Acute and chronic effects of a 24-hour intravenous triglyceride emulsion challenge on plasma lecithin: cholesterol acyltransferase, phospholipid transfer protein, and cholesteryl ester transfer protein activities

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Abstract **Lecithin:cholesterol acyltransferase (LCAT), phospholipid transfer protein (PLTP), and cholesteryl ester transfer protein (CETP) are key factors in remodeling of high density lipoproteins (HDL) and triglyceride-rich lipoproteins. We examined the effect of a large, 24 h intravenous fat load on plasma lipids and free fatty acids (FFA) as well as on plasma LCAT, PLTP, and CETP activity levels in 8 healthy men. The effect of concomitant insulin infusion was also studied, with 1 week between the study days. During Lipofundin^{R} infusion, plasma triglycerides and FFA strongly increased after 8 and 24 h ($P < 0.001$), whereas HDL cholesterol decreased ($P < 0.01$). The increase in triglycerides was mitigated with concomitant insulin infusion ($P < 0.05$ from without insulin). Plasma LCAT activity increased by 17.7 ± 7.7% after 8 h ($P < 0.001$) and by 26.1 ± 11.1% after 24 h ($P < 0.001$), PLTP activity increased by 19.7 ± 15.6% after 24 h ($P < 0.001$), but CETP activity remained unchanged. Concomitant insulin infusion blunted the increase in plasma LCAT activity ($P < 0.05$ from without insulin), but not that in PLTP activity. One week after the first fat load, plasma non-HDL cholesterol ($P < 0.02$), and triglycerides ($P = 0.05$) were increased, whereas HDL cholesterol was decreased ($P < 0.02$). Plasma CETP and PLTP activity levels were increased by 34.8 ± 30.4% ($P < 0.02$) and by 15.9 ± 6.4% ($P < 0.02$), respectively, but LCAT activity was then unaltered. In summary, plasma LCAT, PLTP, and CETP activity levels are stimulated by a large intravenous fat load, but the time course of their responses and the effects of insulin coadministration are different. Changes in plasma LCAT and PLTP activities may be implicated in HDL and triglyceride-rich lipoprotein remodeling under the present experimental conditions.**—Riemens, S. C., A. Van Tol, W. J. Sluiter, and R. P. F. Dullaart. **Acute and chronic effects of a 24-hour intravenous triglyceride emulsion challenge on plasma lecithin:cholesterol acyltransferase, phospholipid transfer protein, and cholesteryl ester transfer protein activities.** ***J. Lipid Res.*** **1999.** 40: **1459–1466.**

Supplementary key words CETP • PLTP • LCAT • triglyceride emulsion • insulin

Apart from lipoprotein lipase and hepatic lipase, lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP) are key factors in intravascular high density lipoprotein (HDL) metabolism (1–13). LCAT esterifies free cholesterol derived from cell surfaces and triglyceride-rich lipoproteins while bound to HDL (2, 3, 8). CETP subsequently transfers cholesteryl ester from HDL to lipoproteins of lower density, partly in exchange for triglycerides (5, 6). PLTP promotes the transfer of phospholipids (7, 9) and free cholesterol (10) between lipoproteins. This lipid transfer protein may also enhance the CETP-mediated lipoprotein cholesteryl ester transfer (9, 11). Moreover, PLTP has been identified as an HDL conversion factor, i.e., it has the ability to convert HDL_3 into smaller and larger HDL particles (12, 13).

Important changes in HDL composition, including increments in HDL free cholesterol, phospholipids, and triglycerides, and in several studies decreases in HDL cholesteryl ester, have been reported to occur during an oral or intravenous fat challenge (14–18). Furthermore, cholesterol concentration in HDL_3, the dense HDL subfraction, decreases after an intravenous fat load (16, 19–21). Such changes in HDL are in part due to shedding of surface fragments originating from triglyceride-rich lipoproteins during their metabolism. Alterations in HDL may also be related to changes in plasma LCAT, affecting cholesterol esterification, and/or lipid transfer proteins influ-

Abbreviations: apo, apolipoprotein; BMI, body mass index; CETP, cholesteryl ester transfer protein; FFA, free fatty acids; HL, hepatic lipase; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; LPL, lipoprotein lipase; MCT, medium-chain triglycerides; PLTP, phospholipid transfer protein; VLDL, very low density lipoproteins.

