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ORIGINAL ARTICLE

Postprandial variations in the cholesteryl ester transfer protein activity, phospholipid transfer protein activity and plasma cholesterol efflux capacity in normolipidemic men

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Abstract

Background and Aim: *Plasma cholesterol efflux capacity is stimulated during postprandial (PP) hypertriglyceridemia. Plasma cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are the key proteins in lipoprotein metabolism and remodelling, but their role during the PP cholesterol efflux process remains indeterminate. The aim of this study was to determine the effect of a fatty meal intake on plasma CETP and PLTP activities, and the capacity of plasma to promote cholesterol efflux, as well as to evaluate the relationship between these three key mechanisms of the reverse cholesterol transport process.*

Methods and Results: *CETP and PLTP activities and the cholesterol efflux capacity of plasma were measured over eight hours following a fatty meal (1000 kcal, 62% fat) in 13 normolipidemic men. CETP activity and the cholesterol efflux capacity of plasma from Fu5AH cells increased after the meal, reaching a maximum after eight hours (respectively 32%, p=0.06, and 6.5%, p=0.045), whereas PLTP activity remained unchanged. CETP and PLTP activities did not correlate with plasma cholesterol efflux capacity in the fasting or PP state. Plasma CETP activity in the fasting state positively correlated with the plasma non-esterified fatty acid (NEFA) levels, but no correlation was found with any lipid or apolipoprotein postprandially. The cholesterol efflux capacity of plasma correlated positively with high-density lipoprotein (HDL) components, the best correlation being with the HDL phospholipid fraction in both the fasting and PP states.*

Conclusions: *These findings suggest that plasma CETP and PLTP activities in healthy normolipidemic subjects are differently regulated in the PP state, and are not correlated with the increased cholesterol efflux capacity of PP plasma. HDL-phospholipid remains the key factor in the regulation of the capacity of plasma to promote Fu5AH cell cholesterol efflux.*

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Introduction

In humans, the postprandial (PP) state represents a major part of the metabolic state due to regular diet intake. The hypothesis that PP hypertriglyceridemia is an independent risk factor for coronary artery disease is supported by a number of studies (1-3), and delayed PP triglyceridemia has been associated with cardiovascular events (2, 3). The potential atherogenic role of PP triglyceride-rich lipopro-

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teins (TGRL) [*ie* chylomicrons and very low-density lipoproteins (VLDL)] was first hypothesised by Zilversmit (4) and has been demonstrated by the accumulation of their remnants in human atherosclerotic lesions (5).

The cholesterol ester transfer protein (CETP) is one of the essential proteins involved in the reverse cholesterol transport process by means of which the cholesterol from peripheral tissues is returned to the liver for reuse or re-excretion. In fasting normolipidemic subjects, CETP promotes the net transfer of cholesteryl esters (CE) from high-density lipoproteins (HDL) to VLDL and low-density lipoproteins (LDL), whereas triglycerides (TG) are transferred to HDL (6). Under conditions of normal hepatic VLDL-CE and LDL-CE clearance through the LDL-receptor pathway, CETP may protect against atherosclerosis by promoting the rapid removal of CE from the plasma compartment, despite a potential lowering effect on HDL-cholesterol (HDL-C). The increased TGRL level in hypertriglyceridemic subjects promotes an increase in CE transfer that contributes to the accumulation of potentially atherogenic particles (7). During the PP phase, the CE transfer rate may be modulated by changes in plasma lipoproteins, and may increase as a result of an increase in CE acceptors and/or CETP concentration (8). There are contrasting data concerning the PP variations in total CE transfer, and CETP activity or concentrations (9-13).

The phospholipid transfer protein (PLTP) promotes the transfer of phospholipids from HDL to other lipoproteins, which accounts for most of the plasma PL transfer between lipoproteins; the rest is due to CETP activity (14). During the PP state, PLTP may play an important role in the catabolism of TGRL under lipoprotein lipase action by transferring surface PL to the plasma HDL fraction. PLTP can also modulate CETP-induced CE exchanges (15). Moreover, like CETP, PLTP can modify HDL particle composition and size, which can influence the cholesterol efflux capacity of plasma (16-20). Furthermore, plasma PLTP and CETP activities are stimulated by a large intravenous fat load in healthy men (21).

