



Changes in Cholesteryl Ester Transfer Protein Activity During Normal Gestation and Postpartum

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Cholesteryl ester transfer protein (CETP) activity was measured in a $d > 1.21$ kg/L plasma fraction collected from healthy women at different times during gestation, postpartum, and in control women. CETP activity was highest in the second trimester of gestation, declined at the third trimester, and was lowest at postpartum. Only the value at the second trimester was significantly different from that of control women. This trend differed from that of circulating lipoproteins: very low-density lipoprotein (VLDL)-lipids, including triglycerides and cholesterol, increased progressively from the first to the third trimester, and then declined at postpartum. Low-density lipoprotein (LDL)-cholesterol levels, like VLDL levels, rose during gestation but then remained elevated at postpartum. High-density lipoprotein (HDL)-cholesterol as well as HDL-phospholipids and apolipoprotein A-I, peaked in the second trimester, remaining elevated in the third trimester and then fell at postpartum. Finally, HDL-triglyceride increased markedly from the first to the second trimester, rose somewhat higher during the third trimester, and declined at postpartum. When all the samples from pregnant women were considered together, CETP activity correlated significantly with HDL-triglyceride levels and the changes in CETP activity during gestation and postpartum paralleled those of the HDL-triglyceride/VLDL-triglyceride ratio. These results suggest that CETP contributes to the exaggerated accumulation of triglycerides in HDL that begins in the second trimester of human gestation.

KEY WORDS: cholesteryl ester transfer protein; gestation; lipoproteins; VLDL; LDL; HDL; triglycerides.

Introduction

Both the number and the lipid composition of lipoprotein particles change in the course of human pregnancy. As pregnancy progresses there is an important increase in total plasma triglycerides, which rapidly decline at postpartum, but the changes in total cholesterol are more moderate (1–3). In examining lipoprotein fractions, increments are noted in both VLDL and LDL lipids, and these, together with the elevated apo B levels, indicate that the number of apo B-containing particles in-

creases progressively from the first to the third trimester (4). These changes have been attributed both to decreased plasma lipolytic activity (5) and to estrogen-stimulated hepatic VLDL production (6). A contrasting biphasic pattern is observed in HDL levels, and both cholesterol and phospholipids rise to a maximum by the second trimester and then gradually decline to pregestational levels at postpartum (4,7). Interestingly, HDL-triglyceride levels increase with gestation, which indicates a profound change in HDL composition in the gestating woman (4).

The plasma of several species contains the so-called cholesteryl ester transfer protein [CETP or neutral lipid transfer protein, LTP-I; (8)], which mediates the heteroexchange of cholesteryl ester for triglyceride between HDL and apo B-containing lipoproteins (9–13). This is the way that cholesteryl esters formed in HDL by the action of lecithin:cholesterol acyltransferase are transferred to both VLDL and LDL, and then cleared through hepatic receptors, thus contributing to reverse cholesterol transport (14). HDL becomes progressively poorer in cholesteryl esters and richer in triglyceride, which is hydrolyzed by either lipoprotein lipase or hepatic lipase (14). CETP is, then, an important factor in HDL metabolism and reciprocal changes in CETP activity and HDL plasma levels are well documented (15–17). CETP activity and mass are elevated in several dyslipidemic states, including both primary hypercholesterolemia and hypertriglyceridemia (18), with the greatest increase being found in severely hyperchylomicronemic patients (19). These observations have led to the hypothesis that hypoalphalipoproteinemia secondary to hypertriglyceridemia is related to HDL enrichment with triglycerides, which is mediated by CETP action (20).

Attempts to correlate CETP activity with other lipidic variables in the general population, have had contradictory results (21–25). In normolipidemic subjects, Marcel *et al.* (24) found that CETP mass was directly correlated to several lipidic parameters including HDL-triglyceride levels. This suggests that the HDL triglyceride content is partially determined by CETP activity, as a reflection of its physiological action (11,12).

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To our knowledge, CETP activity has not previously been measured in gestating women. In the pregnant rabbit, plasma CETP activity decreases progressively throughout gestation, in parallel to the trend observed for cholesterol content in all of the lipoproteins (26). This trend by plasma lipids in the pregnant rabbit is opposite to that in women, as described above. These interspecies differences force us to restrict medical studies of the effect of gestation on the lipoprotein metabolism to women. In the present work we were interested in determining the possible changes of CETP during human gestation and postpartum in order to ascertain whether this protein is implicated in the changes in HDL that take place in this physiological condition.

