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Human plasma phospholipid transfer protein activity is decreased by acute hyperglycaemia: studies without and with hyperinsulinaemia in Type 1 diabetes mellitus

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Abstract

Aims Little is known about the regulation of phospholipid transfer protein (PLTP), that plays a key role in lipoprotein metabolism. PLTP secretion may be up-regulated by glucose *in vitro*, whereas plasma PLTP activity is decreased by exogenous hyperinsulinaemia and glucose-induced hyperinsulinaemia *in vivo*. In the present study, we evaluated the separate effects of hyperglycaemia and hyperinsulinaemia in C-peptide-negative Type 1 diabetic patients.

Methods The protocol was carried out in 16 patients (eight females). In each individual, plasma PLTP mass and activity (measured by enzyme-linked immunosorbent assay and liposome-high density lipoprotein system, respectively) as well as plasma cholesteryl ester transfer protein (CETP) activity, lipids and apolipoprotein levels were determined at the end of four different glucose clamps, each lasting 210 min: standard insulin (30 mU/kg/h) and standard glucose (glucose 5.0 mmol/l) (SI-SG), standard insulin and high glucose (glucose 12 mmol/l) (SI-HG), high insulin (150 mU/kg/h) and standard glucose (HI-SG), and high insulin and high glucose (HI-HG).

Results Plasma lipids and (apo)lipoproteins, measured at the end of the SI-HG, HI-SG and HI-HG clamps, were not significantly different compared with the levels obtained at the end of the SI-SG clamp. Median plasma PLTP mass and activity at the end of the SI-SG clamp were 12.8 mg/l and 13.2 μmol/ml/h, respectively. Median plasma PLTP mass decreased by 9.1% at the end of the HI-HG clamp ($P < 0.01$), whereas the changes at the end of the SI-HG and HI-SG clamps were not significant. Median plasma PLTP activity decreased by 5.7, 4.6 and 8.6% at the end of the SI-HG, HI-SG and HI-HG clamps, respectively (all $P < 0.05$). Median plasma CETP activity was 177 nmol/ml/h at the end of the SI-SG clamp, and decreased by 4.9% ($P < 0.05$) and by 8.3% ($P < 0.05$) at the end of the HI-SG and the HI-HG clamps, respectively. Plasma CETP activity did not change significantly at the end of the SI-HG clamp.

Conclusions The present study demonstrates that plasma PLTP activity is independently decreased by acute hyperglycaemia and hyperinsulinaemia in humans *in vivo*. These data do not support a direct role of short-term hyperglycaemia in up-regulating plasma PLTP levels.

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Keywords diabetes mellitus, hyperglycaemia, hyperinsulinaemia, plasma cholesteryl ester transfer protein, plasma phospholipid transfer protein

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Abbreviations CETP, plasma cholesteryl ester transfer protein; HDL, high-density lipoprotein; HI-HG, high insulin, high glucose; HI-SG, high insulin, standard glucose; LDL, low-density lipoprotein; PLTP, plasma phospholipid transfer protein; SI-HG, standard insulin, high glucose; SI-SG, standard insulin, standard glucose; VLDL, very low density lipoprotein

Introduction

The importance of phospholipid transfer protein (PLTP) in lipoprotein metabolism is increasingly recognized [1–6]. This lipid transfer protein specifically mediates the transfer of phospholipids between lipoproteins, facilitates the transfer of surface fragments from triglyceride-rich lipoproteins to high-density lipoproteins (HDL) during lipolysis, may enhance the cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester transfer from HDL to very low and low-density lipoproteins (VLDL and LDL), and is involved in regulating lipoprotein vitamin E content, thereby affecting lipid oxidation [1–8]. Moreover, PLTP is able to convert the dense HDL_3 subfraction into smaller and larger HDL particles by of particle fusion [9–12]. During this HDL conversion process, small pre-β-HDL particles are produced that are considered to be important initial acceptors of cell-derived cholesterol [13–15]. Indeed, pre-β-HDL formation is positively correlated with PLTP activity in plasma from mice and man [5,16]. Thus, PLTP appears to be one of the key factors in the reverse cholesterol transport pathway, whereby cholesterol is transported from peripheral cells back to the liver where it is metabolized and excreted in the bile [15,17,18]. In addition, it has been recently demonstrated that PLTP deficiency attenuates, whereas PLTP overexpression stimulates, hepatic VLDL secretion in mice [19,20]. PLTP is produced by several cell systems including liver HepG2 cells [21], adipose tissue [22] and macrophages [23].

