Human seminal plasma displays significant phospholipid transfer activity due to the presence of active phospholipid transfer protein

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The lipid composition of germ cell membranes is considerably modified during spermatogenesis, sperm maturation and capacitation. Some of these modifications are caused by exchanges between soluble lipid donors or acceptors and cell membranes. The aim of this study was to assess whether significant lipid transfers between lipoprotein structures are detectable in human seminal plasma. Phospholipid and cholesteryl ester (CE) transfer activities were measured by specific fluorescence and isotopic assays. Seminal plasma samples did not display significant CE transfer. Substantial levels of phospholipid transfer activity were detected in all samples studied, levels were ~25% of the phospholipid transfer activity measured in human blood plasma. Concordantly, CE transfer protein was not detected in seminal plasma, while the presence of the phospholipid transfer protein (PLTP) was confirmed by Western blot analysis. Enzyme-linked immunosorbent assay indicated that seminal PLTP concentrations represented 25% of the concentration measured in blood plasma. Blockade of phosphatidylcholine and phosphatidyl-ethanolamine transfer by a 60 min, 56°C heating step or with anti-PLTP antibody revealed that PLTP accounts for almost 80% of the phospholipid transfer activity present in seminal plasma. As shown by gel-permeation chromatography and Western blot analysis, seminal PLTP activity was partially associated with prostasomes. Significantly higher PLTP activity levels were measured in seminal plasma samples with low seminal vesicle secrections. The latter observation may reflect the sustained secretion of active PLTP that is diluted in a variable volume of PLTP-free seminal vesicle secretion. In conclusion, human seminal plasma displays significant phospholipid transfer activity due to the presence of active PLTP.

Key words: cholesterol/cholesteryl ester transfer protein/phospholipid/phospholipid transfer protein/semen plasma

Introduction

Spermatozoa are surrounded by a limiting plasma membrane that mediates many of the early events of the fertilization process. Spermatozoa membranes, that derive from spermatagonia and spermatocytes, are considerably modified through changes in their cholesterol and phospholipid composition during spermatogenesis, sperm maturation and capacitation (Martinez and Morros, 1996; Nolan and Hammerstedt, 1997; Cross, 1998; Jones, 1998; Flesch and Gadella, 2000; Flesch et al., 2001). Some of these modifications are caused by exchanges between cell membranes and lipid donors or acceptors, raising a considerable interest in identifying the factors that may catalyse lipid transfer in seminal plasma. Recently, human epididymal protein-1 (HE-1), a soluble protein secreted by epididymis in humans and other species, was shown to bind free cholesterol specifically with high affinity. It might be involved in regulation of the lipid composition of the sperm membrane (Kirchhoff et al., 1996; Okamura et al., 1999; Fouchecourt et al., 2000). Other recent studies support a role for seminal plasma phospholipid-binding proteins in mediating the sperm membrane lipid modifications (Desnoyers and Manjunath, 1992; Moreau et al., 1999; Manjunath and Therien, 2002). These single chain, acidic polypeptides, also called bovine seminal plasma (BSP) proteins, are secreted by seminal vesicles, and bind to the sperm surface at ejaculation, stimulating rapid cholesterol efflux in a concentration-dependent manner (Desnoyers and Manjunath, 1992; Moreau et al., 1999; Manjunath and Therien, 2002). These proteins are well characterized in bovine seminal plasma and the presence of similar proteins in human seminal plasma and in other species has been reported (Leblond et al., 1993). Members of the lipid transfer/lipoplysaccharide-binding protein (LT/LBP) family, in particular cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP), are also involved in lipid exchanges in some extracellular fluids. In blood plasma, CETP promotes the exchange of neutral lipids, i.e. cholesteryl esters (CEs) and triglycerides between different lipoprotein classes (Draayna et al., 1987; Bruce et al., 1998a; 1998b). PLTP was first described as a plasma factor that facilitates the transfer of phospholipid between lipoproteins but recent studies have...
reported that PLTP can also exchange a number of other amphipathic compounds including unesterified cholesterol, lipopolysaccharides, diacylglycerol and α-tocopherol (Lagrost et al., 1998; Van Tol, 2002). In addition to their implication in inter-lipoprotein lipid exchanges, both CETP and PLTP were proven to catalyse lipid transfers between lipoproteins and cells, not only in blood plasma but also in other compartments including human cerebrospinal fluid in the case of PLTP (Demeester et al., 2000) and human follicular fluid in the case of CETP (Ravnik et al., 1992). Interestingly, CETP was shown to stimulate sperm cell capacitation (Ravnik et al., 1990; Ravnik et al., 1992). The aim of the present study was to search for the presence of lipid transfer activities in seminal plasma and to assess whether these activities can be related to the presence of PLTP and CETP. To this end we measured CE and phospholipid transfer activities in seminal plasma samples from either healthy volunteers or men consulting at the centre of sterility. The presence of CETP and PLTP was investigated by Western blot analysis using specific monoclonal antibodies. Lipid transfer activities were also analysed in relation to the main markers of accessory glands, i.e. fructose, zin, α-glucosidase, as well as the number of sperm cells.

