Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women

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Abstract To understand the mechanism responsible for maternal hyperlipidemia, 25 healthy pregnant women were studied longitudinally during the three trimesters of gestation and at post-partum, and 11 were studied again at post-lactation. Triglyceride and cholesterol levels increased with gestation in all the lipoprotein fractions. However, the greatest change appeared in low density (LDL) and high density (HDL) lipoproteins, both of which showed an increase in their triglyceride/cholesterol ratio. The proportional distribution of HDL subfractions showed that the HDL_{2b} fraction was the only one that increased with gestation, whereas both HDL_{3a} and HDL_{3b} had the greatest decrease. Cholesteryl ester transfer protein activity increased during the second trimester of gestation. While postheparin lipoprotein lipase activity decreased during the third trimester, postheparin hepatic lipase activity progressively decreased from the first trimester. The 17β-estradiol, progesterone, and prolactin hormones progressively increased from the first trimester of gestation. The lipoprotein-triglyceride values correlated linearly and negatively with the logarithm of either postheparin lipase activities, HDL-triglycerides showing the highest correlation coefficient when plotted against the hepatic lipase values (r = -0.757). It appeared that the highest correlation between any of the HDL subclasses and the activity of the enzymes was for hepatic lipase activity versus HDL_{2b} (r = -0.456) or HDL_{3a} (r = 0.519). A significant lineal correlation also appeared between the postheparin hepatic lipase activity and the logarithm of any of the sex hormones studied, the highest value corresponding to estradiol (r = -0.783). Therefore, during gestation, the effect of estrogen in enhancing very low density lipoprotein (VLDL) production and decreasing hepatic lipase activity plays a key role in the accumulation of triglycerides in lipoproteins of density higher than VLDL.—**Alvarez, J. J., A. Montelongo, A. Iglesias, M. A. Lasunción, and E. Herrera.** Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J. Lipid Res.* 1996. **37:** 299–308.

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Plasma triglyceride levels rise markedly during pregnancy whereas increments in phospholipids and cholesterol are more moderate (1, 2). Although elevated plasma triglycerides are found in all the lipoprotein fractions during late gestation (3–6), the triglyceride/cholesterol ratio remains stable in VLDL despite significant changes in both low density lipoproteins (LDL) and high density lipoproteins (HDL) as gestational time advances (3). Two mechanisms seem to operate either separately or together on these changes. One is the estrogen-induced hepatic synthesis of VLDL-triglycerides (7–9). The abundance of VLDL could support an enhanced conversion to lipoproteins of higher density. The specific enrichment of triglycerides in the latter would require the cholesteryl ester transfer protein (CETP)-mediated transfer of triglycerides from triglyceride-rich lipoproteins to lipoproteins of higher density, and CETP activity has been demonstrated to be enhanced in pregnant women (10, 11). The second mechanism to explain the increase in triglycerides in all the circulating lipoproteins during gestation could be impaired removal of lipoprotein triglycerides by one or two of the lipolytic enzymes, lipoprotein lipase (LPL) or hepatic lipase (HL). LPL is present in the vascular bed of extrahepatic tissues and catalyzes the hydrolysis of triglycerides in VLDL (and in chylomicrons, under fed conditions) and their subsequent conversion into intermediate density lipoproteins (IDL) and LDL, and thus is a key enzyme for the VLDL-IDL-LDL cascade (12). HDL receives some surface constituents from VLDL lipolysis resulting in the conversion of lipid-poor HDL_3 particles to the less dense lipid-enriched HDL_2 particles.

Abbreviations: HL, hepatic lipase; LPL, lipoprotein lipase; CETP, cholesteryl ester transfer protein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; HDL, high density lipoprotein.