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encing phospholipid and neutral lipid transfer. Our knowledge about the effects of a fat challenge on the plasma activity levels of LCAT, CETP, and PLTP, as a measure of their plasma mass concentrations, is still incomplete. Plasma LCAT was reported to correlate positively with fasting triglycerides as well as cholesterol (2), and in vitro studies have shown that free fatty acids (FFA) stimulate the secretion of triglycerides and LCAT by cultured hepatocytes (22). Oral fat loads generally increase cholesterol esterification rates (23–25), probably as a consequence of increases in LCAT substrates. During intravenous fat challenges, the plasma cholesterol esterification rate was reported to be unchanged (19) or increased (26). The response of plasma LCAT activity per se has only been studied after a high fat breakfast (25). Plasma CETP has been found to increase slightly (17, 27, 28) or to remain unchanged (18, 25, 29) after an oral fat load. Plasma PLTP activity was recently reported to remain unchanged after oral fat in normolipidemic non-smoking men (25). No published data are available at present on the plasma LCAT, CETP, and PLTP responses to an intravenous fat challenge. We have recently found that plasma PLTP but not CETP activity decreases in conjunction with a fall in plasma FFA and triglycerides after endogenous (30) and exogenous (31) hyperinsulinemia. This raises the possibility that plasma PLTP is coordinately regulated with plasma FFA and triglycerides.

In the present study, we evaluated the responses of plasma LCAT, CETP, and PLTP activity levels to a large intravenous fat challenge given over 24 h to healthy men. As it is likely that insulin directly inhibits hepatic very low density lipoprotein (VLDL) secretion (32) and thus indirectly influences lipid tolerance, we also tested whether the responses of LCAT and lipid transfer proteins were modified during concomitant hyperinsulinemia.

SUBJECTS AND METHODS

Subjects

Eight non-obese, non-smoking, healthy males participated in the studies. Diabetes mellitus was excluded by a 75 gram oral GTT using a fasting venous blood glucose level <6.1 mmol/l and a 2 h blood glucose level of 10.0 mmol/l as cut-off values. Subjects with a fasting plasma total cholesterol >8.0 mmol/l and/or triglycerides >4.5 mmol/l were excluded. None of the participants had familial hyperlipidemia or suffered from clinically manifest cardiovascular disease. In all of them, the thyreotropin level, liver function tests, and serum creatinine concentration was within the normal range, and blood pressure was $<160/95$ mm Hg. Maximal alcohol intake was 3 beverages per day. Body mass index (BMI) was calculated as weight divided by height squared. The study protocol was approved by the medical ethical committee of the University Hospital Groningen and all participants gave written informed consent.

Study design

The participants were urged to maintain their usual diet and lifestyle 1 week before and between the study days. They did not use alcohol on the day before the studies and were kept fasting from 20.00 h onwards on the days before and during the studies. The subjects were studied on two separate days in random order, with a wash-out period of 1 week in between: *a*) during 24 h infusion of an artificial triglyceride emulsion (Lipofundin^R) and *b*) during 24 h of infusion of Lipofundin^R plus insulin. On the study days, the subjects consumed a 1000 kcal diet consisting of 37% carbohydrate, 38% fat, and 24% protein divided over breakfast, lunch, and dinner. They remained in the supine position after 8.00 h on the study days. The subjects were admitted at 07.00 h both for the Lipofundin^R study and the Lipofundin^R plus insulin study. One hand vein was cannulated and the cannula was kept open with a saline drip (NaCl, 154 mmol/l, 30 ml/h). This hand was placed in a thermoregulated box with an ambient temperature of 55°C to obtain arterialized venous blood (33). After 1.5 h of supine rest, baseline blood samples for measurement of blood glucose, insulin, lipids, (apo) lipoproteins, FFA, LCAT, CETP, and PLTP activity levels were obtained. Further blood samples were taken after 8 and 24 h.

During the Lipofundin day, Lipofundin^R (containing a fat emulsion with 50 g/l soya oil, 50 g/l medium-chain triglycerides (MCT), 25 g/l glycerol, 12 g/l soya phospholipids, and 0.25 g/l cholesterol (21)) was infused for 24 h in a dose of 0.2 g of triglycerides/kg per h via a second cannula, inserted in a contralateral antecubital vein.

During the Lipofundin plus insulin day, Lipofundin^R was infused for 24 h at a similar rate, in combination with intravenous insulin (Velosulin, Novo Nordisk, Denmark) administered at a rate of 30 mU/kg per h, preceeded by an insulin bolus of 5 mU/kg. Blood glucose was measured at 10-min intervals and was maintained at its fasting level with a variable dextrose infusion (20% w/w) with potassium chloride (20 mmol per liter of dextrose) to prevent hypokalemia.