Knowledge about the regulation of PLTP is incomplete. In man, plasma PLTP activity, assayed by methods that measure the activity of plasma PLTP independently from the endogenous lipoproteins involved, is elevated in conditions associated with insulin resistance, including obesity, Type 2 diabetes mellitus and smoking [24–30]. There appears to be a metabolic interrelationship between insulin, plasma triglyceride and PLTP regulation [25,31]. An intravenous triglyceride challenge increases plasma PLTP activity [32]. In turn, 3–24 h of exogenous hyperinsulinaemia as well as acipimox administration lowers plasma PLTP activity, together with a decrease in plasma free fatty acids and triglycerides in healthy subjects [27,28]. A decrease in plasma PLTP activity has also been observed in healthy subjects in response to short-term hyperglycaemia [33], but the interpretation of this observation is hampered because the effects of hyperglycaemia per se and that of the resulting hyperinsulinaemia cannot be separated. None of these studies documented plasma PLTP concentration. Using a molecular biological approach, it has been demonstrated that PLTP is subject to genetic regulation via responsive elements for the peroxisome proliferator activated receptor-α (PPAR-α) [34], the farnesoid X-activated receptor [35] and liver X receptors [36]. Of interest, one report showed that human PLTP transcription in HepG2 cells is up-regulated by glucose via nuclear hormone receptors in the promotor region of the PLTP gene [37]. This *in vitro* experiment appears to be in contrast with the *in vivo* observation showing that plasma PLTP activity is decreased in response to hyperglycaemia [33]. Thus, it is of interest to measure human plasma PLTP activity levels in an experimental *in vivo* design that documents the effects of hyperinsulinaemia and hyperglycaemia separately.

The purpose of the present study was to evaluate the effects of short-term hyperglycaemia and hyperinsulinaemia, as separate interventions as well as in combination, on plasma PLTP activity and concentration by performing clamp studies in men and women with Type 1 diabetes mellitus.

Patients and methods

The protocol was approved by the medical ethics committee of the Groningen University and all patients provided written informed consent. Eligible patients were recruited by advertisement in a local newspaper. Diabetes mellitus was diagnosed applying World Health Organization criteria [38]. All subjects had ketosis-prone diabetes mellitus and were considered to suffer from Type 1 diabetes mellitus on clinical grounds. Insulin deficiency was confirmed by a plasma C-peptide concentration < 0.2 nmol/l after glucagon stimulation. Age at onset of disease was < 30 years and disease duration was > 10 years in all participants. Exclusion criteria were clinically manifest cardiovascular disease, smoking, hypertension (systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg), a urinary albumin excretion rate > 20 μg/min (pre)proliferative retinopathy, peripheral neuropathy, liver function test abnormalities, thyroid dysfunction, a body mass index (BMI) > 27 kg/m^2 and pregnancy. Retinopathy was quantified by routine fundoscopy performed by an ophthalmologist. Background retinopathy was defined as a few scattered retinal haemorrhages and microaneurysms. No medication other than insulin and oral contraceptives were used. In the female patients, all studies were carried out between day 4 and 21 of their menstrual cycle.