As modifications in lipoprotein particle levels or composition, and in enzymatic activities during the PP phase, may modulate the extra-hepatic cholesterol efflux process (22-25), the aim of this study was to determine the effect of a fatty meal intake on plasma CETP and PLTP activity, as well as on the capacity of plasma to promote cholesterol efflux. We also evaluated the relationship between these three key mechanisms of the reverse cholesterol transport process during PP lipidemia in healthy normolipidemic subjects.

Methods

Subjects

A group of normolipidemic males (n=13) living in a religious community were selected on the basis of their fasting plasma lipid values (cholesterol <210 mg/dL, TG <110 mg/dL, HDL-C >35 mg/dL). None of the participants was obese (body mass index <27 Kg/m^2), diabetic or a smoker. Table 1 shows the mean fasting lipid profile of the study group.

Dietary protocol

The dietary protocol included an initial 16-week stabilisation period during which 35% of total daily energy intake was in the form of fat (94 g/day): 11% of saturated fatty acids (SFA) (31 g/day), 5% of polyunsaturated fatty acids (PUFA) (12 g/day of linoleic acid and 1.5 g/day of alpha-linolenic acid), and 16% of oleic acid (43 g/day). The daily cholesterol supply was 300 mg. Daily energy and lipid intakes were evaluated by means of a seven-day dietary enquiry in order to obtain the calculated level of the dietary components, and verified by means of chemical analyses of the related meals.

The fatty study meal (1000 Kcal, 67 g fat: 32 g monounsaturated fatty acids, 13 g PUFA, 22 g SFA and 300 mg cholesterol) was administered at 7:00 a.m. after a 12-hour overnight fast and consisted of solid foodstuffs.

Blood samples were collected on ice before and every two hours for eight hours after the test meal in sterile ethylenediaminetetraacetic acid (EDTA)-containing tubes. After plasma separation by means of centrifugation at

TABLE 1
Characteristics of the study group.

Body mass index (Kg/m^2)	22±4
Mean age (years)	45±22
Plasma glucose (mmol/L)	4.60±0.5
Plasma cholesterol (mmol/L)	4.65±0 9
Plasma triglycerides (mmol/L)	0 78±0.21
Plasma phospholipids (g/L)	2 49±0.55
Plasma HDL-C (mmol/L)	1 26±0.25
Plasma LDL-C (mmol/L)	2 94±0 62
Plasma Apo AI (g/L)	1.50±0 29
Plasma Apo B (g/L)	0.82±0.12

Mean values±SD (n=13) Apo. apolipoprotein; HDL-C high-density lipoprotein cholesterol; LDL-C low-density lipoprotein cholesterol

3500 rpm for 35 min at 4°C, the samples were immediately aliquoted and stored at -80°C until further analysis, except for the aliquots kept at 4°C for chylomicrons separation within two hours. The protocol was approved by the Human Ethics Committee of Hôpital Henri Mondor, Créteil, France.

Isolation of lipoproteins

Chylomicrons were separated from 2.5 ml of fresh plasma layered with 0.5 ml 0.15 M NaCl, 1 mM EDTA solution (d=1.006) by means of centrifugation at 18000 rpm for 35 min at 12°C in a Sorvall ST21 centrifuge (Sorvall, Les Ulis, France) equipped with a SL50T rotor. For the CETP and PLTP assays, VLDL+IDL+LDL (1.006<d<1.063) and HDL (1.063<d<1.21) were isolated at 4°C by means of sequential ultracentrifugation in a Kontron centrikon T-1065 ultracentrifuge equipped with a 50Ti rotor.