Laboratory measurements

Blood was collected into EDTA (1.5 mg/ml)-containing tubes and was placed on ice immediately. Plasma was separated from blood cells within 30 min by centrifugation at 3000 rpm for 15 min at 4°C. Samples were frozen and kept at −80°C before assay.

Blood glucose was measured with an APEC Glucose analyzer (APEC Inc., Danvers, MA). Plasma free insulin was assayed by radioimmunoassay (Novo Nordisk Immunochemical Department, Copenhagen, Denmark). Plasma FFA were measured enzymatically using a kit from Wako Chemicals (FRG, cat. no. 994-75409).

Lipids were measured in plasma and in the HDL-containing supernatant fraction after removal of apolipoprotein B (apoB)-containing lipoproteins with polyethylene glycol-6000 (34). Non-HDL lipids were calculated as the difference between plasma and the HDL fraction. Total cholesterol and triglycerides were measured enzymatically. Triglyceride concentrations were corrected for glycerol. Apos A-I and B were assayed by immunoturbidimetry with kits obtained from Boehringer Mannheim (FRG, cat. no. 726478 and 726494, respectively).

Plasma activity levels of LCAT, CETP, and PLTP were assayed in duplicate as described (35–37). Plasma LCAT activity was assayed using excess exogenous substrate containing [^{3}H]cholesterol (37). The plasma LCAT activity is linearly related to the amount of plasma in the incubations and is regarded as a measure of its mass (38). CETP activity was determined after removal of non-HDL lipoproteins from each sample by precipitation with Mg^{2+}/phosphotungstate (36, 37). The isotope assay measures the transfer of cholesteryl ester between exogenous cholesteryl-[1-^{14}C]oleate-labeled LDL and an excess of unlabeled pooled normal HDL. LCAT was inhibited with dithiobis-2-nitrobenzoic acid. CETP activity was calculated as the bidirectional transfer between labeled LDL and HDL. The plasma CETP activity level obtained by this method is strongly correlated with CETP mass (39) and is independent of the endogenous plasma lipoproteins.

Plasma PLTP activity was assayed with a phospholipid vesicles–HDL system (36, 37). Plasma samples were incubated with [^{3}H]phosphatidylcholine-labeled liposome vesicles and an excess of pooled normal HDL. Subsequently, the vesicles were precipitated with a mixture of NaCl, $MgCl_2$, and heparin (final concentrations of 230 mmol/l, 92 mmol/l, and 200 U/ml, respectively). Plasma PLTP activity levels are linearly related to the amount of plasma added to the incubation system. The method is specific for PLTP and is not influenced by the phospholipid transfer promoting properties of CETP (36). The measurements of these factors were performed in one run using the same batches of substrates. The within-assay coefficients of variation were 4.5%, 2.7%, and 3.5% for LCAT, CETP, and PLTP, respectively. The plasma activity levels of these factors were related to the activity levels in human pool plasma, which was included in each run, and are expressed in arbitrary units (AU, corresponding to the percentage of the activities in the pool plasma). In the present series of experiments, a plasma LCAT, CETP, and PLTP activity of 100 AU corresponds to 65.6 nmol esterified cholesterol/ml per h, 214.6 nmol cholesteryl ester/ml per h, and 18.5 μmol phosphatidylcholine/ml per h, respectively. Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined in plasma 20 min after intravenous injection of 50 U/kg of heparin as described previously (40). This test was performed 1 to 3 weeks before the first fat challenge.

Statistical analysis

Data are expressed as mean ± SD unless stated otherwise. Changes in parameters from baseline were evaluated by Friedman's two-way analysis of variance and by paired Wilcoxon tests where appropriate. Comparison of changes in parameters during Lipofundin^R alone and Lipofundin^R plus insulin was carried out using paired Wilcoxon tests. Bivariate correlations coefficients were calculated by Spearman's rank test (r_s). A two-sided P value <0.05 was taken as significant.

RESULTS

Mean age of the study participants was 53 ± 8 years, BMI was 24.3 ± 2.8 kg/m^2, and median alcohol intake was 0.2 (range 0–2) consumptions per day. LPL and HL activities in postheparin plasma were 152 ± 55 U/l and 389 ± 129 U/l, respectively.