Materials and methods

Ethics

Investigations were conducted in accordance with the World Medical Association Declaration of Helsinki ethical principles for medical research involving human subjects. All patients gave informed written consent.

Samples

Human semen samples were obtained by masturbation from four healthy volunteers and were used for Western blot analysis, time-dependent lipid transfer activities and gel-permeation chromatography. For determining mean PLTP activities, mean PLTP concentrations and the association of PLTP with seminal plasma parameters, semen samples were obtained from 51 men consulting at the centre of sterility (Dijon, Hôpital du bocage) after 3–5 days of sexual abstinence. Ejaculates were allowed to liquefy at 37°C for at least 30 min. After liquefaction, standard semen parameters were obtained according to the World Health Organization guidelines (World Health Organization, 1999). The 51 men were subdivided according to their spermogrammes (as recommended by World Health Organization, 1999): normozoospermia (n = 16), oligozoospermia (n = 10), asthenozoospermia (n = 28), teratozoospermia (n = 22) and oligoasthenoteratozoospermia (n = 7). Blood samples were collected from volunteers into sterile lithium-heparinate containing tubes and immediately centrifuged at 3000 g for 15 min at 4°C.

Biochemical analysis

Seminal plasma samples were centrifuged at 3000 g for 10 min within 2 h after sampling. The supernatant was carefully removed and stored at –20°C before biochemical analysis. Zinc in seminal plasma samples was measured by a colorimetric assay using 1-(2-pyridylazo)-2-naphtol (Fuentes et al., 1982). Fructose and alpha-glucosidase activity were determined using commercially available kits (Roche Diagnostics S.A., Meylan, France). Cholesterol and protein concentrations were measured using commercially available kits on a Cobas Integra 700™ analyser (Cobas Integra total cholesterol™ Ref. 07 6301 2, Cobas Integra total protein™ Ref. 07 3761 5; Roche Diagnostics S.A.)

Measurement of CE transfer activity

Fluorescence assay

CE transfer activity was measured with a commercially available fluorescence assay using synthetic liposomes enriched with nitrobenz-oxadizol (NBD)-labelled CEs and very low-density lipoprotein (VLDL) (Roar Biomedical, New York, USA). The fluorescent CE is present in a self-quenched state when associated with the donor. The fluorescence intensity of the donor decreases as the concentration of acceptor lipid increases. The fluorescent counter (PerkinElmer Life Sciences) for a 30 min period, with a 465 nm excitation and a 535 nm emission wavelength. PLTP activity in seminal plasma (increase in fluorescence/min) was calculated by dividing the increase in fluorescence in the samples between 0 and 20 min.

Measurement of phospholipid transfer activity

One-phase fluorescence assay

Phospholipid transfer activity was measured using a commercially available fluorescence activity assay (Cardiovascular targets, New York, NY, USA) following the instructions provided by the manufacturer. The PLTP Activity Kit includes donor and acceptor particles. Incubation of donor and acceptor with PLTP source results in the PLTP-mediated transfer of fluorescent phospholipid. The fluorescent phospholipid (NBD-labelled phospholipid) is present in a self-quenched state when associated with the donor. PLTP-mediated transfer is determined by the increase in fluorescence intensity as the fluorescent lipid is removed from the donor and transferred to the acceptor. Briefly, plasma samples (5 μl), fluorescent-labelloed donors (3 μl) and unlabelled acceptors (50 μl), were incubated at 37°C in a final volume of 200 μl of Tris buffered saline (TBS) (Tris, 10 mmol/l; NaCl, 150 mmol/l, pH 7.4) in 96 well microplates. Changes in fluorescence were monitored for a 2 h period using a Victor3™ fluorescent counter (PerkinElmer Life Sciences, Boston, MA, USA), with 465 nm excitation and 535 nm emission wavelengths.