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HDL_2 particles are degraded by HL (13, 14), which catalyzes the hydrolysis of HDL phospholipids and triglycerides (15–17) and thus, HDL_2 are again converted to lipid-poor HDL_3 (12). Both LPL and HL activities in postheparin plasma have been shown to be decreased in pregnancy, the change being more striking for HL than for LPL (18) and increments in HDL_2 rather than HDL_3 seem to be responsible for the HDL-cholesterol level seen during late gestation in women (5, 6, 19). After the development of nondenaturating gradient gel electrophoresis (GGE), it has recently been shown that during late gestation there is a specific increase in the HDL_{2b} subclass (10). Estrogen administration has been shown to decrease HL activity in postmenopausal women with minor effects on LPL (20), and, as well, it also produces an enrichment of triglycerides in HDL and raises the level of in HDL_2-cholesterol (21). The possibility then exists that the exaggerated increase in plasma estrogens during gestation is responsible for the reduced HL activity and its subsequent effect of modifying the lipoprotein profile, and, more specifically, shifting the HDL subclass distribution to the larger and more buoyant species. Because, to our knowledge, no single report combines information on all these parameters during pregnancy, the present paper studies them in healthy pregnant women who were followed longitudinally during the three trimesters of gestation, at postpartum and at post-lactation, the latter values taken as their own nonpregnant controls.

MATERIAL AND METHODS

Subjects and blood sampling

After a 12-h overnight fast, basal venous blood samples from 25 pregnant healthy women were taken in tubes containing 1.0 mg/ml of Na_2 EDTA at first (9–12 weeks), second (21–24 weeks), and third trimester (32–35 weeks) of gestation and at 2–4 weeks after parturition (postpartum). Eleven of these women lactated for 2–6 months and were studied again approximately 60 days after the end of the lactation period, when spontaneous menses had resumed (postlactation). All subjects gave informed consent and they were included in the study under the basis of the following criteria: normal course and outcome of the pregnancy, no endocrine abnormalities, single pregnancy, term delivery, and no family history of lipid or carbohydrate disorders. The women included in the study were 29.5 ± 0.7 years old, had a body mass index before pregnancy of 22.6 ± 0.33 kg/m^2 and a body weight increment with gestation of 9.25 ± 0.13 kg (means ± SE).

After the basal blood samples were taken, heparin was injected intravenously in a dose of 50 IU/kg, and 10 min later a second blood sample was taken into dry glass tubes kept on ice. Plasma was separated immediately.

Lipoprotein isolation and lipid and apolipoprotein determinations

An aliquot of plasma from basal samples was frozen at -80°C for hormone and apolipoprotein measurements, whereas another aliquot was subjected immediately to sequential ultracentrifugation in a Ti50 Beckman rotor (Palo Alto, CA). VLDL were floated at 45,000 rpm for 18 h at d 1.006 g/ml. The infranatant was brought to d 1.063 g/ml with solid KBr and subjected to ultracentrifugation at 45000 rpm for 20 h for isolation of LDL; the last infranatant was brought to d 1.21 g/ml with KBr and ultracentrifuged at 47,000 rpm for 44 h for isolation of HDL. Floating supernatants were recovered by tube slicing and, after proper dilution, aliquots were used for triglyceride and cholesterol measurements with a Hitachi 705 autoanalyzer (Boehringer Mannheim, Mannheim, Germany).

HDL subfractionation by nondenaturing gradient gel electrophoresis (GGE)

Aliquots of isolated HDL were kept frozen at -20°C until processed for GGE subfractionation following a modification of the procedure described by Verdery et al. (22). Preliminary experiments that processed aliquots of the same HDL sample, fresh and after 3 weeks of freezing, demonstrated no difference in the subfractionation pattern. Precast slab 4–20% polyacrylamide gels in 0.375 mol/L Tris-HCl were obtained from Bio-Rad Laboratories (Richmond, CA) and samples were processed in a Mini-Protean II electrophoresis chamber from the same source at 4°C for 20 min at 70 V and 16 h at 125 V. Reference proteins used to determine particle sizes included thyroglobulin (8.5 nm), ferritin (6.1 nm), catalase (5.2 nm), lactate dehydrogenase (4.08 nm), and bovine albumin (3.55 nm) from the HMW Electrophoresis Calibration Kit, Pharmacia LKB (Uppsala, Sweden). Gels were stained with Coomassie G-250 and scanned at 540 nm using computer-assisted densitometry (Densitometer E-C 910, Apparatus Corporation, St. Petersburg, FL), with a Videophoresis II program (EC-934 software). Data from each scan were encoded as the optical density at each pixel and transferred to an ASCII text file. These text files were imported in an interactive computer program (22) that used data from external standards to automatically calculate the apparent Stoke's radius of the center of Gaussian curves. The scan of each HDL gradient gel was always modeled with six Gaussian curves having their corresponding peaks within the following ranges: HDL_{3c} (3.86–3.94 nm), HDL_{3b} (4.16–4.24 nm), HDL_{3a} (4.46–4.54 nm), HDL_{2a} (4.73–4.87 nm), HDL_{2b}