Marked intraindividual variations in baseline plasma (apo)lipoproteins, PLTP, and CETP activity levels were observed between the two study days. To evaluate a possible carry-over effect, the baseline lipid parameters before and 7 days after the first Lipofundin^R challenge were compared. On the first occasion, six subjects received Lipofundin^R without and two with concomitant insulin infusion. As shown in **Table 1**, plasma total cholesterol, non-HDL cholesterol, and triglycerides increased, whereas HDL cholesterol decreased when measured 7 days after the first Lipofundin^R challenge. Plasma PLTP and CETP activity increased by 15.9 ± 6.4 and 34.8 ± 30.4%, but LCAT activity was not altered. The relative increments in plasma PLTP and CETP activities were greater than the change in LCAT activity. The relative changes in plasma PLTP (r_s = 0.81, $P < 0.05$) and CETP activity (r_s = 0.93, $P < 0.01$) were correlated with the changes in non-HDL cholesterol, but the relationships between the changes in these lipid transfer factors with the change in plasma triglycerides did not reach significance (r_s = 0.14, n.s. and r_s = 0.64, P = 0.10, respectively). Furthermore, the relative change in HDL cholesterol was correlated with the relative change in plasma CETP activity (r_s = −0.86, $P < 0.02$), but not significantly with the relative changes in plasma triglycerides (r_s = −0.55, n.s.) and PLTP activity (r_s = −0.52, n.s.).

With Lipofundin^R alone, blood glucose was 4.7 ± 0.4, 4.7 ± 0.5, and 5.0 ± 0.4 mmol/l at baseline and after 8 and 24 h (n.s.), whereas plasma insulin levels were 9.3 ± 5.6, 28.7 ± 33.0 ($P < 0.05$), and 14.9 ± 9.6 mU/l (n.s.), respectively. This increase in plasma insulin after 8 h was expected after the meal at 12.30 h. With Lipofundin^R plus insulin, infusion blood glucose was 4.1 ± 1.0, 4.3 ± 0.3, and 4.5 ± 0.3 at these time points (n.s.). Plasma insulin rose from 7.6 ± 4.1 mU/l at baseline to 48.4 ± 22.1 ($P < 0.02$) and 39.2 ± 9.7 mU/l ($P < 0.02$) at 8 and 24 h ($P < 0.05$ vs. Lipofundin^R alone at 8 and 24 h). The mean coefficient of variation of the glucose level during the 24 h insulin clamp was 12.3 ± 2.1%. This coefficient of variation was relatively high due to the blood glucose-increasing effects of the three meals.

TABLE 1. Baseline plasma (apo)lipoproteins and plasma PLTP, CETP, and LCAT activity in 8 healthy subjects before and 7 days after Lipofundin^R administration

	Before Lipofundin^R	7 Days after Lipofundin^R	% Change
Plasma total cholesterol (mmol/l)	4.85 ± 0.45	6.14 ± 0.82	26.7 ± 13.9[b]
Non-HDL cholesterol (mmol/l)	3.76 ± 0.49	5.16 ± 0.90	37.6 ± 19.1[b]
HDL cholesterol (mmol/l)	1.09 ± 0.22	0.93 ± 0.27	−15.7 ± 12.8[b]
Plasma triglycerides (mmol/l)	0.91 ± 0.45	1.28 ± 0.91	29.4 ± 37.5[a]
Plasma FFA (μmol/l)	831 ± 271	783 ± 273	−1.5 ± 32.2
Plasma apolipoprotein A-I (g/l)	1.16 ± 0.11	1.11 ± 0.16	−4.8 ± 6.9
Plasma apolipoprotein B (g/l)	0.82 ± 0.10	0.96 ± 0.17	17.7 ± 24.2
Plasma PLTP activity (AU)	85.2 ± 9.5	98.5 ± 10.2	15.9 ± 6.4[b,c]
Plasma CETP activity (AU)	86.9 ± 23.9	113.8 ± 26.6	34.8 ± 30.4[b,d]
Plasma LCAT activity (AU)	90.9 ± 12.2	94.4 ± 12.2	4.1 ± 6.9

Data given as mean ± SD. On the first study day, six subjects received Lipofundin^R alone and two received insulin concomitantly.

[a] $P = 0.05$; [b] $P < 0.02$ from before Lipofundin^R.

[c] $P < 0.05$; [d] $P < 0.01$ compared to % change in plasma LCAT activity.