Patients, materials, and methods

PATIENTS

Eleven healthy pregnant women were studied. Their ages ranged from 20 to 37 years (mean \pm SD, 30.9 ± 4.0). The patients were attended at La Paz Hospital, Madrid, by Dr. Luis F. Pallardo. During the first (9–10 weeks), second (21–23 weeks), and third (32–34 weeks) trimester of gestation and 2–4 weeks postpartum (nonlactating), venous blood samples were collected after an overnight fast, in Na₂-EDTA-containing tubes (1 mg/mL), cooled in ice, and then the plasma was promptly separated by low speed centrifugation. The control group was 29 healthy volunteers from the medical center personnel, age 34.6 ± 6.4 years (not significantly different from pregnant group). All the patients and controls were informed of the study and gave their consent.

ISOLATION OF LIPOPROTEINS AND LIPID ANALYSIS

Lipoproteins were isolated by sequential ultracentrifugation from 10 mL of plasma, as described (4). The lipoprotein-containing supernatants (VLDL, LDL, and HDL) were then analyzed without any further manipulation. The infranatant of $d = 1.21$ kg/L (lipoprotein-free plasma) was stored at -70°C until processing for CETP activity. Under these storage conditions, CETP activity is stable for up to 16 months. Prior to the CETP assay, these fractions were exhaustively dialyzed against 0.15 M NaCl, 1 mmol/L Na₂·EDTA, and brought to the initial plasma volume (10 mL) with this medium.

Lipids were measured in whole plasma and in the lipoprotein fractions. Total cholesterol and triglyceride (Menarini Diagnostici, Firenze, Italy), and choline-containing phospholipids (Boehringer Mannheim, Mannheim, Germany) were measured enzymatically in an autoanalyzer Technicon RA-1000 (Technicon Ltd., Dublin, Ireland). Apo A-I was determined by means of the Array Beckman nephelometer (Beckman Instruments, Palo Alto, CA, USA) using the respective reagent kit.

ASSAY OF CHOLESTERYL ESTER TRANSFER PROTEIN

CETP was determined in lipoprotein-free plasma as the activity that transfers ³H-cholesteryl oleate from prelabelled apolipoprotein E-free HDL to exogenous VLDL. Lipoproteins (VLDL and HDL) were isolated from pooled human plasma by ultracentrifugation as above and the HDL were labelled with [1,2,6,7-³H]cholesteryl oleate (New England Nuclear, Bad Homburg, Germany) by incubation for 15 h at 37°C , in the presence of lipoprotein-free human plasma, as previously described (13). The ³H-HDL were reisolated by ultracentrifugation, dialyzed against 0.05 M NaCl, 5 mM Tris-HCl, pH 7.4, then supplemented with solid MnCl₂ to give a final concentration of 25 mM and loaded into chromatographic columns containing heparin-Sepharose CL-6B (Pharmacia Fine Chemicals, Uppsala, Sweden) at approximately 1 mg HDL-cholesterol/3 mL of gel. After equilibration for 60 min at 4°C , elution was initiated with 0.05 M NaCl, 5 mM Tris-HCl, pH 7.4, containing 25 mM MnCl₂, at a flow rate of 24 mL/h. Only fractions corresponding to this first peak were collected, pooled, and dialyzed against 0.15 M NaCl, 1 mmol/L Na₂·EDTA. This HDL-fraction, called HDL-A, has been chemically characterized elsewhere (13,27) and contains apo E-poor lipoprotein particles. This ³H-HDL-A preparation was finally passed through 0.45- μm pore-size filters, stored at 4°C , and used within 1 month. A single batch of ³H-HDL-A was used for all samples.

For the assay, 20 μL of sample (lipoprotein-free plasma) were mixed with 150 μL saline (0.15 M NaCl), 25 μL Tris-HCl, 0.25 M, pH 7.4, containing dithionitrobenzoic acid 18 mM, 50 μL VLDL (153 μg triglyceride), and 50 μL ³H-HDL-A (13.5 μg esterified cholesterol, radioactivity $>70,000$ dpm). In some tubes, VLDL was omitted and substituted by an equal volume of 0.15 M NaCl. The tubes were incubated in a shaking bath (60 cycles per min) at 37°C for 8 h. For control, other tubes were not incubated but immediately processed after the addition of ³H-HDL-A. For the isolation of the newly formed ³H-VLDL, the tubes were placed in ice, and then 200 μL of human plasma, followed by 50 μL of a solution containing 10 g/L dextran sulfate (M_w 50,000, from Sochibo, Vélizy Villaloublay, France) and 0.32 mol/L MgCl₂ were added to each tube. The tubes were vortexed, incubated for 15 min at room temperature, and centrifuged at $1500 \times g$ for 30 min. The clear supernatant, containing no VLDL, was carefully transferred to scintillation vials, mixed with OptiPhase HiSafe II cocktail (Pharmacia LKB Biotechnology) and used for ³H-radioactivity counting in a Beckman 3800 liquid-scintillation counter (Beckman). Radioactivity incorporated into VLDL was calculated as the difference between the value obtained in the supernatant of the tube that contained no VLDL and the value in the tube that contained VLDL. After correcting for specific radioactivity of ³H-HDL-A, cholesteryl ester transfer activity was expressed as