Lipid, apolipoprotein and hormone measurements

Plasma cholesterol, TG, PL, unesterified cholesterol and non-esterified fatty acids (NEFA) were quantified enzymatically using commercial kits (Biochem Pharma Inc., France; Boehringer Mannheim, Germany; bioMérieux, France; and Wako Chemicals Gmbh, Germany). HDL-C was determined after phosphotungstate precipitation of the plasma samples (bioMérieux, France) and LDL-C was calculated using Friedewald's formula. The plasma levels of apolipoproteins AI and B were measured by means of immunonephelometry using a Behring BNII and Dade-Behring antibodies according to ARCOL French Committee recommendations. Insulin was measured by means of a radioimmunology assay (RIA) using a Phasedeph kit (Pharmacia/Upjohn, Uppsala, Sweden).

Measurement of plasma cholesterol efflux capacity

The capacity of plasma to promote cholesterol efflux from cultured Fu5AH hepatoma cells was quantified using the De la Llera Moya method (26). Briefly, the cells were seeded in 12-well plates in Eagle's minimal essential medium (MEM) (Eurobio Laboratories, France) supplemented with 5% new-born calf serum (Gibco Life Technologies, France), 1% glutamin and 0.75% penicillin-streptomycin (Eurobio Laboratories, France) for two days. They were then radiolabeled by adding 2µCi of [1,2-^{3}H] cholesterol (NEN Life Science Products, Paris, France) per well to the medium and incubating them for three days in order to obtain confluent monolayers. The cells were then incubated with 0.5% bovine serum albumin (Sigma Aldrich, France) in MEM for 18 hours. The plasma samples kept at -80°C were quickly thawed and diluted to 5% in cell medium before being added to the wells containing the radiolabelled cells and incubated for four hours. Each sample was measured in triplicate. At the end of the incubation, the medium from each well was removed, centrifuged to precipitate floating cells, and aliquot radioactivity was determined by means of liquid scintillation. Cell lipid radioactivity was measured after lipid extraction by isopropanol. Standard plasma prepared from a pool of normolipidemic plasma was used as a reference for inter-assay homogeneity. Fractional cholesterol efflux was calculated as the amount of label released into the medium in relation to the total label in each well, expressed as a percentage, and divided by the standard efflux value.

CETP activity assay

Cholesteryl ester transfer activity was measured in the fasting and PP diluted plasma samples by means of an indirect method (8), which accurately reflects CETP mass in normal and hyperlipoproteinemic subjects (27). Briefly, exogenous cholesterol donors (3H HDL-CE) and cholesterol acceptors (LDL+VLDL) were obtained from a fresh human normolipidemic plasma pool. More than 95% of the radioactivity was in the esterified form in the final preparation of 3H-labelled HDL-CE. Unlabelled HDL was used to adjust specific activity to 2000 dpm/µg of CE. The reaction was conducted as follows: 15 µl of plasma samples were incubated in the presence of 10 µg HDL-CE (20000 dpm) and 250 µg LDL-CE+VLDL-CE in a final volume of 250 µl Tris 50 mM, NaCl 150 mM and EDTA 2 mM, pH 7.4. Each sample was measured in triplicate over two hours at 37°C, after which the LDL+VLDL fraction was precipitated with 0.1% 50000 mol. wt dextran sulphate, MgCl2 50 mM (final concentrations). The supernatants containing HDL particles (150 µl) were then collected and counted for radioactivity. Tubes without plasma samples were used as blanks to evaluate non-specific, spontaneous CE transfer (5-11%). The CE transfer rate in a control plasma pool was determined in each experiment and remained linear within a range from 5 to 20 µl. The CETP-dependent transfer of CE was determined as the loss of radioactivity in the HDL particles after incubation, and calculated as the ratio between the difference in the radioactivity transferred in the presence or absence of CETP and total initial radioactivity. CETP activity was estimated as the CE transfer rate expressed as nmol of CE transferred/hour/ml of plasma.