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TABLE 1. Plasma lipoprotein lipids and apolipoproteins in women during pregnancy, postpartum, and postlactation

	1st Trimester	2nd Trimester	3rd Trimester	Postpartum	Postlactation
			mg/dl		
Total triglyceride	60 ± 5^a	117 ± 9^b	184 ± 14^c	81 ± 9^a	63 ± 7^a
Total cholesterol	170 ± 5^a	234 ± 8^b	254 ± 9^c	234 ± 9^b	192 ± 14^a
VLDL-Tg	22 ± 4^a	44 ± 6^a	83 ± 11^b	38 ± 8^a	31 ± 6^a
VLDL-Chol	4 ± 1^a	8 ± 1^a	18 ± 2^b	7 ± 1^a	6 ± 1^a
LDL-Tg	20 ± 2^a	44 ± 3^b	62 ± 5^c	28 ± 2^d	19 ± 2^a
LDL-Chol	89 ± 5^a	136 ± 8^b	153 ± 8^c	155 ± 9^c	119 ± 13^b
HDL-Tg	12 ± 2^a	26 ± 2^b	29 ± 2^c	8 ± 1^d	6 ± 1^d
HDL-Chol	68 ± 3^a	82 ± 3^b	71 ± 3^a	66 ± 3^a	56 ± 4^a
ApoA-I	136 ± 5^a	163 ± 7^b	162 ± 6^b	138 ± 5^a	135 ± 4^a
ApoB-100	83 ± 5^a	126 ± 6^b	158 ± 9^c	125 ± 9^b	94 ± 9^a

Values are means ± SE of 25 women studied throughout gestation and postpartum; 11 were also studied postlactation. VLDL (d < 1.006 g/ml), LDL (d 1.006–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) were separated by ultracentrifugation at their respective density ranges. Statistical analysis was performed using one-way repeated measures analysis of variance and paired multiple comparisons between groups at each of the gestational or postgestational stages studied; the same letter within one parameter means no statistical differences between the groups, whereas different letters indicate significant differences between the corresponding groups ($P < 0.05$).

(5.31–5.49 nm), and HDL_1 (5.89–6.11 nm). These Gaussian curves sufficed to model any HDL pattern with a normalized squared sum of deviations between the original scan and the model that was always less than 0.15% and a Gaussian model equal to 100 ± 0.3% of the total area of the scan. The amount of each subfraction was calculated both as a percentage of total scan area and as mg cholesterol/dl, based on calculated chromogenicities (22) and total HDL cholesterol.

Lipase activities, cholesteryl ester transfer protein activity, and hormone determinations

Hepatic lipase activity in postheparin plasma was determined according to Huttunen et al. (23), using gum arabic-stabilized [^{3}H]triolein (DuPont/New England Nuclear, Boston, MA) as a substrate. Lipoprotein lipase activity was measured using human VLDL prelabeled with [^{3}H]triolein as substrate, as previously described (24). Briefly, for labeling, glycerol tri(8,9n-^{3}H)oleate (New England Nuclear) dissolved in dimethysulfoxide was incubated with the fraction of d > 1.006 g/ml from human plasma at 37°C for 1 h. Human plasma VLDL isolated by ultracentrifugation (d < 1.006) were added and the mixture was incubated further at 37°C for 17 h. The ^{3}H-labeled VLDL were isolated by ultracentrifugation. For the assay, postheparin plasma samples were mixed with [^{3}H]VLDL (final concentration, 1.5 mmol/L VLDL-triglyceride, radioactivity > 60.000 dpm) in Tris-HCl (pH 8.2) containing 0.15 mol/L NaCl and 18% fatty acid-free bovine serum albumin. LPL activity was calculated as the difference of total [^{3}H]VLDL hydrolysis and that observed in the presence of 1 mol/L NaCl (interassay CV, 5%) expressed in nKat of triglyceride/L plasma. Cholesteryl ester transfer protein (CETP) activity was measured in lipoprotein-free plasma samples (d > 1.21 g/ml) using apoE-free HDL labeled with [^{3}H]cholesteryl oleate as substrate and exogenous VLDL as acceptor, as previously described (11, 24).