picomoles cholesteryl ester transferred to VLDL/min/mL plasma.

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM. Statistical comparisons between trimesters and postpartum were performed with the dependent Student's *t*-test. Statistical comparisons versus controls were performed by the independent Student's test. Regression analyses, linear model ($y = a + bx$), were done between CETP activity and lipid variables using Pearson's method. All calculations were done using the Statgraphics statistical package (Statistical Graphics corporation STSC Inc, Tockville, MD, USA).

Results

PLASMA LIPID AND CETP ACTIVITY IN HUMAN GESTATION

Healthy, normolipidemic women were longitudinally studied at the first, second, and third trimesters of gestation and then at postpartum. Table 1 summarizes the concentrations of lipids and apo A-I at these times. During gestation both total triglyceride and cholesterol increased progressively attaining their highest values at the third trimester and declining at postpartum. VLDL levels increased with gestational time as indicated by both the triglyceride and the cholesterol contents of the fraction. In fact, the triglyceride/cholesterol mass ratio in VLDL remained stable through gestation and postpartum, and ranged between 5.5 and 6.1, which indicates that the number of VLDL particles increased with gestation. The LDL-cholesterol plasma concentration rose like the VLDL values during gestation, but it remained elevated at postpartum in comparison to the first trimester and controls (Table 1). The lipid profile of pregnant women at the first trimester of gestation was very similar to that of

control women, none of the lipid parameters studied being significantly different between these groups.

As also shown in Table 1, the pattern of HDL differed from that of VLDL or LDL. HDL-cholesterol and -phospholipids, as well as apo A-I, increased approximately 12% from the first to the second trimester, then remained elevated in the third trimester, and declined at postpartum to values that did not differ from those at the first trimester. None of these values differed significantly from controls. HDL-triglyceride levels increased twofold from the first to the second trimester and rose somewhat higher at the third trimester. The values at the second and the third trimesters were significantly higher than in controls.

To better understand the changes in the composition of HDL with gestation, several mass ratios were calculated. As shown in Table 2, the phospholipid/cholesterol mass ratio in HDL, as well as the ratios of apo A-I to HDL-cholesterol or to HDL-phospholipids did not significantly change with gestation or at postpartum. Although these ratios were similar in women at the first trimester as compared to controls, the HDL-phospholipid/cholesterol ratio was slightly higher in pregnant women at the second and third trimester and postpartum than in controls. The increases in HDL lipids and apo A-I concentrations from the first to the second trimester described above probably reflect the greater number of HDL particles with a similar composition. With regard to the triglyceride content of HDL, both the cholesterol/triglyceride ratio in HDL and the apo A-I/HDL-triglyceride mass ratio significantly decreased in the second and third trimesters and then recovered at postpartum (Table 2). These changes reflect the triglyceride enrichment in the HDL particles at mid- and late gestation.

CETP activity was measured in the lipoprotein-free plasma fraction and the results are summarized in Table 3. CETP activity values were very similar in the first trimester and at postpartum and did not differ from those of controls. In the second trimester CETP activity was slightly elevated (18%) as com-

