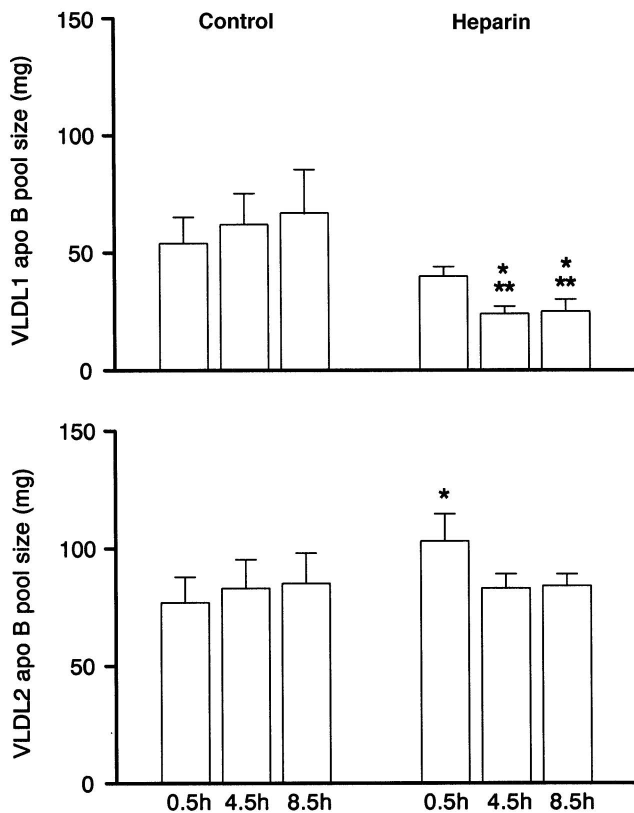

Fig. 4. Pool sizes of VLDL1 and VLDL2 apo B. * $P<0.05$ vs. control value at same time point. ** $P<0.05$ vs. value at 0.5 h.

4. Discussion

This study shows that heparin increases rapidly the catabolism of VLDL1 and VLDL2 apo B simultaneous with a rise in plasma circulating LPL and HL activity. However, although the FFA levels were increased twofold during the heparin study compared with the control study, the production of total VLDL apo B was not significantly increased. Consequently the concentration of plasma triglyceride showed a significant drop.

The catabolism of VLDL1 apo B may have been even higher than we could detect. We suspect that heparin induced lipolysis was so rapid that it is possible that some VLDL1 released from the liver was converted to VLDL2 before sampling could take place from a peripheral vein. Evidence for this is provided by the immediate increase in fatty acid levels within a short period of giving heparin and the reduced triglyceride in VLDL1 at 0.5 h. The alternative explanation that heparin administration actually increases VLDL2 production in the liver is unlikely given the observations in previous studies that altering FFA levels effects VLDL1 but not VLDL2 production [23]. Some rapid lipolysis of VLDL2 may also occur but we are unable to detect it given the current methodology. Heparin had only a modest influence on the production of total VLDL apo B.

LPL serves also as a ligand for the receptors of VLDL [31] and LDL related protein (LRP) [32]. Zambon et al. [33] have shown that in pre- and postheparin plasma samples most of the lipoprotein lipase dimers are associated with VLDL particles, when ex vivo lipolytic activity is inhibited, which supports the hypothesis that, in vivo, lipoprotein lipase may affect the receptor-mediated removal of these particles. It is possible that also changes in remnant particle return to the liver may have affected the production rates of VLDL apo B. VLDL particles and chylomicron remnants have been shown to stimulate VLDL apo B synthesis when added in the culture media [34,35]. Thus it is possible that the catabolism of the particles was increased through two mechanisms, enhanced lipolysis and increased receptor uptake of the particles.

Eight healthy normolipidemic subjects participated in the study. Apo E2 allele carriers were exluded from the study. By chance, an over-representation of apo E4 allele was observed. The differences in the catabolism of E3 and E4 allele carriers have earlier been shown by Demant et al. [36]. However, because of the small number of subjects we were not able to study the possible effect of apo E alleles. For reasons not known, two subjects had serum glycerol values higher in the beginning of the control study compared with the heparin study. However, the FFA concentrations of these subjects were comparable.