All hormones were measured in total plasma using commercially available RIA kits following the protocols given by the manufacturers as follows: prolactin (Allegro PRL, Nichols Institute, San Juan Capistrano, CA), 17β-estradiol (Sorin Biomedica S.p.A., Saluggia, Italy), and progesterone (INC Biomedicals Inc., Irvine, CA). The interassay CV was < 9% for all hormone determinations. ApoA-I and apoB were measured by kinetic immunonephelometry (Array Protein System, Beckman).

Statistics

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way repeated measured analysis of variance and the paired multiple comparison Student-Newmans-Keuls test. These statistical analyses as well as Pearson correlation coefficients were calculated with a SigmaStat Statistical Analysis System v.

TABLE 2. Plasma lipoprotein triglyceride/cholesterol mass ratios in women during pregnancy, postpartum, and postlactation

	1st Trimester	2nd Trimester	3rd Trimester	Postpartum	Postlactation
VLDL Tg/Chol	5.88 ± 0.29^a	6.08 ± 0.38^a	4.78 ± 0.25^b	6.29 ± 0.36^a	$5.14 \pm 0.36^{a,b}$
LDL Tg/Chol	0.22 ± 0.01^a	0.33 ± 0.01^b	0.40 ± 0.02^c	0.19 ± 0.01^a	0.17 ± 0.01^a
HDL Tg/Chol	0.18 ± 0.01^a	0.31 ± 0.02^b	0.42 ± 0.02^c	0.13 ± 0.01^a	0.11 ± 0.01^a

Values are given as means ± SE. Statistical analysis was performed using one-way repeated measures analysis of variance and paired multiple comparisons between groups at each of the gestational or postgestational stages studied. The same letter within one parameter means no statistical differences between groups; different letters indicate significant differences between the corresponding groups ($P < 0.05$).

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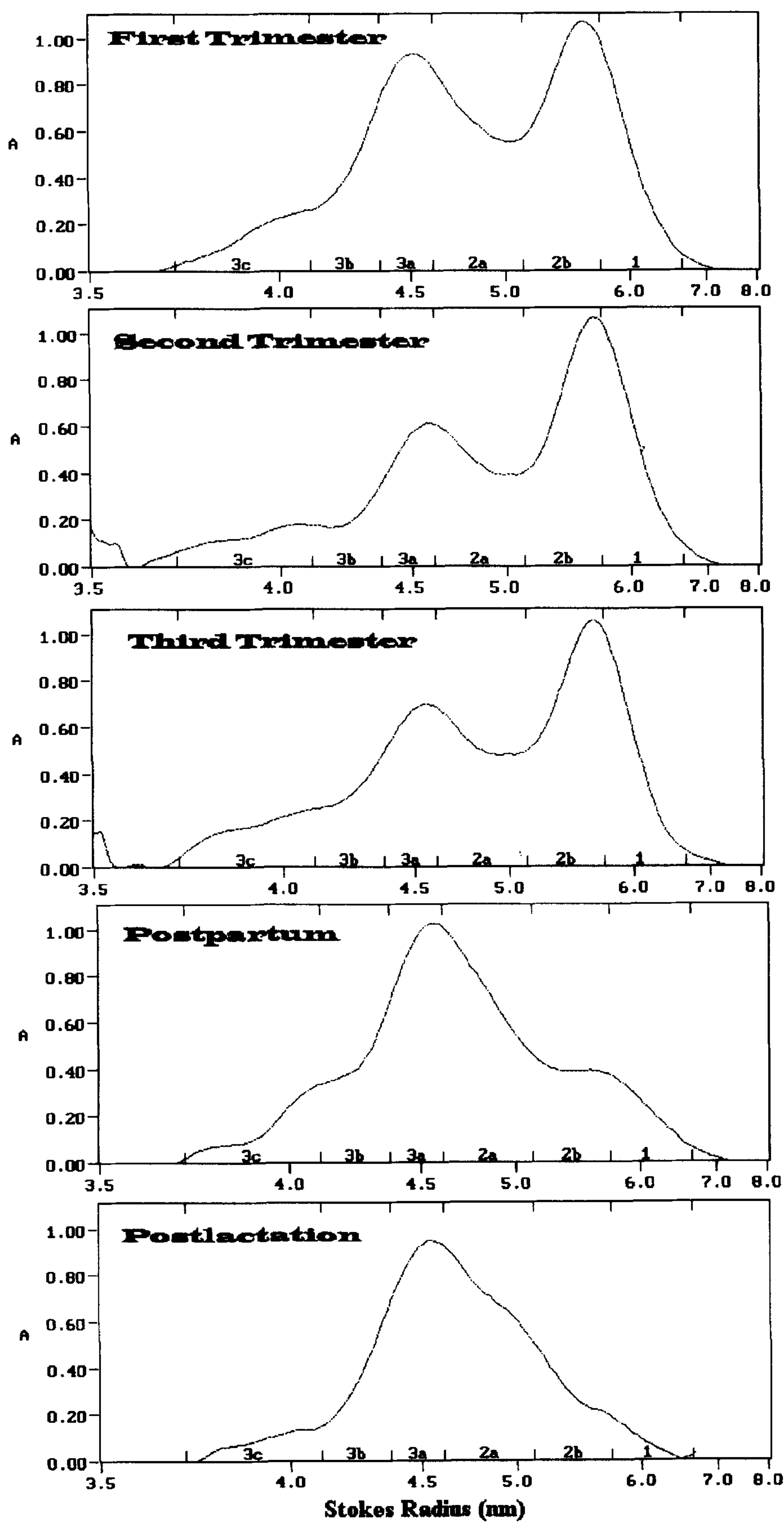