The relative changes in plasma lipids, PLTP, LCAT, and CETP activities during Lipofundin^R without and with concomitant insulin infusion were compared to their baseline levels on each study day. Plasma triglycerides, FFA, and non-HDL cholesterol concentrations rose profoundly during Lipofundin^R ($P < 0.01$ for all after 8 h and 24 h, **Fig. 1 A–C**). Concomitant administration of insulin blunted the increments in plasma triglycerides and non-HDL cholesterol ($P < 0.05$ for both after 24 h), but did not affect the increase in plasma FFA. HDL cholesterol decreased during Lipofundin^R ($P < 0.02$ after 8 h and $P < 0.001$ after 24 h) and its fall tended to be smaller with Lipofundin^R plus insulin ($P < 0.07$, Fig. 1D). The relative increase in plasma triglycerides after 8 h was negatively correlated with plasma LPL activity ($r_s = -0.76$, $P < 0.05$ without and $r_s = -0.69$, $P < 0.10$ with insulin infusion), but not after 24 h of Lipofundin^R administration ($r_s = -0.26$, n.s. without and $r_s = -0.31$, n.s. with insulin infusion). The triglyceride responses were not correlated with HL activity (data not shown). The relative fall in HDL cholesterol was correlated with the relative rise in plasma triglycerides without insulin, but not significantly with insulin infusion (combined data at 8 and 24 h, $r_s = 0.73$, $P < 0.01$ and $r_s = 0.46$, $P < 0.10$, respectively).

With Lipofundin^R, plasma LCAT activity increased by 17.7 ± 7.7% after 8 h ($P < 0.001$) and by 26.1 ± 11.1% after 24 h ($P < 0.001$). Its rise was blunted by concomitant insulin infusion ($P < 0.05$, **Fig. 2A**). Plasma PLTP activity increased only after 24 h of Lipofundin^R infusion (by 19.7 ± 15.6%, $P < 0.001$) and its rise was not significantly modified by insulin (Fig. 2B). In contrast, plasma CETP activity did not significantly change with Lipofundin^R alone or in combination with insulin at 8 h or 24 h (Fig. 2C). The relative increments in plasma PLTP and LCAT activity were