PLTP activity assay

PLTP activity was measured using Damen *et al*'s radiolabelled assay (28) with certain modifications. Briefly, 70 µl of

liposomes containing 125 nmoles L-phosphatidylcholine and trace-labelled with [14C]1-dipalmitoyl phosphatidylcholine were combined on ice with HDL (0.5 µmoles of phospholipids), iodoacetate 1.5 M and 20 µl of plasma, and completed to 250 µl with TSEA buffer (10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1% sodium azide). This reaction mixture was incubated for 15 min at 37°C, and then placed on ice. The liposomes were then precipitated by adding 500 µl TSEA, 100 µl of 1% 50000 mol. wt dextran sulphate and 500mM MgCl2 according to Murdoch *et al* (29). After 30 min on ice, the samples were centrifuged at 3000 rpm for 30 min at 4°C, and radioactivity was measured in 600 µl of the supernatant in order to determine the amount of radioactivity transferred from the radiolabelled liposomes to the HDL during the incubation period. The plasma samples were assayed in duplicate. PLTP activity was expressed as nmoles of PL transferred/hour/ml of plasma. For each assay, the background transfer to HDL in the absence of plasma (blank 37°C) was subtracted from the activity measured in the presence of plasma. A pool of human plasmas was included in each assay as a control in order to correct the inter-assay variation (intra-assay CV=7%; inter-assay CV=3%). The PL transfer was linear between 5 and 30 µl of plasma after 15-min incubation, and between 10 and 30 min for each dose of plasma. Under these conditions, with 20 µl plasma and 15-min incubation, 14% of total 14C PL radioactivity is transferred to HDL.

Statistical analysis

Data are presented as mean values±SD. Student's paired *t* test was used to estimate the statistical differences between parameters. The PP incremental areas under the curves above baseline (iAUC) were calculated using trapezoidal formulae. The associations between variables were tested by means of Pearson's correlation coefficient and stepwise multiple regression analysis. All of the statistical procedures were performed using Microsoft Excel 0.7 software and the Systat statistical package.

Results

PP lipidemia

Table 2 shows that, in comparison with baseline, plasma TG levels increased by 116% after two hours ($p<0.001$) and 114% after four hours ($p<0.0001$), and then progressively decreased until returning to baseline values after eight hours. Peak plasma PL (10%, $p<0.001$) and NEFA levels

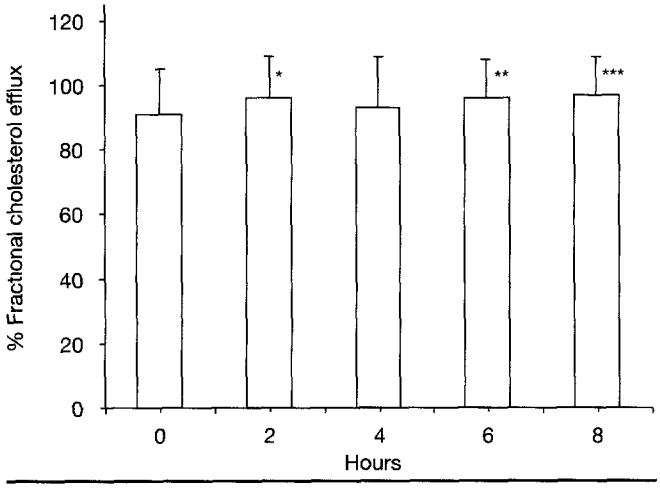
TABLE 2

Variations in plasma lipid parameters in fasting state and during PP phase.