Fig. 1. A representative series of nondenaturating gel scans of HDL subpopulation changes found in one woman during the three trimesters of gestation, at postpartum and postlactation.

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TABLE 3. Proportional distribution of plasma HDL subfractions in women during pregnancy, postpartum, and postlactation

	1st Trimester	2nd Trimester	3rd Trimester	Postpartum	Postlactation
			%		
HDL_1	14.8 ± 3.7[a]	13.3 ± 3.3[a]	9.3 ± 2.6[a,b]	4.5 ± 0.7[b]	3.4 ± 0.7[b]
HDL_{2b}	25.8 ± 2.0[a]	31.3 ± 2.2[b]	31.7 ± 2.1[b]	21.5 ± 1.5[c]	18.6 ± 2.1[d]
HDL_{2a}	19.2 ± 1.5[a]	17.1 ± 0.9[a]	14.4 ± 0.9[b]	19.7 ± 1.1[a]	20.2 ± 1.4[a]
HDL_{3a}	21.9 ± 1.8[a]	21.1 ± 1.7[a]	20.3 ± 1.2[a]	29.0 ± 1.3[b]	29.0 ± 2.5[b]
HDL_{3b}	13.0 ± 2.2[a]	12.3 ± 1.4[a]	15.6 ± 1.2[a,b]	18.3 ± 1.4[b]	22.2 ± 2.8[c]
HDL_{3c}	5.3 ± 1.1[a]	5.0 ± 0.6[a]	8.7 ± 1.0[b]	7.1 ± 0.7[a,b]	6.6 ± 1.0[a]

Values are means ± SE. HDL obtained by ultracentrifugation were processed by polyacrylamide gradient gel electrophoresis for HDL subfraction. Gels were stained with Coomassie G-250 and densitometric areas were expressed as percent of the total area of the scan. Statistical analysis was performed using one-way repeated measures analysis of variance and paired multiple comparisons between groups at each of the gestational or postgestational stages studied. The same letter within one parameter means no statistical differences between groups; different letters indicate significant differences between the corresponding groups ($P < 0.05$).

1.0 (Jandel Scientific GmbH, Erkrath, Germany).

RESULTS

Plasma lipid, lipoprotein, and apolipoprotein concentrations

As shown in **Table 1,** plasma triglyceride and cholesterol concentrations appear similar at the first trimester of gestation and at postlactation; they progressively increase at the second and third trimester of gestation to decline at postpartum; and these changes are sharper for triglycerides than for cholesterol (Table 1). As regards ultracentrifugally isolated lipoproteins, VLDL-triglycerides and -cholesterol increase slightly from the first to the second trimester of gestation, and they increase significantly at the third trimester of gestation, declining again at postpartum to values that did not differ from those at postlactation. LDL-triglycerides increase more than LDL-cholesterol as gestational time advances, reaching their highest level at the third trimester of gestation. HDL-triglyceride levels already appeared higher at the first trimester of gestation than at postlactation, and they further increased at the second and third trimester of gestation, to decline at postparturition to values similar to those at postlactation. HDL-cholesterol levels only increased at the second trimester of gestation. Plasma apoA-I and B-100 concentrations increased at the second trimester of gestation, but, whereas apoA-I levels remained stable at the third trimester and declined at postparturition to values found at postlactation, apoB-100 levels continued to increase up to the third trimester and, although they declined slightly at postparturition values, still remained higher than those at postlactation.