TABLE 1
Plasma Lipids in Human Gestation, Postpartum and Controls

	Trimester (n = 11)			Postpartum (n = 11)	Controls (n = 29)
	First	Second	Third		
Total triglyceride	0.68 ± 0.10	1.27 ± 0.11	2.05 ± 0.28 ^a	1.20 ± 0.33	1.00 ± 0.10
Total cholesterol	4.47 ± 0.18	5.93 ± 0.27 ^a	6.56 ± 0.39 ^a	6.20 ± 0.57 ^a	4.93 ± 0.15
VLDL-triglyceride	0.25 ± 0.09	0.39 ± 0.08	0.819 ± 0.23	0.63 ± 0.26	0.52 ± 0.08
VLDL-cholesterol	0.10 ± 0.03	0.15 ± 0.03	0.34 ± 0.10	0.23 ± 0.13	0.18 ± 0.05
LDL-cholesterol	2.83 ± 0.18	4.19 ± 0.18 ^a	4.50 ± 0.36 ^a	4.57 ± 0.52 ^a	3.23 ± 0.15
HDL-triglyceride	0.14 ± 0.01	0.27 ± 0.02 ^a	0.32 ± 0.02 ^a	0.11 ± 0.01	0.14 ± 0.01
HDL-cholesterol	1.55 ± 0.13	1.76 ± 0.15	1.63 ± 0.10	1.47 ± 0.18	1.63 ± 0.08
HDL-phospholipid	1.33 ± 0.10	1.59 ± 0.13	1.49 ± 0.08	1.36 ± 0.12	1.40 ± 0.05
Plasma apo A-I ^b	1.41 ± 0.07	1.61 ± 0.13	1.69 ± 0.11	1.43 ± 0.09	1.50 ± 0.05

Plasma lipids in mmol/L. Statistical comparisons versus controls by independent Student's *t*-test.

^a Statistically significant differences ($p < 0.05$).

^b g/L plasma.

TABLE 2
HDL Mass Ratios in Human Gestation, Postpartum, and Controls

	Trimester (<i>n</i> = 11)			Postpartum (<i>n</i> = 11)	Controls (<i>n</i> = 29)
	First	Second	Third		
HDL-phospholipid/ HDL-cholesterol (mmol/mmol)	0.85 ± 0.50	0.90 ± 0.05 ^a	0.91 ± 0.05 ^a	0.95 ± 0.05 ^a	0.80 ± 0.05
HDL-cholesterol/ HDL-triglyceride (mmol/mmol)	11.79 ± 1.13	6.80 ± 0.68 ^a	5.44 ± 0.45 ^a	14.28 ± 2.76	12.9 ± 0.68
A-I/HDL-cholesterol (g/mmol)	0.97 ± 0.08	0.98 ± 0.08	1.05 ± 0.04	1.01 ± 0.12	1.05 ± 0.04
A-I/HDL-triglyceride (g/mmol)	10.52 ± 0.87	7.02 ± 1.14 ^a	5.53 ± 0.52 ^a	13.16 ± 1.75	11.40 ± 0.88
A-I/HDL-phospholipid (g/mmol)	1.08 ± 0.08	1.10 ± 0.8	1.16 ± 0.07	1.07 ± 0.08	1.24 ± 0.08

Statistical comparisons versus controls by independent Student's *t*-test.

^a Statistically significant differences (*p* < 0.05).

pared to the first trimester, and this value was significantly higher than postpartum and control values. Those in the third trimester were intermediate between the second trimester and postpartum values (Table 3).

Because the changes in CETP activity paralleled the changes in plasma lipoproteins, the linear correlations between these parameters were calculated separately in all pregnant and postpartum samples and controls. When these calculations were performed for each trimester separately, there was no significant correlation (data not shown) between the parameters in any of the pregnant or nonpregnant groups. When all the samples from gestating women were computed together, a significant correlation was noted for both HDL-triglyceride and A-I/HDL-triglyceride ratio. Even though these values were statistically significant, the *r* values observed were low, indicating that CETP activity was responsible for approximately 10% of the HDL-triglyceride variability in these women.

Discussion

The present study describes the changes in CETP activity during gestation in normolipidemic, healthy women for the first time. The major change consisted of the elevation of CETP activity at mid-

gestation as compared to postpartum and controls values. In a previous study with rabbits, Quig and Zilversmit found that plasma lipid transfer activity decreased dramatically at day 28 of pregnancy and recovered at postpartum (26). This drastic change in lipid transfer activity paralleled the progressive hypocholesterolemia observed in the pregnant rabbit, and the association between plasma lipid levels and neutral lipid transfer activity was therefore obvious (26). Our observations in women are opposite to the results in the rabbit because an increase was found in CETP activity in women at mid-gestation rather than the decrease found in rabbits. It should be noted, however, that gestation in women is accompanied by hyperlipidemia (1,3,4), whereas in the rabbit it is accompanied by hypolipidemia (26). So there is a parallelism of sorts between CETP activity and plasma lipids in the two species. In the present work, as in the report of Quig and Zilversmit (26), CETP was assayed in the *d* > 1.21 kg/L plasma fraction, so that the activity values obtained would not be influenced by the endogenous plasma lipoproteins of density ≤HDL.