The study was designed as a randomised crossover trial. All subjects were studied on four separate days, during which four different glucose-clamps were performed in random order. At least 3 days separated each clamp. Subjects arrived at the clinic after an overnight fast, without using their morning insulin dose. Glucose was measured (YSI Model 23A, Yellow Springs, OH, USA) every 5 min in blood samples from a catheter positioned in an antecubital vein of the right forearm which was positioned in a heated box in order to achieve arterialized blood. A primed insulin infusion (Velosulin, Novo Nordisk, Denmark) at a fixed rate for each clamp, which was combined with a variable

glucose infusion (20%) to which 20 mmol/l potassium chloride was added to prevent hypokalaemia, was administered in an antecubital vein of the contralateral arm. Insulin was infused at 30 mU/kg/h (standard insulin, SI) or at 150 mU/kg/h (high insulin, HI) in two clamps each. For both SI and HI schedules, glucose was clamped at a target level of 5.0 mmol/l (standard glucose, SG) during one clamp and at 12.0 mmol/l (high glucose, HG) during the other clamp. The standard insulin, standard glucose (SI-SG) clamp served as reference to study the effects of hyperinsulinaemia and hyperglycaemia. The duration of the clamps was 210 min. At the beginning of each clamp, insulin administration was started at the predetermined infusion rate. The first 150 min were used to achieve target glucose levels, after which glucose was maintained at this level for 60 min. At the end of each clamp, blood samples for determination of plasma free insulin (apo)lipoproteins, PLTP mass and activity and CETP activity were obtained. All 16 patients completed all four glucose clamps.

Laboratory analysis

Arterialized venous blood was collected into tubes containing ethylene diaminetetraacetic acid (1.5 mg/l) and was placed on ice immediately. Plasma was obtained by centrifugation at 3000 r.p.m. for 15 min at 4°C. Plasma aliquots were stored at −80°C until analysis. HDL cholesterol was assayed by a homogeneous method [39] using a commercially available assay system (Abbott Inc., cat no. 30–3064/R3, Abbott Park, Ill, USA). VLDL + LDL cholesterol was calculated as the difference between plasma total cholesterol and HDL cholesterol. Cholesterol and triglycerides were measured enzymatically. Apo AI and B were assayed by immunoturbidimetry using commercially available kits (Boehringer Mannheim, Germany, cat nos. 7266478 and 726494, respectively).

Plasma PLTP activity was assayed in a liposome vesicles-HDL system as described [40]. Plasma samples were incubated with [^{3}H]-phosphatidylcholine labelled vesicles and an excess of pooled normal HDL. Subsequently, the liposomes were precipitated with a mixture of NaCl, $MgCl_2$ and heparin at final concentrations of 230 mmol/l, 92 mmol/l and 200 U/ml, respectively. The method is not affected by the phospholipid transfer promoting action of cholesteryl ester transfer protein [40]. The PLTP activity so measured is linearly related to the amount of plasma used in the incubations and is not influenced by the endogenous lipoproteins in plasma. Plasma PLTP activity is expressed in µmol/ml/h. Plasma PLTP concentration was measured with a sandwich enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies (mAb113 and mAb114) raised against recombinant human PLTP as described [41]. The within-assay coefficients of variation of plasma PLTP activity and PLTP mass amount to 3.5 and 3.0%, respectively. Plasma CETP activity was assayed after removal of VLDL + LDL from each sample as described [24]. The assay system measures the transfer of [^{14}C-oleate]-cholesteryl ester from labelled LDL to an excess of unlabelled pooled normal HDL, while lecithin: cholesterol acyltransferase is inhibited with dithiobis-2-nitrobenzoic acid. CETP activity is calculated as the bi-directional transfer of cholesteryl esters between labelled LDL and HDL. Plasma CETP activity is expressed in nmol/ml/h. The within-assay coefficient of variation is 2.7%.

Blood glucose was measured with an YSI glucose analyser (Model 23A, Yellow Springs, OH, USA). Plasma-free insulin was assayed by radioimmunoassay (Pharmacia & Upjohn, Uppsala, Sweden). Glycated haemoglobin (HbA_{1c}) was measured by high performance liquid chromatography (Bio-Rad, Veenendaal, the Netherlands; reference range in adults: 4.6–6.1%).