The nonparallel changes between the triglyceride and cholesterol contents in the different lipoprotein fractions prompted us to determine how their triglyceride-to-cholesterol ratio was modified during gestation. As shown in **Table 2,** the triglyceride/cholesterol ratio in VLDL remained stable throughout gestation and postpartum, whereas in both LDL and HDL the ratio progressively increased with the advance of gestation, with a striking decline at postpartum. The proportional enrichment in triglycerides with gestation was even higher in HDL than in LDL, as a comparison between the triglyceride/cholesterol ratio at the third trimester of gestation and that at postlactation shows that HDL had increased by 358% whereas LDL had increased 241% during pregnancy.

HDL subclass patterns

Figure 1 shows a representative series of nondenaturating gel scans of HDL subpopulation changes during pregnancy in one woman throughout pregnancy, postparturition, and postlactation, and **Table 3** summarizes the percent distribution of the HDL subclasses in all women studied. As would be expected, at postlactation

TABLE 4. Plasma cholesteryl ester transfer activity (CETP) and postheparin lipase activities in women during pregnancy, postpartum, and postlactation

	1st Trimester	2nd Trimester	3rd Trimester	Postpartum	Postlactation
CETP activity	211 ± 10[a,b]	226 ± 9[a]	207 ± 7[a,b]	202 ± 9[b]	178 ± 9[b]
LPL activity	407 ± 36[a]	454 ± 39[a]	273 ± 29[b]	439 ± 39[a]	418 ± 51[a]
HL activity	485 ± 47[a]	283 ± 25[b]	212 ± 31[b]	788 ± 56[c]	919 ± 82[d]

Values are means ± SE. Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were determined in postheparin plasma and values are given as pKat/ml. Cholesteryl ester transfer protein (CETP) activity was measured in lipoprotein-free plasma (d > 1.21 g/ml) and values are given as nmol cholesteryl ester transferred per 8 h/ml plasma. Statistical analysis was performed using one-way repeated measures analysis of variance and paired multiple comparisons between groups at each of the gestational or postgestational stages studied. The same letter within one parameter means no statistical differences between groups; different letters indicate significant differences between the corresponding groups ($P < 0.05$).

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TABLE 5. Plasma estradiol, progesterone, and prolactin in women during pregnancy, postpartum, and postlactation

	1st Trimester	2nd Trimester	3rd Trimester	Postpartum	Postlactation
β-Estradiol (pg/ml)	1600 ± 217[a]	9277 ± 969[b]	16397 ± 1307[c]	55 ± 12[d]	55 ± 11[d]
Progesterone (ng/ml)	28.0 ± 2.2[a]	56.0 ± 3.8[b]	132.5 ± 9.3[c]	0.4 ± 0.1[d]	1.3 ± 0.8[d]
Prolactin (ng/ml)	33.3 ± 4.1[a]	101.9 ± 6.7[b]	125.7 ± 5.6[c]	47.4 ± 7.0[a]	6.3 ± 0.6[d]

Values are means ± SE. Statistical analysis was performed using one-way repeated measures analysis of variance and paired multiple comparisons between groups at each of the gestational or postgestational stages studied. The same letter within one parameter means no statistical differences between groups; different letters indicate significant differences between the corresponding groups ($P < 0.05$).

the most abundant HDL is HDL_{3a} and the least abundant is HDL_1. This proportion is, however, greatly altered during gestation when HDL_{2b} becomes the most abundant HDL subfraction whereas HDL_{3c} becomes the least abundant. The proportion of both HDL_1 and HDL_2 was already higher during the first trimester of gestation than during postlactation and remained at this level throughout the three trimesters of gestation. At the third trimester of gestation the percentage of HDL_{2a} decreased and that of HDL_{3c} increased when compared to values either at earlier stages or at postlactation. The percentage of both HDL_{3a} and HDL_{3b} appeared lower throughout gestation than at postlactation. Correcting these percent values in the individual HDL subclasses by the total cholesterol present in HDL showed that the greatest change appeared in the HDL_{2b} fraction, which was significantly higher during the three trimesters of gestation as compared to both postpartum and postlactation, while none of the other HDL subfractions showed any meaningful modification during gestation (data not shown).