FFAs for VLDL-triglyceride synthesis may be derived from four sources; directly from plasma FFAs, from cytoplasmic triglyceride stores, from de-novo lipogenesis and from triglycerides derived from incoming lipoproteins. Only a small proportion of FFAs taken up from plasma are used directly for triglyceride synthesis, over 70% are re-esterified to cytoplasmic triglyceride stores and then hydrolyzed for use in VLDL triglyceride production. Using perfused rat livers Heimberg showed in 1962 that increased availability of FFA stimulated hepatic triglyceride secretion [37]. In line, oleate increases the production of triglycerides when added to culture medium of HepG2 cells [9,10] and rat hepatocytes [11]. Whether exogenous FFAs increase the amount of secreted apo B and thus the number of produced VLDL particles in in vitro studies is more controversial. Some studies failed to show an increase in apo B production [10,12] while others [13,14] showed an increase in apo B production when oleate was added to culture medium of Hep G2 cells and rat hepatocytes. Gibbons et al. [38] have suggested that utilization of cytosolic triglycerides, rather than plasma FFA, as the direct source of VLDL provides a buffer through which VLDL secretion rates may, in the short-term, be controlled independently of plasma FFA levels. Thus, it is possible that increase in FFA supply to the liver during the heparin infusion may not acutely increase the syn-

Table 3
VLDL1 and VLDL2 components (mg/dl)

	Control study			Heparin study		
	0.5 h	4.5 h	8.5 h	0.5 h	4.5 h	8.5 h
*VLDL*1						
Triglyceride	33.6 ± 6.2	34.7 ± 6.4	35.7 ± 8.4	21.6 ± 1.8	11.3 ± 1.5	13.6 ± 2.0[b,c]
Free cholesterol	3.0 ± 0.6	3.2 ± 0.7	3.3 ± 0.9	2.4 ± 0.1	1.2 ± 0.2	1.4 ± 0.2[b,c]
Cholesterol ester	3.2 ± 0.7	3.2 ± 0.7	3.7 ± 1.1	2.5 ± 0.4	1.3 ± 0.2	1.5 ± 0.3[a]
Phospholipid	9.0 ± 1.8	9.6 ± 2.0	10.2 ± 2.7	6.7 ± 0.5	3.5 ± 0.5	4.0 ± 0.6[b,c]
Protein	4.7 ± 0.8	4.8 ± 0.9	5.0 ± 1.1	2.7 ± 0.3	1.6 ± 0.1	1.9 ± 0.4[a]
*VLDL*2						
Triglyceride	11.0 ± 1.4	11.8 ± 1.5	12.2 ± 1.7	14.2 ± 1.5	10.7 ± 0.7	11.2 ± 0.9
Free cholesterol	2.0 ± 0.3	2.2 ± 0.3	2.3 ± 0.3	3.0 ± 0.4	2.4 ± 0.2	2.8 ± 0.5
Cholesterol ester	4.0 ± 0.7	4.0 ± 0.7	4.3 ± 0.8	5.2 ± 0.8	4.2 ± 0.5	4.3 ± 0.5
Phospholipid	5.7 ± 0.8	5.9 ± 0.8	6.2 ± 0.9	7.8 ± 0.9	6.1 ± 0.5	6.1 ± 0.4
Protein	3.6 ± 0.5	3.8 ± 0.5	3.9 ± 0.6	4.5 ± 0.5	3.5 ± 0.2	3.6 ± 0.3

[a] $P<0.05$ during the heparin study,
[b] $P<0.01$ during the heparin study,
[c] $P<0.05$ the heparin study compared with the control study (reANOVA) (Results are means ± SE).

thesis of VLDL particles. This concept is supported by findings showing that when oleate is added in perfusate of rat liver, apo B secretion is increased in the fasted but not in the fed state [39].

In the study by Lewis et al. [8], heparin and Intralipid together increased the production of VLDL apo B and triglycerides in healthy men. In that study FFAs were increased similarly two-fold as in the present study. However, plasma free glycerol increased much more in their study (to 546 ± 68 μmol/l) compared with the present study (167 ± 25 μmol/l). It is possible that Intralipid, through its effect on free glycerol availability, may have an additional effect on VLDL apo B production. The study design did not allow the determination of catabolic rates and thus the impact of production and catabolism on the increase of plasma triglycerides seen in the study can not be evaluated.