Hours	0	2	4	6	8
Total triglycerides (mmol/L)	0.76±0.21	1 64±0.71***	1.63±0.58***	1.32±0.49**	0.84±0 29
Total cholesterol (mmol/L)	4.65±0 86	4.42±0 67*	4.55±0.78	4 60±0.75	4.55±0.62
Unesterified cholesterol (mmol/L)	1 19±0.21	1.40±0 23**	1.37±0.21*	1 40±0.18**	1 27±0 16
Esterified cholesterol (mmol/L)	3.47±0 91	3±0.54**	3.18±0.70**	3 21±0.67*	3.31±0.49*
Phospholipids (mmol/L)	2 49±0.54	2 52±0 43	2 67±0.48**	2.76±0.43**	2 71±0 39*
Non esterified fatty acids (g/L)	0.10±0.07	0 09±0.03	0.11±0 04	0.18±0 06*	0 20±0.07*
Apo AI (g/L)	1.50±0 28	1.48±0.25	1.47±0.24	1.47±0 23	1 43±0.23
HDL-C (mmol/L)	1.34±0 36	1.29±0.34*	1 27±0.36*	1.32±0 34	1 29±0.34
HDL-TG (mmol/L)	0 08±0.05	0 09±0 03	0.12±0.03*	0.13±0.05***	0 13±0.05*
HDL-PL (mmol/L)	1 19±0.28	1 19±0.25	1.25±0.25*	1 33±0.23**	1.32±0 21*
LDL-C (mmol/L)	2.95±0.59	2.38±0 52***	2.51±0.59***	2.68±0 57**	2.90±0.44
Plasma insulin (pmol/L)	35±14	81±37	38±13	26±12*	27±12*
Plasma glucose (mmol/L)	4.94±0 56	4.50±0 83	5.05±0.50	5.05±0 67	5.05±0.44

Values are mean±SD (n=13) Significant difference between postprandial *vs* fasting values calculated by Student's *t* test * $p<0$ 01, ** $p<0.001$, *** $p<0$ 0001.
Apo apolipoprotein, HDL-C high-density lipoprotein cholesterol, LDL-C low-density lipoprotein cholesterol, PL phospholipids, PP. postprandial, TG· triglycerides

FIGURE 1
Postprandial (PP) variation in plasma cholesterol efflux capacity in male normolipidemic subjects estimated by the release of 3H-cholesterol from Fu5AH cells incubated for four hours with fasting and PP human plasma diluted to 5% in the cell medium The fractional cholesterol efflux is expressed as the % of a control plasma pool tested under the same conditions. Each data point is the average of triplicate determinations. Mean values±SD (n=13). Significant changes (paired Student's *t* test) in PP *vs* fasting values are indicated by * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



(105%, $p<0.01$) were reached after respectively six and eight hours. During the early PP phase (2h-PP), total cholesterol, CE and LDL-C levels respectively decreased by 5% ($p<0.02$), 13% ($p<0.001$) and 20% ($p<0.0001$), whereas UC levels showed a 19% increase ($p<0.001$) that was maintained until six hours PP. HDL composition was modified throughout the PP period: during the early PP phase, HDL-C slightly but significantly decreased by 4% after two hours ($p<0.01$) and 5.7% after four hours ($p<0.0001$): after eight hours, HDL-TG had significantly increased by 56% ($p<0.01$) and HDL-PL by 11.5% ($p<0.01$). Plasma insulin levels increased by 128% ($p=0.0001$) after two hours, and then rapidly decreased to significantly lower levels in comparison with the fasting level (26% at 6h, $p=0.007$; and 23% at 8h, $p=0.01$). There were no significant changes in plasma glucose levels between the second and eighth PP hours.

PP plasma cholesterol efflux capacity

Plasma cholesterol efflux capacity was stimulated during the PP phase and was significantly higher than baseline after two (5.8%, $p<0.05$), six (5.3%, $p<0.05$) and eight hours (6.5%, $p<0.05$) (Fig 1). Table 3 shows that plasma cholesterol efflux capacity positively correlated with HDL-PL, HDL-C and Apo-AI at baseline and throughout the PP phase. Multiple stepwise regression analysis with cholesterol efflux as the dependent variable and plasma components as independent variables showed that HDL-PL is always the main predictor of cholesterol efflux.

PP evolution of plasma CETP activity

The assay used to determine CETP-dependent CE transfer, which is based on an exogenous labelled donor and an excess of exogenous CE acceptors, has been shown to reflect CETP mass (27). As whole plasma samples were used, CETP activity may have been modified by endogenous factors in the samples themselves (30).