Plasma cholesteryl ester transfer protein (CETP) and postheparin lipase activities

As shown in **Table 4,** the activity of CETP does not differ at the different time points studied with the exception of a significant increase at the second trimester of gestation when the CETP activities were higher than at either postpartum or postlactation. The activities of postheparin lipases are also shown in Table 4. Whereas lipoprotein lipase activity was significantly decreased only at the third trimester of gestation, the hepatic lipase activity already appeared decreased at the first trimester of gestation and further decreased at the second and the third trimester of gestation when compared to values at either postpartum or postlactation.

Plasma hormone concentrations

Table 5 summarizes the changes observed in 17β-estradiol, progesterone, and prolactin at the three trimesters of gestation and postpartum. Of these hormones, both the 17β-estradiol and the progesterone levels show

TABLE 6. Lineal semilogarithmic regressions between the changes in plasma lipoprotein triglyceride content and the changes in lipase activities in women during pregnancy, postpartum, and postlactation

	Log HL	Log LPL
VLDL-TG		
n	76	65
r	-0.466	-0.351
P	< 0.001	0.004
LDL-TG		
n	78	66
r	-0.652	-0.398
P	< 0.001	0.001
HDL-TG		
n	74	63
r	-0.757	-0.344
P	< 0.001	0.006

Lineal semilogarithmic regression analysis formula, Y = a + b (logX). The values correspond to the differences between two consecutive periods (2nd minus 1st trimester, 3rd minus 2nd trimester, postpartum minus 3rd trimester, and postlactation minus postpartum).

TABLE 7. Lineal regressions between the changes in HDL subfractions and the changes in lipase activities in women during pregnancy, postpartum, and postlactation

	HL	LPL
HDL_1		
n	82	70
r	-0.077	0.076
P	< 0.492 (NS)	0.531 (NS)
HDL_{2b}		
n	82	70
r	-0.456	-0.169
P	0.001	0.161 (NS)
HDL_{2a}		
n	82	70
r	0.415	0.284
P	0.001	0.017
HDL_{3a}		
n	82	70
r	0.519	0.220
P	< 0.001	0.068 (NS)
HDL_{3b}		
n	82	70
r	0.123	0.052
P	0.271 (NS)	0.668 (NS)
HDL_{3c}		
n	82	70
r	-0.234	-0.162
P	0.034	0.182 (NS)

Lineal regression analysis formula, Y = a + b X. The values correspond to the differences between two consecutive periods (2nd minus 1st trimester, 3rd minus 2nd trimester, postpartum minus 3rd trimester, and postlactation minus postpartum).

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TABLE 8. Lineal semilogarithmic regressions between the changes in lipase activities and the changes in hormones in women during pregnancy, postpartum, and postlactation

	log Estradiol	log Progesterone	log Prolactin
HL			
n	81	81	83
r	-0.783	-0.765	-0.526
P	< 0.001	< 0.001	< 0.001
LPL			
n	69	69	71
r	-0.332	-0.401	-0.143
P	0.005	0.001	0.233 (NS)

Lineal semilogarithmic regression analysis formula, Y = a + b(logX). The values correspond to the differences between two consecutive periods (2nd minus 1st trimester, 3rd minus 2nd trimester, postpartum minus 3rd trimester, and postlactation minus postpartum).

the greatest change, with their values already being highly augmented at the first trimester of gestation and progressively increasing up to the third trimester before declining sharply at postpartum. Plasma prolactin levels also appeared higher at the first trimester of gestation than at postlactation, and they progressively increased as gestational time advanced.

Lineal correlations between the changes in plasma level of lipoprotein-triglycerides and postheparin lipase activities

Table 6 summarizes the lineal correlations between the changes in plasma lipoprotein-triglyceride levels and the logarithm of the changes in postheparin activity of lipoprotein lipase and of hepatic lipase. It can be seen that all of the correlation coefficients are negative and significant, and that values for the correlation coefficients corresponding to hepatic lipase are higher than those for lipoprotein lipase activity, the highest being those that correspond to HDL-triglycerides versus hepatic lipase. On the basis of this finding it was decided to determine which of the changes in the different HDL subclasses correlated better with the changes in the postheparin lipase activities. As shown in **Table 7,** the most significant correlations appeared between hepatic lipase activity and either HDL_{2b} (negative) or HDL_{3a} (positive). The changes in CETP activity did not correlate with any of the lipoprotein values commented upon above (data not shown).