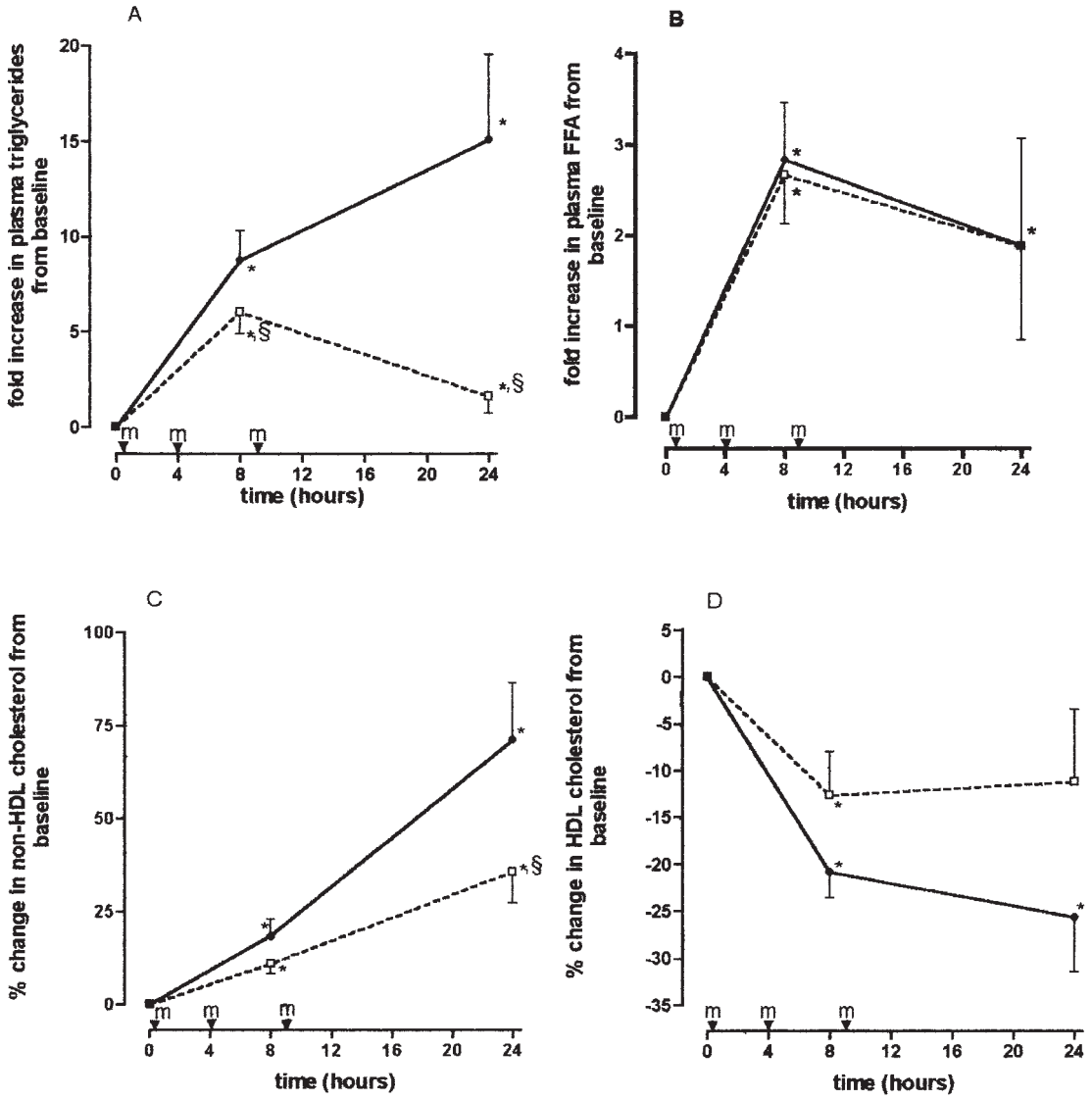

Fig. 1. Fold increase in plasma triglycerides from baseline (A), fold increase in plasma FFA from baseline (B), % change in non-HDL cholesterol from baseline (C), and % change in HDL cholesterol from baseline (D) during 24 h of Lipofundin^R infusion (●) or Lipofundin^R + insulin infusion (□) in healthy non-obese subjects. Data represented are mean values and SEM. * $P << 0.05$ from baseline; § $P < 0.05$ from without insulin; m indicates meal intake.