The changes in lipid and lipoprotein plasma levels during gestation and postpartum observed herein were as would be expected in healthy women. There was a progressive increase in the concentrations of both total cholesterol and triglycerides, which was

TABLE 3
Plasma Cholesteryl Ester Transfer Protein Activity in Human Gestation, Postpartum, and Controls

	Trimester (<i>n</i> = 11)			Postpartum (<i>n</i> = 11)	Controls (<i>n</i> = 29)
	First	Second	Third		
CETP-activity	410 ± 23	500 ± 41 ^a	435 ± 25	385 ± 27	412 ± 12

Picomoles cholesteryl ester transferred/min/mL plasma. Statistical comparisons versus controls by independent Student's *t*-test.

^a Statistically significant difference (*p* < 0.05).

proportionally greater for the latter lipid. Both VLDL and LDL-lipid levels progressively increased until the third trimester of gestation, probably as a result of both the augmented hepatic production of VLDL particles (28,29) and the diminished plasma lipolytic activity (30) that are observed at late gestation.

The trend of HDL was markedly different from that of apo B-containing lipoproteins, insofar as HDL-cholesterol reached a high point in the second trimester, remained elevated in the third trimester, and declined at postpartum. These results agree with previous findings (2-4). The fact that the level of the changes in both plasma apo A-I and HDL-phospholipids in relation to HDL-cholesterol were of similar magnitude, suggests to us that at mid- and late gestation there are more HDL particles circulating in plasma than in the first trimester and at postpartum. Other studies have demonstrated that HDL₂ rather than HDL₃ was responsible for the enhancement of HDL during gestation (2). The mechanism responsible for this phenomenon is not known, although both the reduction of hepatic lipase activity (30), as a factor that accelerates HDL catabolism (31), and the elevation of estrogens (4,6), as stimulators of apo A-I synthesis (32), could contribute.

A striking feature was the triglyceride enrichment of HDL triglyceride. We observed that the HDL-triglyceride plasma concentration doubled between the first and the second trimester, and this increase was proportionally much higher than that of the other HDL components. Actually, the only HDL mass ratios that change with gestation were those affecting triglycerides, which demonstrates that HDL particles accumulating at mid- and late gestation in women are enriched in this lipid. This is also true when compared to controls. At present, the origin of the triglyceride excess in HDL is not known and the metabolic steps that lead to the HDL accumulation are not established. It can be speculated, however, that the elevated CETP activity observed at mid-gestation is causally related to this phenomenon. First, the HDL-triglyceride concentration in the gestating women studied was directly correlated with CETP activity, and a similar relationship in the general, normolipidemic population has previously been found by others (24). A significant correlation between CETP activity and HDL-triglyceride was not found in our relatively small group of controls probably due to the reduced number of individuals. This indicates that CETP is not the only determinant of HDL-triglyceride levels; the factors such as plasma lipolytic activities are probably more important. Second, it is well documented that the physiological action of CETP is to transfer triglycerides from VLDL to HDL in exchange for cholesteryl esters (11,12). In correlation with this, HDL from species that do not naturally have CETP, like the rat or pig, become enriched in triglycerides after incubation with exogenous CETP and VLDL (33,34), whereas the *in vivo* administration of antibodies

against CETP to rabbits, which do have CETP, results in a decrease of triglycerides in HDL (35). Hence, not excluding a role for plasma lipolytic activities, we propose that the concomitant changes of triglycerides in HDL and CETP activity observed herein at mid-gestation are mechanistically related. In this interaction, obviously, the augmented availability of VLDL-triglyceride constitutes the driving force for the transfer of triglyceride to HDL. However, it is interesting to note that the HDL-triglyceride/VLDL-triglyceride ratio was highest in the second trimester of gestation and lowest at postpartum, and these changes paralleled CETP activity (Table 1).

In summary, we describe the increase of CETP activity at mid-gestation for the first time in normal women. CETP directly correlated with HDL-triglyceride levels when all the samples were considered together and the trend of CETP activity during gestation and postpartum paralleled that of the HDL-triglyceride/VLDL-triglyceride ratio. These results allow us to suggest that CETP contributes to the increased accumulation of triglycerides in HDL that occurs in the second trimester of human pregnancy. Further studies are required to determine which factors, hormonal, dietary, or others, are responsible for the increase in CETP activity that we have observed.

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