We have earlier shown that when FFAs are suppressed using an antilipolytic agent, acipimox, the production of VLDL1 apo B is decreased and that of VLDL2 increased [23]. Suppressing the FFAs with acipimox did not affect the amount of total produced VLDL particles. Similarly, in the present study, the acute increase in FFAs did not affect the number of secreted particles. This is in line with the concept of buffer mechanism of cytosolic triglycerides between the plasma FFAs and triglyceride production as discussed above. Insulin, however, is able to suppress acutely the production of total number of VLDL particles [4,23]. We have previously shown, that insulin has a direct suppressive effect on the production of VLDL in the liver, an effect which is independent of the FFA availability [23]. This effect is defective in patients with non-insulin-dependent diabetes mellitus [22]. We speculate that FFA availability regulates the production of VLDL triglycerides in longer-term by increasing the cytosolic triglyceride stores, which in turn serve as a buffer against acute changes in VLDL production. The function of insulin in regulation of production of VLDL particles is more rapid. Insulin suppresses the production of VLDL particles postprandially, when the lipolytic routes are needed for chylomicrons. In longer-term hyperinsulinemic states, insulin may enhance the synthesis of triglycerides in the liver leading to increased VLDL production seen in in vivo [40] and in vitro [41,42] studies.

In conclusion, acute heparin administration changes profoundly the metabolism of VLDL subclasses in fasted state. It increases the catabolism of VLDL particles markedly, while the effect on VLDL apo B production

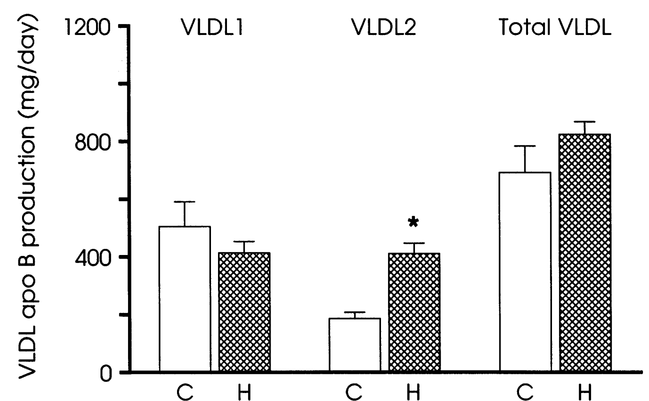

Fig. 5. Production rates of VLDL1 and VLDL2 apo B. * $P<0.05$ heparin vs. control study. Values are calculated from parameters at 4.5 h.

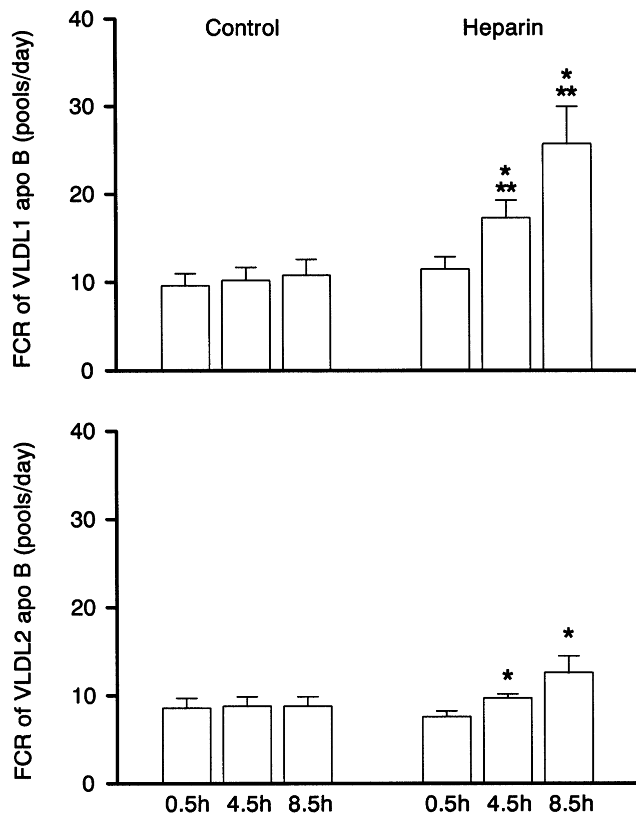

Fig. 6. Fractional catabolic rates of VLDL1 and VLDL2 apo B. * $P < 0.05$ vs. control study value at the same time point, ** $P < 0.05$ vs. value at 0.5 h. (Wilcoxon signed rank test).

is only trivial. Chronic administration of heparin may alter the metabolism of VLDL particles both in the fasted and the fed states but so far the exact influence of heparin is not known.

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