Isotopic assay

CE transfer activity was determined by measuring the transfer of radiolabelled CEs from biosynthetically labelled 3H-CE-low-density lipoprotein (LDL) to unlabelled acceptor high-density lipoprotein (HDL) (Lagrost et al., 1994). LDL was biosynthetically labelled according to the procedure previously described. Briefly, a d > 1.13 g/ml plasma fraction obtained after ultracentrifugation of 20 ml of blood plasma was dialysed against TBS and then incubated for 24 h at 37°C with 10 nmol of [1tr, 2α, 3-H] cholesterol to allow cholesterol esterification by lecithin:cholesterol-acyltransferase. Subsequently, a 0.195 < d < 1.055 g/ml fraction obtained from 10 ml of plasma was added to the incubated mixture. The incubation was then prolonged for 6 h to allow the transfer of radiolabelled CEs from HDL to LDL. Finally, radiolabelled LDL (14C-CE-LDL) was recovered by sequential ultracentrifugation. For the measurement of CE transfer activity, 2.5 nmol of 14C-CE-LDL and 10 nmol of unlabelled HDL were incubated for 3 h at 37°C in the presence of 10 μl of sample (seminal plasma or blood plasma) in a final volume of 50 μl. Following the incubation, the d < 1.068 g/ml and the d > 1.068 g/ml fractions were separated by ultracentrifugation and the percentage of radioactivity transferred from LDL to HDL was measured. CE transfer activity was calculated as follows:

\[ T = \frac{P(3H-CE) \times Q_t}{t \times v} \]

where T is the CE transfer activity (nmol/ml/h), P(3H-CE) is the proportion of labelled CE in the d > 1.068 g/ml fraction, Q_t is the total amount of CE present in the LDL per sample (nmol), t is the incubation time (h), v is the volume of plasma added per sample (ml).

Two-phase isotopic and fluorescence assays

Phospholipid transfer activity was determined as the rate of phospholipid transfer from labelled liposomes toward HDL after selective precipitation of lipoproteins. Briefly, 10 μmol of egg phosphatidylcholine (PC) containing 10 nmol of 14C-PC (14C-PC-labelled liposomes) or 100 nmol of NBD-phosphatidyl-ethanolamine (NBD-PE-labelled liposomes) was dried under a stream of nitrogen and recovered in 1 ml of TBS. 14C-PC- and NBD-PE-labelled liposomes were then incubated at 37°C for 30 min in the presence of 14C-PC liposomes or NBD-PE liposomes (125 nmol of phospholipids), human HDL (100 nmol of cholesterol) and iodoacetate (75 nmol) in a final volume of 50 μl in TBS. Phospholipid
liposomes were subsequently precipitated by the addition of 350 µl of TBS and 300 µl of a 500 mmol/l NaCl, 215 mmol MnCl₂, 445 U/ml heparin solution. Successively, tubes were vortexed, left for 10 min at room temperature and finally, the precipitate was removed by a 10 min centrifugation at 9000 g in an Eppendorf centrifuge and the supernatant submitted to radioactivity or fluorescence counting. Phospholipid transfer activity was calculated after deduction of the blank control value (liposomes and HDL incubated at 37°C without plasma samples) (Lagrost et al., 1994). Phospholipid transfer activity was calculated as follows:

\[ T = \left[ P(\% PL) \times Q \right] / (t \times v) \]

where \( T \) is the phospholipid transfer activity (nmol/µg/h), \( P(\% PL) \) is the proportion of labelled phospholipid present in the supernatant, \( Q \) is the total amount of phospholipid present in the liposomes per sample (nmol), \( t \) is the incubation time (h), \( v \) is the volume of plasma added per sample (µl). Specific phospholipid transfer activity (nmol/µg/h) is calculated by dividing the phospholipid transfer activity by PLTP concentration.

**Measurement of PLTP concentration by enzyme-linked immunosorbent assay (ELISA)**

PLTP concentrations were determined using a competitive ELISA adapted to a Biomek 2000™ Laboratory Automation Workstation (Beckman, Palo Alto, CA, USA.) as previously described (Desrumaux et al., 1997). The antibody was a polyclonal PLTP antiserum that was obtained by injecting a rabbit with synthetic PLTP fragments.