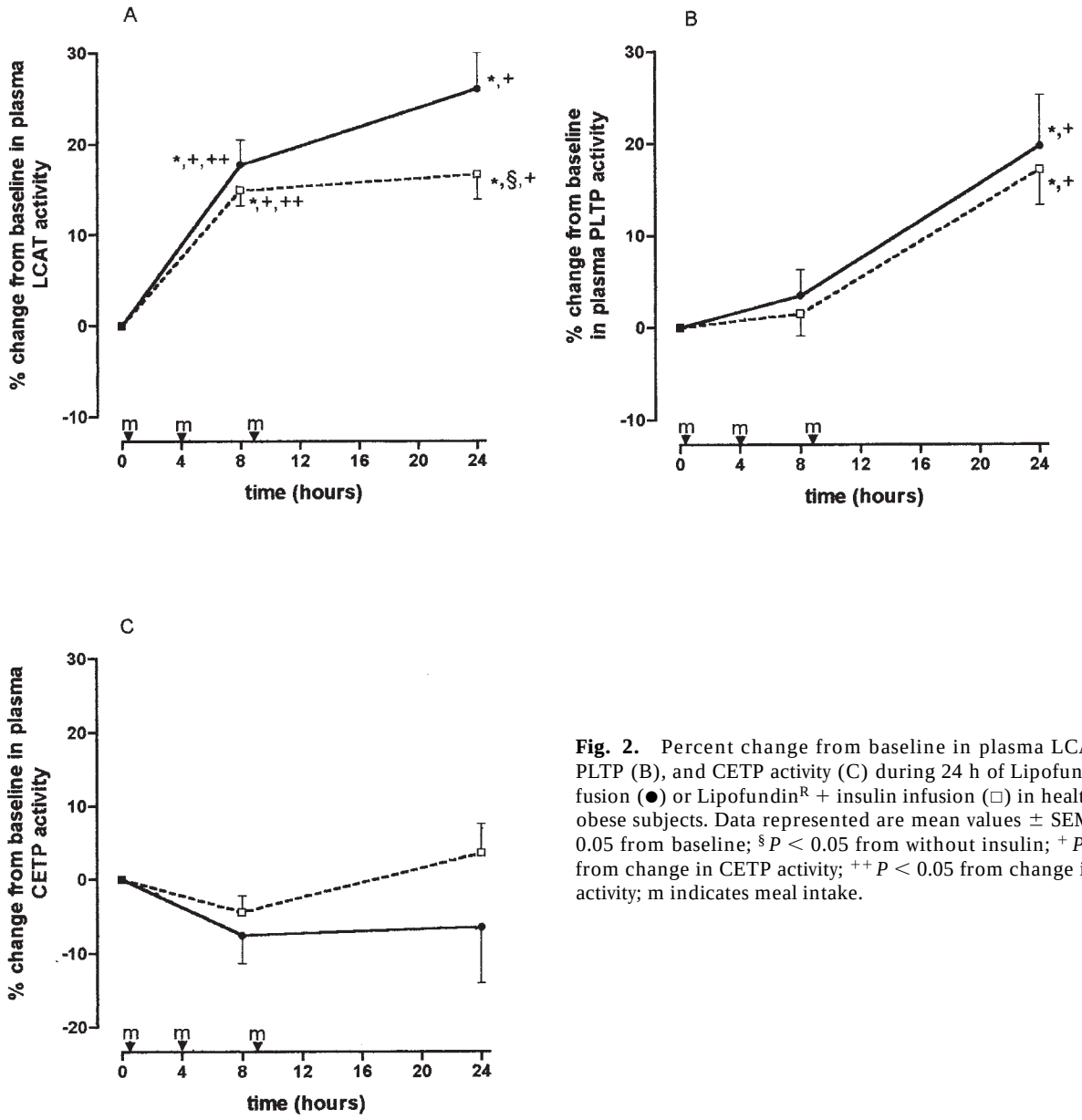

Fig. 2. Percent change from baseline in plasma LCAT (A), PLTP (B), and CETP activity (C) during 24 h of Lipofundin^R infusion (●) or Lipofundin^R + insulin infusion (□) in healthy nonobese subjects. Data represented are mean values ± SEM. $^*P < 0.05$ from baseline; $^§P < 0.05$ from without insulin; $^+P < 0.05$ from change in CETP activity; $^{++}P < 0.05$ from change in PLTP activity; m indicates meal intake.

greater ($P < 0.05$ for both) than the minor change in plasma CETP activity levels at 8 and 24 h, both without and with concomitant insulin infusion.

DISCUSSION

To our knowledge this is the first study demonstrating that the plasma activity levels of LCAT and PLTP increase in response to a large 24-h intravenous fat challenge. These findings support the hypothesis that the plasma availability of FFA, triglycerides, or other lipids is involved in the acute regulation of LCAT and PLTP. The 24-h time course of the plasma LCAT and PLTP response was different, and concomitant administration of insulin mitigated the LCAT, but not the PLTP increase after 24 h. This suggests that the mechanisms responsible for the acute regulation of LCAT and PLTP are not identical. An unexpected finding of our study was that plasma levels of non-HDL cholesterol and triglycerides were higher when measured 1 week after the first fat challenge. Plasma CETP and PLTP activities were increased in association with these lipoprotein changes, suggesting a chronic effect on lipid transfer protein regulation.

In the present experiments, we used a large and prolonged intravenous fat load for several reasons. First, it was expected that an effect on plasma LCAT and lipid transfer proteins could be more readily demonstrated under such circumstances. Second, sustained elevations in plasma FFA and triglycerides were necessary to be able to evaluate the effect of concomitant prolonged insulin infusion on the possible LCAT and lipid transfer protein responses. Furthermore, it should be noted that the assays that were used to measure plasma LCAT, CETP, and PLTP activities reflect the amounts of active proteins in plasma. Although plasma LCAT and CETP activities so measured

strongly correlate with their mass (38, 39), an increase in their specific activity cannot be excluded.

The relative increase in plasma triglycerides after 8 h of Lipofundin^R was negatively correlated with basal LPL activity in postheparin plasma. This finding is in keeping with the notion that intravascular triglyceride lipolysis is a determinant of the triglyceride response to a fat challenge (41). After 24 h no such relationship was observed, suggesting a contribution of other mechanisms to the plasma triglyceride increment at that time point. Coadministration of insulin diminished the increase in plasma triglycerides, which is likely explained by insulin stimulation of intravascular triglyceride lipolysis through activation of LPL (42) and by a direct inhibitory effect of insulin on hepatic triglyceride release (32, 43). The increment in plasma FFA after the intravenous fat challenge was not modified by concomitant insulin infusion. The lack of effect of insulin on plasma FFA under these experimental conditions may have been due to an increase in FFA consequent to stimulated intravascular lipolysis offsetting the fall in FFA which would be expected as a consequence of insulin-induced inhibition of adipose tissue lipolysis (44).

The increases in plasma LCAT and PLTP activities observed within 24 h after intravenous Lipofundin^R may be related to the magnitude of the hyperlipidemia induced as well as to the type of fat load, with Lipofundin^R containing large quantities of MCT, phospholipids, and glycerol. In comparison, plasma LCAT and PLTP activities do not increase within 8 h after a fat-rich mixed meal causing a modest elevation in triglycerides (25). The increase in plasma LCAT is in accord with the concept that FFA, derived from intravascular hydrolysis of the artificial triglyceride emulsion, enhance hepatic LCAT secretion together with triglycerides (22). The blunted plasma LCAT response and the less pronounced rise in triglycerides when insulin was coadministered would suggest that insulin may have, in part, prevented LCAT release from liver in conjunction with a diminished triglyceride secretion (32, 43). The increment in plasma PLTP activity after 24 h of Lipofundin^R infusion lends further support to the hypothesis that the regulation of plasma PLTP is linked to FFA and triglyceride metabolism (30, 31). PLTP is synthesized in a variety of tissues (7, 45), and the predominant source of PLTP in human plasma is unknown. The stimulation of plasma PLTP after increasing plasma lipids and FFA as well as the lowering of plasma PLTP, FFA and triglycerides by insulin and Acipimox^R (unpublished observations) raise the possibility of regulation in liver or adipose tissue, but we cannot rule out effects on PLTP secretion or catabolism by other tissues.