Statistical analysis

Data are given in median (interquartile range). Friedman's non-parametric two-way analysis of variance was used to evaluate the changes in variables at the end of the SI-HG, the HI-SG and the HI-HG clamps with the values at the end of the SI-SG clamp as reference. Duncan's method was applied to correct for multiple comparisons. Previous data from our laboratory showed a day-to-day variation in PLTP activity, expressed as a Pearson's coefficient of correlation for repeated measurements, of 0.90 [28]. The standard deviation in PLTP activity was 15% of the mean value [28]. To detect a 5% change in PLTP activity ($\alpha = 0.05$, $\beta = 0.20$) a group size of 16 participants was calculated. Two-sided P-values < 0.05 were considered significant.

Results

Sixteen Type 1 diabetic patients, eight females and eight males, were selected for the study. Their clinical characteristics and HbA_{1c} levels are provided in Table 1.

Plasma total cholesterol, HDL cholesterol, VLDL + LDL cholesterol, triglycerides, apo AI and apo B concentrations obtained at the end of the SI-HG, the HI-SG and the HI-HG clamps were not significantly different from the levels at the end of the SI-SG clamp (Table 2). Median plasma PLTP mass and PLTP activity levels at the end of the SI-SG clamp are shown in Table 3. Median plasma PLTP mass significantly decreased by 9.1% (interquartile range −21.5 to −0.9%) at the end of the HI-HG clamp ($P = 0.002$). The changes in PLTP mass at the end of the SI-HG ($P = 0.168$) and HI-SG ($P = 0.663$) clamps were not significant. Median plasma PLTP activity decreased significantly at the end of the SI-HG (−5.7%, interquartile

Table 1 Clinical and laboratory parameters of the study participants

	Median (interquartile range)
Age (years)	25 (21–28)
Diabetes duration (years)	14 (10–17)
Body mass index (kg/m^2)	22.3 (20.7–25.3)
Systolic blood pressure (mmHg)	131 (121–135)
Diastolic blood pressure (mmHg)	80 (74–82)
Urinary albumin excretion rate (µg/min)†	4 (3–6)
Retinopathy‡ (*n*)	14; 2; 0
HbA_{1c} (%)	7.7 (7.1–8.4)
Serum creatinine (µmol/l)	81 (75–90)
Total cholesterol (mmol/l)	4.3 (3.8–4.9)
Triglycerides (mmol/l)	0.9 (0.5–1.1)
HDL cholesterol (mmol/l)	1.2 (1.0–1.4)

†Average excretion rate obtained in three consecutive overnight urine collections immediately before the first glucose clamp.
‡Absent; background; (pre)proliferative.

Table 2 Glucose clamp characteristics and plasma (apo)lipoproteins

	SI-SG (standard insulin, standard glucose)	SI-HG (standard insulin, high glucose)	HI-SG (high insulin, standard glucose)	HI-HG (high insulin, high glucose)
Insulin infusion rate (mU/kg/h)	30	30	150	150
Plasma free insulin† (mU/l)	23 (21–30)	20 (19–29)	114 (92–139)	108 (90–140)
Blood glucose‡ (mmol/l)	4.7 (4.6–5.0)	11.7 (11.4–12.0)	4.7 (4.5–5.0)	11.8 (11.6–11.9)
Glucose infusion rate‡ (mg/kg/min)	3.2 (2.6–4.6)	5.7* (3.8–8.9)	10.4*** (7.1–12.4)	16.1*** (12.1–24.6)
Total cholesterol† (mmol/l)	4.1 (3.7–4.5)	4.1 (3.5–4.7)	4.2 (3.6–4.5)	4.0 (3.5–4.6)
HDL cholesterol† (mmol/l)	1.1 (0.9–1.3)	1.0 (0.8–1.4)	1.1 (1.0–1.3)	1.1 (0.9–1.3)
VLDL + LDL cholesterol† (mmol/l)	3.0 (2.5–3.8)	2.9 (2.4–3.7)	3.1 (2.7–3.3)	2.7 (2.4–3.6)
Triglycerides† (mmol/l)	0.6 (0.4–0.8)	0.5 (0.4–0.7)	0.5 (0.4–0.7)	0.5 (0.3–0.7)
Apo AI† (g/l)	1.52 (1.37–1.63)	1.33 (1.27–1.61)	1.47 (1.35–1.58)	1.41 (1.22–1.53)
Apo B† (g/l)	0.87 (0.70–1.05)	0.87 (0.64–0.96)	0.81 (0.69–1.01)	0.80 (0.68–0.96)