**Western blot analysis**

After treatment in non reducing conditions, the samples were resolved by 10% SDS–PAGE and transferred onto a nitrocellulose membrane (Hybond™; Amersham-Pharmacia Biotech, Freiburg, Germany). After blocking for 1 h with 5% fat-free dry milk, the resulting blots were developed by successive incubations with specific primary antibodies (1h) and horseradish peroxidase-coupled second antibodies (1/3000) (1 h). All incubations were performed in phosphate buffer saline (PBS) (Na₂HPO₄, 80 mmol/l; NaH₂PO₄, 20 mmol/l; NaCl, 137 mmol/l; pH 7.4) containing 0.1% (v/v) Tween 20. Finally, the blots were revealed using a chemiluminescent substrate (ECL™; Amersham-Pharmacia Biotech). The antibodies were TP2 mouse monoclonal antibody for PLTP (2 µg/ml); Amersham-Pharmacia Biotech). The antibody was specific monoclonal CETP antibody (Figure 2).

**Presence of phospholipid transfer activity in human seminal plasma**

The ability of blood and seminal plasma to promote the transfer of phospholipids was investigated using a one-phase fluorescence assay that measured the transfer of labelled phospholipid from liposomes to unlabelled acceptors (see Materials and methods). Blood plasma showed high phospholipid transfer activity. After only 5 min, the transfer rate decreased probably due to equilibration of the fluorescent-labelled phospholipids between donor and acceptor particles (Figure 3). In contrast to CE transfer activity that was detected only in blood plasma, significant phospholipid transfer activity levels were also measured in seminal plasma samples, with a gradual rise in fluorescence that was observed across the 20 min incubation period studied (Figure 3). Based on the initial phospholipid transfer rates (Figure 3), phospholipid transfer activity was ~5-fold weaker in seminal plasma than in blood plasma from the same healthy

\[ P \text{< 0.05, Mann–Whitney).} \]

Materials and methods, Figure 1 shows typical CE transfer curves that were obtained with blood and seminal plasma samples from one healthy subject. As predicted from a number of previous studies, blood plasma showed a time-dependent CE transfer that developed gradually over the 2 h incubation at 37°C (Figure 1). In contrast, no significant CE transfer activity was detected in seminal plasma under identical incubation conditions (Figure 1). Consistent observations were made with seminal plasma samples from 10 different men (healthy volunteers, fluorescent NBD-CE-labelled liposomes (5 µl) and unlabelled VLDL acceptors (5 µl) were incubated at 37°C in a final volume of 200 µl of TBS buffer in 96 well microplates (see Materials and methods). All values are mean ± SD of triplie determinations (*, significantly different from blank and seminal plasma samples, } P \text{< 0.05, Mann–Whitney).}
Whether seminal PLTP is also able to transfer other phospholipid (non-immune rabbit immunoglobulins) (Figure 5). In order to assess inhibition as compared with samples treated with control antibody seminal plasma with anti-PLTP polyclonal antibody produced an 80% contribution of PLTP to this process, the effect of its blockade by either a 60 min, 56°C inhibited PC transfer activity by 75%. Preincubation of seminal plasma with anti-PLTP polyclonal antibody produced an 80% inhibition as compared with samples treated with control antibody (non-immune rabbit immunoglobulins) (Figure 5). In order to assess whether seminal PLTP is also able to transfer other phospholipid molecules (i.e., PE), the ability of anti-PLTP polyclonal antibody to inhibit the transfer of PE induced by seminal plasma was measured. An 80% inhibition of the transfer of PE was observed, indicating that PLTP present in seminal plasma is also able to transfer other phospholipid molecules (Figure 5).

**Distribution of PLTP in seminal plasma**

A pool of four seminal plasma samples was fractionated by gel-permeation chromatography on a Superose 6-HR column (see Materials and methods). Cholesterol and protein concentrations as well as PLTP activity were measured in eluted fractions. As shown in Figure 6, the majority of cholesterol and PLTP activity coeluted in early fractions that contained large, protein-poor components with an apparent molecular weight >600 kDa. On the basis of structural and compositional analyses (high cholesterol content and high molecular weight), the components of the earlier, PLTP-containing fraction present the main features of prostasomes that have been previously characterized in seminal plasma (Ronquist and Brody, 1985; Arienti et al., 1999). These data suggest that PLTP is associated with prostasomes and not with HDL 3-like particles that were previously characterized in seminal plasma (Grizard et al., 1992).