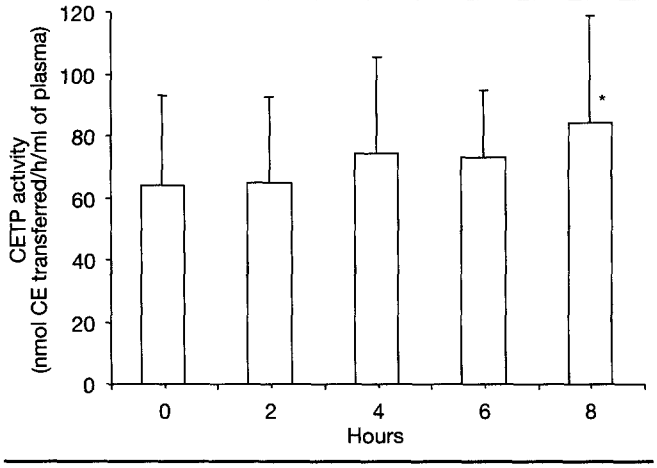
TABLE 3
Relationship between plasma cholesterol efflux capacity and plasma parameters during postprandial phase

Hours	0	2	4	6	8
Correlation between plasma cholesterol efflux capacity and the following parameters					
HDL-C	0,76**	0,59*	0,80**	0,64*	0,68**
HDL-PL	0,89***	0,77**	0,92***	0,88***	0,88***
HDL-TG	0,68**	NS	NS	NS	NS
Apo AI	0,74**	0,64*	0,72**	0,70**	0,58*
NEFA	0,60*	NS	NS	NS	NS
CETP activity	NS	NS	NS	NS	NS
PLTP activity	NS	NS	NS	NS	NS

Values represent Pearson's correlation coefficient (r) for n=13 Statistical significances are indicated as *$p<0.05$, **$p<0.01$, ***$p<0.0001$ Apo apolipoprotein, CETP cholesteryl ester transfer protein; HDL-C high-density lipoprotein cholesterol, NEFA non-esterified fatty acids, NS not significant; PL. phospholipids; PLTP phospholipid transfer protein, TG triglycerides

FIGURE 2

PP variation of plasma CETP activity in male normolipidemic subjects assayed by incubating 15 µl of plasma samples with ^{3}H-cholesterol labelled exogenous HDL-CE and exogenous acceptors (VLDL+LDL) for 2h at 37°C in 50 mM Tris, 150mM NaCl, 2mM EDTA, pH 7.4, in a final volume of 250 µl. CETP activity was determined as the loss of ^{3}H-CE from HDL after incubation, and calculated as the ratio between the difference in radioactivity transferred in the presence or absence of CETP and total initial radioactivity. The CE transfer rate is expressed as nmol of ^{3}H-HDL-CE transferred/hour/ml of plasma. Mean values±SD (n=13) Significant change at 8h-PP (paired Student's *t* test) are indicated by * $p<0.05$ *vs* 2h-PP values. CE: cholesteryl esters; CETP: cholesterol ester transfer protein; EDTA: ethylenediaminetetraacetic acid; HDL: high-density lipoproteins; LDL: low-density lipoproteins; PP: postprandial; VLDL: very low-density lipoproteins.



Mean CETP activity progressively increased throughout the PP phase, reaching a peak after eight hours (84.5±34 nmol/h/ml). This value was not significantly different from baseline (32%, $p=0.06$) because of the inter-individual heterogeneity of fasting levels, but it was significantly different from the value measured after two hours (30%, $p=0.04$) (Fig 2). The fasting values significantly and negatively correlated with the PP-incremental iAUC ($r=-0.56$, $p<0.05$), thus suggesting that the fasting CETP level may determine the magnitude of the PP response. Plasma CETP activity in the fasting state positively correlated with plasma NEFA levels ($r=0.70$, $p<0.01$). No correlations were found between CETP and the other tested parameters (Table 3), but multiple stepwise regression analysis with CETP activity as the dependent variable revealed a positive correlation with NEFA ($p<0.001$) and a negative correlation with HDL-C ($p<0.004$) only in the fasting state, thus indicating that NEFA and HDL-C levels were the main predictors of fasting CETP activity.