*$P < 0.05$ vs. SI-SG; ***$P < 0.001$ vs. SI-SG.
Data are expressed as median (interquartile range).
†Determined at the end of each glucose-clamp. ‡Obtained over the last hour of the clamp.

Table 3 Plasma PLTP mass, PLTP activity and CETP activity at the end of the glucose clamp

	SI-SG (standard insulin, standard glucose)	SI-HG (standard insulin, high glucose)	HI-SG (high insulin, standard glucose)	HI-HG (high insulin, high glucose)
PLTP mass (mg/l)	12.8 (8.1–14.1)	11.3 (8.4–14.0)	11.8 (9.2–14.6)	10.7 (7.8–12.8)**
PLTP activity (μmol/ml/h)	13.2 (11.6–14.5)	12.6 (11.2–13.5)*	13.0 (11.7–14.0)*	12.7 (10.9–13.3)**
CETP activity (nmol/ml/h)	177 (165–183)	165 (151–207)	170 (157–186)*	165 (147–175)**

*$P < 0.05$ vs. SI-SG; **$P < 0.01$ vs. SI-SG.
Data are expressed as median (interquartile range).

range −14.4 to −1.7%, $P = 0.020$), the HI-SG (−4.6%, interquartile range −8.9 to +1.9%, $P = 0.021$), and the HI-HG clamps (−8.6%, interquartile range −11.9 to −4.3%, $P = 0.002$). Median plasma CETP activity (Table 3) decreased at the end of the HI-SG (−4.9%, interquartile range −11.6 to +1.7%, $P = 0.012$) and the HI-HG (−8.3%, interquartile range −13.9 to −3.5%, $P = 0.005$) clamps. Median plasma CETP activity did not significantly change at the end of the SI-HG clamp ($P = 0.085$).

Discussion

The present experiments were carried out in C-peptide-negative subjects with Type 1 diabetes mellitus to separate the effects of hyperglycaemia and hyperinsulinaemia on the regulation of plasma PLTP mass and activity. It was found that both short-term hyperglycaemia per se and hyperinsulinaemia decrease plasma PLTP activity. In addition, plasma CETP activity was significantly lowered in response to hyperinsulinaemia, either alone or in combination with hyperglycaemia. The current experiments therefore extend studies in healthy subjects and Type 2 diabetic patients showing that plasma PLTP and to some extent CETP is decreased by exogenous insulin [27,28,42]. Our finding that plasma PLTP activity is decreased by hyperglycaemia without concomitant hyperinsulinaemia is novel and makes it unlikely that the high plasma PLTP activity levels found in both Type 1 and Type 2 diabetes mellitus [26,43] are directly related to a high ambient blood glucose level.