In order to confirm these results, prostasomes were isolated by centrifugation and gel-filtration chromatography as previously described (Arienti et al., 1999). The presence of PLTP in the prostasome-containing fraction was assessed by Western blot analysis. As shown in Figure 7, PLTP was detected in the prostasome fraction confirming that seminal PLTP is at least partially associated with prostasomes. In contrast to what was observed with gel-filtration chromatography, significant amounts of PLTP were also retrieved in the fraction deprived of prostasomes.
Relationship of PLTP activity to sperm parameters

Seminal plasma is a complex biological fluid with testicular, epididymal, prostatic and seminal vesicle secretions contributing to its final composition. In a first attempt to evaluate whether seminal PLTP secretion can be attributed to specific tissue sites, PLTP activity in seminal plasma samples was compared with accessory sex gland secretion markers, including fructose as a seminal vesicle marker, zinc as a prostatic marker and α-glucosidase as an epididymal marker. The number of sperm cells was used as a marker of exocrine testicular function. Two-group comparisons of PLTP activity levels were conducted after the population studied was divided in two distinct groups (‘normal’ versus ‘abnormal’) according to the WHO standards (i.e. abnormal fructose <13 μmol/ejaculate; abnormal zinc <2.4 μmol/ ejaculate; abnormal α-glucosidase <20 mIU/ejaculate; abnormal spermatozoa <40 millions/ejaculate). No statistically significant differences were observed between the ‘normal’ and ‘abnormal’ subgroups for α-glucosidase, zinc and number of sperm cells. In contrast, PLTP activity was significantly higher in the samples with low fructose values as compared with samples with normal fructose values (P < 0.05) (Table II).

Table I. PLTP concentration and activity in two series of seminal plasma samples

<table>
<thead>
<tr>
<th></th>
<th>PLTP concentration (n = 15) (mg/l)</th>
<th>PLTP transfer activity (n = 30) (nmol/ml/h)</th>
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</thead>
<tbody>
<tr>
<td>Mean values</td>
<td>1.51 ± 1.50</td>
<td>179 ± 62</td>
</tr>
<tr>
<td>Range</td>
<td>0.36-6.3</td>
<td>79-302</td>
</tr>
</tbody>
</table>

Seminal samples were obtained from men consulting at the centre of sterility. PLTP concentration was determined by specific ELISA as described in Materials and methods. PLTP activity was obtained by measuring the transfer of 14C-PC from radiolabelled liposomes to unlabelled HDL acceptors.

Figure 4. Anti-PLTP Western blot analysis of human seminal plasma samples. Samples from healthy volunteers (seminal plasma, 2 μl (~50 μg of protein); human plasma, 0.5 μl (~25 μg of protein) and partially purified PLTP (4 μg of protein) were treated as described in Materials and methods. The samples were resolved by 10% SDS–PAGE and finally blotted onto nitrocellulose membrane. The resulting blots were successively incubated with monoclonal PLTP antibody, anti-mouse IgG peroxydase-conjugated immunoglobulins and finally revealed using a chemiluminescent substrate. The samples are: 1 and 2, seminal plasma; 3, blood plasma; 4, purified PLTP; 5, biotinylated molecular weight markers.

Figure 5. Effect of anti-PLTP polyclonal antibody (A and B) and effect of a pre-heating step (C) on phospholipid transfer activity in human seminal plasma. (A) Mixtures containing 14C-PC-radiolabelled liposomes, HDL, iodoacetate and seminal plasma from one healthy volunteer (10 μl) were incubated at 37°C in a final volume of 100 μl in the presence of polyclonal PLTP antibody (100 μg) (+ anti-PLTP antibody’ samples) or in the presence of control immunoglobulins (100 μg) (‘control’ samples). Phospholipid transfer activity was determined as described in Materials and methods. Each bar represents the mean ± SD of triplicate determinations (*, significantly different from control sample, P < 0.05, Mann–Whitney). (B) Mixtures containing NBD-PE-labelled liposomes, HDL, iodoacetate and seminal plasma from one healthy volunteer (10 μl) were incubated at 37°C in a final volume of 100 μl in the presence of polyclonal PLTP antibody (100 μg) (+ anti-PLTP antibody’ samples) or in the presence of control immunoglobulins (100 μg) (‘control’ samples). Phospholipid transfer activity was determined as described in Materials and methods. Each bar represents the mean ± SD of triplicate determinations (*, significantly different from control sample, P < 0.05, Mann–Whitney). (C) Mixtures containing 14C-PC-radiolabelled liposomes, HDL and iodoacetate were incubated at 37°C in a final volume of 100 μl in the presence of 10 μl of preheated (1 h at 56°C) or non-heated seminal plasma from one healthy volunteer (‘heated’ and ‘control’ samples respectively). Phospholipid transfer activity was determined after a 30 min incubation period as described in Materials and methods. Each point represents the mean ± SD of triplicate determinations (*, significantly different from control sample, P < 0.05, Mann–Whitney).
Discussion