Whereas the acute increases in plasma LCAT and PLTP activity in response to Lipofundin^R are likely to be associated with alterations in FFA and lipid metabolism, the increments in plasma CETP and PLTP activity observed 1 week after the first fat load may be related to different mechanisms. The increases in these lipid transfer protein activities were positively correlated with the increments in non-HDL cholesterol levels, but not significantly with the changes in plasma triglycerides. Both CETP and PLTP gene expression is stimulated by dietary cholesterol feeding (45–47). It seems, therefore, possible that the chronic increases in plasma CETP and PLTP activity were, at least in part, secondary to higher non-HDL cholesterol levels induced by the fat challenge. Lipoprotein-X, which often accumulates during infusion of high doses of lipid emulsions, was not measured in the present study. This lipoprotein particle was shown to be cleared from the circulation within 2 to 4 days in the neonate (48), but lipoprotein-X may still be present in the non-HDL fraction even 1 week after the fat challenge in adults.

During intravascular lipolysis of triglyceride-rich lipoproteins, large amounts of phospholipids and free cholesterol are shed from the surface of such lipoproteins allowing them to be catabolyzed to smaller particles (3, 14, 49). These lipoprotein constituents are transferred to HDL, increasing the free cholesterol and phospholipid content in this lipoprotein fraction (14, 16, 18). Both LCAT and PLTP have been suggested to facilitate this transfer of lipoprotein surface constituents (8–10). The increases in plasma LCAT and PLTP activity in response to the fat load may thus play an integrative role in enhancing the remodeling of triglyceride-rich lipoproteins and HDL under the current experimental conditions. However, the large and prolonged fat challenge and the nonphysiological composition of the triglyceride emulsion used indicate that one should be cautious when extrapolating the implications of the present findings to in vivo lipoprotein metabolism after a fat-rich meal. The fall in HDL cholesterol during the Lipofundin^R infusion is in agreement with previous observations after an oral and intravenous fat challenge (16–21), and has been attributed to an increase in the rate of transfer of cholesteryl ester from HDL to (artificial) triglyceride-rich particles (16, 18, 50, 51). Indeed, the acute decrease in HDL cholesterol during the fat load was correlated with the increase in plasma triglycerides. However, 1 week after the first fat challenge, the fall in HDL cholesterol was correlated with the increase in plasma CETP activity. This underscores the role of both plasma triglyceride-rich lipoproteins, that can accept cholesteryl ester from HDL, and the activity level of CETP per se in the process of cholesteryl ester transfer (11, 52, 53). In addition, increases in plasma PLTP activity may further enhance the cholesteryl ester transfer process (9, 11).

Hypertriglyceridemia that occurs in the postprandial phase is assumed to be atherogenic, as triglyceride-rich particles are metabolized to remnant particles that can deposit cholesteryl ester in the arterial wall (54, 55). The ability of LCAT to esterify free cholesterol in the HDL fraction is considered to represent an early step in the process of reverse cholesterol transport (56), whereby cholesterol is transported back to the liver where it is metabolized and excreted in the bile. During PLTP-mediated HDL remodeling, preβ-HDL particles are generated that have the ability to accept free cholesterol from cell surfaces (57, 58). Accordingly, overexpression of PLTP in mice increases preβ-HDL (59, 60) and enhances hepatic HDL uptake (60). Moreover, efflux of free cholesterol from cells in culture to human plasma is positively corre-

lated with plasma PLTP activity (61). Keeping the above-mentioned caveats in mind, it is tempting to speculate that high levels of plasma LCAT and PLTP activity in association with hypertriglyceridemia (37) could represent a defense mechanism against atherosclerosis by increasing preβ-HDL and activating the LCAT reaction.

In conclusion, plasma LCAT and PLTP activity acutely increase in response to a large intravenous fat load, whereas plasma CETP activity responds later in association with changes in non-HDL cholesterol. Such changes in LCAT and lipid transfer proteins may be implicated in the metabolism of triglyceride-rich lipoproteins and HDL under the present experimental conditions.

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