The mechanisms responsible for the acute lowering effects of hyperglycaemia per se and hyperinsulinaemia on plasma PLTP activity are unknown. Theoretically, this decrease in plasma PLTP could be as a result of diminished release of tissue-derived PLTP in the plasma compartment, enhanced PLTP

clearance or a combination of these mechanisms. Obviously, the results of the current experiments cannot be directly compared with recent data showing that PLTP activity secreted by HepG2 cells is 65–96% stimulated by *in vitro* incubation with 12.5 or 25 mmol/l glucose, respectively [37]. In that study, there was also a small (about 30%) up-regulation of PLTP mRNA levels. The latter was only observed with 25 mmol/l of glucose and osmolarity-controlled experiments were not carried out. It is possible that the presently employed duration of hyperglycaemia was too short or that the degree of hyperglycaemia was not high enough to alter PLTP expression in tissues. This relatively moderate degree of hyperglycaemia was chosen to mimic blood glucose levels as regularly encountered in diabetic subjects.

Several further methodological aspects of our study need to be mentioned. First, the Type 1 diabetic patients were studied during eu- and hyperglycaemia with a plasma insulin concentration of about 25 mU/l. This plasma insulin level is comparable with that encountered during daily life in this patient category, but higher than that observed in fasting healthy subjects. Such an insulin level is necessary to render Type 1 diabetic patients euglycaemic. Indeed, as a possible manifestation of insulin resistance, plasma triglycerides did not significantly decrease in response to hyperinsulinaemia, which contrasts with findings in healthy subjects [27,33]. To circumvent the possible drawback of a modestly high plasma insulin level, a completely different study design would have been required by using somatostatin administration in healthy subjects to prevent the occurrence of hyperinsulinaemia in response to hyperglycaemia. However, the possibility that somatostatin affects PLTP regulation cannot be excluded and this consideration would have strongly limited the interpretation of the data. Second, it can be argued that plasma dilution consequent to the glucose clamp procedures is in part responsible for the decreases in plasma PLTP. A relevant effect of volume loading on the plasma PLTP lowering is very unlikely because there was no significant decrease in plasma (apo)lipoproteins in response to hyperglycaemia and hyperinsulinaemia in the present study. Moreover, in previous studies we have shown that the drop in plasma PLTP activity after hyperglycaemia-induced hyperinsulinaemia as well as after exogenous insulin infusion remains evident when compared with a similar volume load [27,33].

In contrast with the strong correlation of CETP activity with its plasma concentration [44], equivocal data have been reported regarding the relationship between plasma PLTP mass and PLTP activity. It has been proposed that PLTP in human plasma exists in two forms, one active and one inactive, with active and inactive PLTP present in different macromolecular complexes [45–47]. It is therefore of interest to measure both plasma PLTP activity and its mass when evaluating factors involved in plasma PLTP regulation [18]. The decrease in plasma PLTP mass in response to combined hyperglycaemia and hyperinsulinaemia is novel and provides complementary information compared with measurement of plasma PLTP activity alone.

The stimulatory effect of PLTP on several steps in the reverse cholesterol transport pathway [6,13–15,17,18] can be interpreted to be anti-atherogenic. Alternatively, *in vivo* studies in hyperlipidaemic mouse models show that PLTP deficiency attenuates, whereas PLTP overexpression accelerates atherosclerosis development [19,48]. Recent data have demonstrated that plasma PLTP activity is positively and independently related to prevalent coronary artery disease, with a 13% higher plasma PLTP activity in coronary patients [49]. Increments in plasma PLTP activity of approximately 15% compared with healthy subjects have been observed in Type 1 diabetic patients and are thought to largely explain diabetes-related differences in plasma apo AI levels and altered HDL size distribution [50]. Thus, relatively minor differences in plasma PLTP activity may have pathophysiological relevance. In the present study, a median drop in plasma PLTP activity of 5.7 and 4.6% was found after hyperglycaemia and hyperinsulinaemia, respectively. In comparison, a decrease in plasma PLTP activity of about 6–10% was previously observed after short-term hyperinsulinaemia in obese and non-obese healthy subjects [27].

In conclusion, plasma PLTP activity is decreased by acute hyperglycaemia as well as by hyperinsulinaemia in humans *in vivo*. These results do not support the possibility that short-term moderate hyperglycaemia up-regulates plasma PLTP.

Competing interests

None declared.

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