The present study reports for the first time that human seminal plasma displays significant phospholipid transfer activity. In contrast, CE transfer activity and CETP were not detectable in human seminal plasma. Although CETP and PLTP were first identified and characterized in blood plasma, their presence was recently reported in some other extracellular fluids, including human follicular fluid for CETP (Ravnik et al., 1992) and human cerebrospinal fluid for PLTP (Demeester et al., 2000). In contrast to CETP production that is restricted to a relatively limited number of organs or cell types (Drayna et al., 1987), PLTP displays a wider spectrum of expression, with significant amounts of PLTP reported in ovary, placenta and testis (Albers et al., 1995). In the present study, significant amounts of phospholipid transfer activity could be measured in all seminal plasma samples, and the presence of PLTP was confirmed by Western blot analysis and by specific ELISA. Both the activity and the concentration of PLTP in seminal plasma was ~25% of those found in blood plasma (Desrumaux et al., 1997). Specific phospholipid transfer activity was similar in both fluids, indicating that PLTP from either blood or seminal plasma is functionally equivalent. In addition to PLTP, other specific proteins which are susceptible to bind and transport lipids in seminal plasma have been characterized. HE-1, a soluble protein secreted by the epididymis, is able to bind free cholesterol with high affinity (Kirchhoff et al., 1996; Okamura et al., 1999; Foucheeour et al., 2000). Other lipid-binding proteins are the BSP proteins which are small acidic proteins (15–30 kDa), abundant in bovine seminal plasma (Desnoyers and Manjunath, 1992; Moreau et al., 1999; Manjunath and Therien, 2002). These proteins are able to stimulate phospholipid and cholesterol efflux from sperm cell membranes. They were shown to interact with HDL, just as plasma PLTP does (Moreau et al., 1999). It was therefore important to determine to what extent PLTP contributes to the phospholipid transfer activity of seminal plasma, one key point for the future assessment of its real physiological impact. In this respect, both heat-inactivation of PLTP and specific inhibition by polyclonal anti-PLTP antibody brought consistent responses, with up to 80% of the seminal phospholipid transfer activity attributable to PLTP and not to other seminal phospholipid-binding proteins. In these experiments, lipid transfer was measured using exogenous lipid donors and acceptors. Therefore, the ability of seminal PLTP to transfer molecules present in semen (free cholesterol, vitamin E, etc.) associated with endogenous lipidic structures (prostasomes, spermatozoa membranes, etc.) remains to be established.

In seminal plasma, lipids are mainly associated in prostasomes and in HDL-like structures (Ronquist and Brody, 1985; Arienti et al., 1999; Grizard et al., 1992). Prostasomes are vesicular organelles (~200 nm diameter) that are produced by the prostate gland. They contain few proteins, and their lipid moiety shows a high cholesterol to phospholipid ratio (Ronquist and Brody, 1985; Arienti et al., 1999). HDL-like structures present lower cholesterol to phospholipid ratios than prostasomes, but unlike plasma HDL they are devoid of CEs, apo A-I or apo E (Grizard et al., 1992). In the present study, gel-filtration chromatography and Western blot analysis of purified prostasomes revealed that seminal PLTP is partially associated with prostasomes. This localization is in good agreement with known structural properties of plasma PLTP that, unlike CETP, was shown to interact