PP evolution of plasma PLTP activity

Plasma PLTP activity did not significantly vary during the PP period (Fig 3). There were no significant correlations between fasting PLTP activity and plasma lipid components, or plasma insulin or glucose levels. PLTP activity did not correlate with CETP activity or plasma cholesterol efflux capacity.

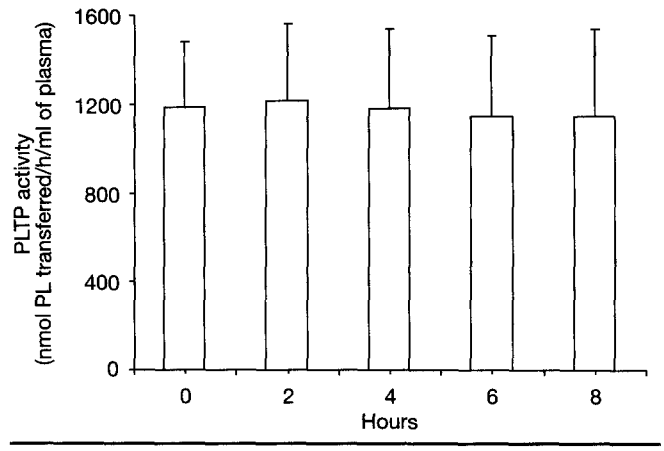
Discussion

We evaluated the impact of PP modifications on three key steps in the reverse cholesterol transport process by measuring the capacity of cholesterol efflux and variations in CETP and PLTP activities in PP plasma samples taken from normolipidemic subjects after a 1000 Kcal test meal consisting of usual solid foodstuffs and containing a moderate amount of fat (67 g) and 300 mg of cholesterol.

As expected, the PP changes in lipidemia during the initial PP phase (2h-4h PP) were characterised by a peak increase in plasma TG levels, whereas there was a slight but

FIGURE 3

Postprandial variation of plasma PLTP activity in male normolipidemic subjects assayed by incubating 20 µl of plasma in the presence of 70µl liposomes containing 125 nmoles L-phosphatidylcholine and trace labelled with [^{14}C]1-dipalmitoyl phosphatidylcholine and exogenous HDL at 37°C for 15 min in TSEA buffer (10mM Tris, 150mM NaCl, 1mM EDTA, 0.1% sodium azide) in a final volume of 250 µl. PLTP activity was determined as the amount of radiolabelled PL transferred from liposomes to HDL. PLTP activity is expressed as nmoles of PL transferred/hour/ml of plasma. Mean values±SD (n=13). EDTA: ethylenediaminetetraacetic acid; HDL: high-density lipoproteins; PLTP: phospholipid transfer protein.



significant reduction in plasma cholesterol fractions (CE, LDL-C and HDL-C). During the later PP phase (6-8h), there was a large and significant increase in plasma NEFA, HDL-TG and HDL-PL levels, whereas TG levels decreased to baseline levels (as indicators of enhanced lipolysis, and CETP- and PLTP-mediated transfers).

Plasma cholesterol efflux capacity in the PP phase was studied using whole plasma (including chylomicrons and remnants) in order to maintain the *in vivo* physiological situation of humans who spend almost 80% of their lives in the PP state.

Cholesterol efflux from Fu5AH cells was stimulated throughout the 8h-PP period, and peaked eight hours after the fatty meal. Plasma cholesterol efflux capacity in the fasting and PP states positively correlated with HDL-C, HDL-PL and Apo-AI levels, but HDL-PL proved to be the best predictor of plasma cholesterol efflux capacity at each PP timepoint. These results confirm the importance and efficiency of HDL-PL in promoting cellular cholesterol efflux, as has been reported in the case of PL-enriched PP HDL from humans (25) and serum from rats or humans (30) using the same cell culture model. Moreover, Fu5AH cells express high levels of the SR-BI receptor, which mediates cell cholesterol efflux in a HDL-PL dependent manner (31).

CETP is the key protein involved in the redistribution of CE and TG between the different lipoprotein fractions. In our experiments, CETP activity increased during the late PP period. This may have been due to an increase in CETP mass or a modulation in protein activity induced by the compositional and structural variations in plasma lipoprotein substrates occurring during PP hypertriglyceridemia. Our results agree with those of other PP studies using high-fat test meals including 150-600 mg of dietary cholesterol (10-12, 32), but the results of PP studies using test meals associated with dietary cholesterol levels of less than 150 mg (13, 33, 34) indicate a lack of effect on CETP. These diverging results may be due to the influence of different levels of dietary fat and cholesterol on CETP synthesis as a consequence of an increase in CETP gene expression in response to the high-fat, high-cholesterol content of the diets (35). If so, the PP increase in plasma CETP observed in our subjects after a meal containing a moderate quantity of fat and more than 150 mg of cholesterol may have been due to the effect of dietary cholesterol on CETP expression.

CETP activation during the late PP period (6h-8h) was accompanied by a large increase in plasma NEFA, thus suggesting the possible role of lipoprotein-bound NEFA in enhanced CETP-mediated CE transfer between the different lipoproteins (36-38). Stimulated CETP activity may also have been due to increased protein binding to phospholipid-enriched HDL (10), because the phospholipid fraction of HDL particles concomitantly increased in the later PP phase. However, despite the positive fasting correlation between CETP activity and NEFA levels, and the increases in CETP activity, NEFA and HDL-PL, during the later PP phase no correlations were found between these parameters at any PP time in terms of absolute concentrations or PP-incremental values (data not shown).

Both CETP activity and plasma cholesterol efflux capacity were stimulated during the PP phase, which may support the previously observed stimulation of reverse cholesterol transport under the influence of PP lipolysis (36, 39). However, no correlation was found between these two processes, which suggests that the rate of CETP-mediated CE transfer between TGRL and HDL did not directly modulate the rate of cell cholesterol efflux.

The importance of PLTP in lipoprotein metabolism has been underlined by a number of recent studies. Given the proposed role of PLTP in PL transfer between TGRL and HDL during PP lipolysis, it can be hypothesised that the increase in TGRL levels during the PP phase may enhance PLTP-mediated PL transfer to HDL. Nevertheless, the question of the interrelations between PP plasma PLTP activity, plasma cholesterol efflux capacity and plasma lipid metabolism remains open, and so it was thought to be interesting to measure PLTP activity and investigate its relationship with PP plasma cholesterol efflux capacity and lipids in the same group of normolipidemic subjects. PLTP activity measured using this technique is related to PLTP mass, especially its catalytically active entity (40).

Fasting PLTP activity did not correlate with CETP activity, plasma cholesterol efflux capacity or plasma lipids. The absence of a relationship between PLTP activity and plasma cholesterol capacity is in line with the results obtained in the control group of another study using the same cell model (41). Furthermore, the absence of a significant relationship between PLTP activity and plasma lipid parameters has also been previously reported (42-44).

During PP lipidemia, plasma PLTP activity did not vary and did not correlate with plasma lipid components, particularly plasma TGRL (which may enhance PL transfer during PP lipolysis) and HDL-TG and NEFA levels, which have been shown to facilitate interactions between HDL and PLTP (20, 45). Our data therefore indicate that PLTP activity (or mass) does not seem to limit PL transfer during PP lipidemia and that variations in HDL composition do not influence PLTP activity. Furthermore, there do not seem

to be any correlations between plasma PLTP activity and plasma cholesterol efflux capacity.

We therefore conclude that, after the ingestion by normolipidemic subjects of a single fatty meal containing moderate amounts of fat and cholesterol, increased plasma cholesterol efflux capacity is accompanied by increased CETP activity but no change in PLTP activity, thus indicating that these proteins are controlled by a different set of regulatory signals during the PP phase. The capacity of plasma to promote PP cellular cholesterol efflux is not related to the lipid transfer activities of CETP and PLTP, thus indicating that these may not necessarily modulate the initial step of cell cholesterol efflux. Furthermore, the activities of these two proteins are not interrelated. HDL-PL remains the key factor in the regulation of the capacity of plasma to promote cholesterol efflux during PP lipidemia.

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