Table II. Association of PLTP activity with sperm parameters

<table>
<thead>
<tr>
<th>Fructose</th>
<th>Zinc</th>
<th>α-Glucosidase</th>
<th>Spermatozoa</th>
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<tbody>
<tr>
<td>&lt;13 mmol</td>
<td>&gt;13 mmol</td>
<td>&lt;2.4 mmol</td>
<td>&gt;20 mIU</td>
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<tr>
<td>(n = 4)</td>
<td>(n = 47)</td>
<td>(n = 47)</td>
<td>(n = 4)</td>
</tr>
<tr>
<td>PLTP activity</td>
<td>12.5 ± 6.5*</td>
<td>5.4 ± 2.0</td>
<td>5.4 ± 1.5</td>
</tr>
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</table>

PLTP in sperm samples with abnormal values of one of the selected markers (fructose, alpha-glucosidase, zinc and sperm cells) were compared with PLTP activities in samples presenting normal values of the same marker. (*, significantly different from PLTP activity in samples with fructose >13 μmol, P < 0.05, Mann–Whitney).
readily with large phospholipid-containing liposomes (Lagrost et al., 1994).

In humans, PLTP is known to be produced by a wide variety of organs and cells (Albers et al., 1995) and the source of seminal PLTP remains to be identified. In a first attempt to investigate whether PLTP is secreted by one or several glands that contribute to seminal plasma production, PLTP activity levels in seminal plasma samples were compared with prostate, epididymis or seminal vesicle markers. PLTP activity was not diminished in seminal plasma samples containing abnormally low amounts of epididymal or prostatic secretions. Only a significant increase in PLTP levels was observed when seminal vesicle secretion was low as assessed by measuring fructose concentration. Since seminal vesicle secretion constitutes the major sperm fluid, the least dilution of PLTP in a lower volume of a PLTP-free fluid might account for the observed rise in phospholipid transfer activity. Although the testis was shown to express high levels of PLTP mRNA (Albers et al., 1995), PLTP activity was not significantly different in patients with oligospermia. However, it cannot be excluded that PLTP of seminal plasma might be produced, at least in part, by testis, and the identification of cell types that are responsible for the testicular production of PLTP will constitute a major step during this quest. Another hypothesis holds that seminal PLTP might derive from blood plasma, as might be the case for albumin (Lindholm et al., 1974). However, albumin and PLTP behave differently in this context, in spite of similar molecular weights. Indeed, the substantial levels of PLTP found in seminal plasma, ~25% of the levels in blood plasma, are in contrast with the trace amounts of albumin that did not exceed a few per cent of blood plasma levels (Lindholm et al., 1974).

The physiological function of seminal PLTP remains to be established and is beyond the scope of the present study. CETP, which is closely related to PLTP (Bruce et al., 1998a; Bruce et al., 1998b), has been shown to stimulate sperm cell capacitation, that involves a sterol depletion of the spermatozoa membrane (Cross et al., 1998; Flesch and Gadella, 2000). Interestingly, PLTP is also able to stimulate cellular cholesterol efflux (Nishida et al., 1997), and its implication in inter lipoprotein cholesterol and phospholipid exchanges has been clearly established (Wolbauer et al., 1999). Therefore, it is tempting to speculate that, like BSP proteins and CETP (Ravnik et al., 1992; Manjunath and Therien, 2002), PLTP may be involved in the capacitation process. PLTP may either inhibit or stimulate capacitation, depending on the presence of cholesterol donors or acceptors.

In fact, PLTP is a multifunctional protein and transfers a number of amphiphatic compounds, including not only phospholipids and unesterified cholesterol, but also diacylglycerides, lipopolysaccharides and \( \alpha \)-tocopherol, i.e. the main isomer of vitamin E (Desrumaux et al., 1998; Jiang et al., 2002). Interestingly, it has long been recognized that spermatogenesis and sperm cells are highly sensitive to vitamin E depletion (Bensoussan et al., 1998; Therond et al., 1996) and significant decreases in total antioxidant capacity of seminal plasma were associated with infertility in humans (Lewis et al., 1999). Recently, PLTP was shown to exchange vitamin E between plasma lipoproteins and cells, leading to substantial alterations in the antioxidant defence of lipid structures (Desrumaux et al., 1998; Jiang et al., 2002). If such a PLTP-mediated vitamin E transfer process was also proven to occur in seminal plasma, PLTP might well arise as an important factor in the control of the \( \alpha \)-tocopherol content of sperm cell membranes. These two hypotheses will need further investigation. Finally, it remains possible that PLTP functions in the testis or in accessory organs and has no physiological role in seminal plasma. In this case, seminal phospholipid transfer activity and PLTP itself could be considered as functional markers of testis or accessory sex glands.

References


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