Lineal correlation between changes in postheparin lipase activities and changes in sex hormone levels

As the possibility exists that some of the observed findings could be initially induced by the action of estrogens on hepatic lipase activity, the relationship between the changes in these parameters was also established. As shown in **Table 8,** there was a highly significant negative lineal correlation between the changes in postheparin hepatic lipase activity and the logarithm of the changes of 17β-estradiol, progesterone, and prolactin, with the correlation coefficient value being highest for the former. The correlation coefficient for the changes in postheparin lipoprotein lipase activity and the logarithms of the changes of estradiol and progesterone also appeared significant but their values were

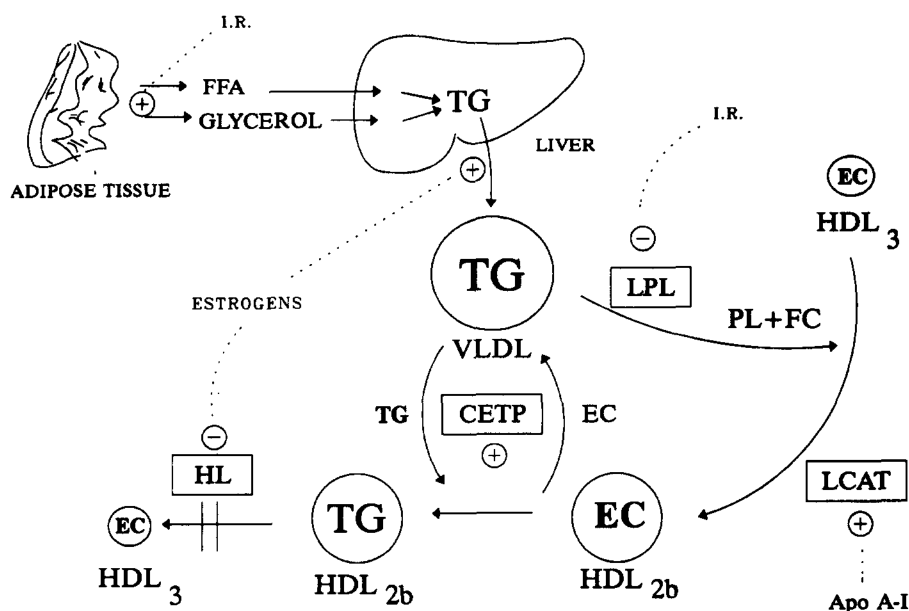

Fig. 2. Schematic representation of major interactions of lipoprotein metabolism during gestation. Activated steps (+) and inhibited steps (-); I.R., insulin resistance; PL, phospholipids; FC, free cholesterol; EC, esterified cholesterol; TG, triglycerides; LPL, lipoprotein lipase; HL, hepatic lipase; CETP, cholesteryl ester transfer protein; LCAT, lecithin cholesterol acyltransferase. See text for additional explanations.

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much lower than the one corresponding to hepatic lipase, indicating a more secondary relationship between these parameters.

DISCUSSION

Although several previous reports from different investigators, including ourselves (3, 5, 6, 10, 11, 18, 19, 25), have addressed the question of lipoprotein profile, sex hormone levels, and enzyme activities related to lipoproteins during gestation in women, the present report is the first to study all these parameters in a longitudinal manner at the three gestational trimesters, postpartum, and postlactation. This strategy has allowed us to determine the correlation between these parameters in order to establish their approximate relationships. The present results show that maternal hypertriglyceridemia has several components, one of which is the increase in circulating VLDL. This change only becomes significant at the third trimester of gestation, coinciding with the decline in postheparin lipoprotein lipase activity, which suggests that the enzyme activity and circulating VLDL may be related. This relationship is also suggested by the significant and negative correlation found when these two particular parameters, from all the individual values studied, are plotted one against the other. It cannot be discounted that enhanced VLDL production would also contribute to the increase in circulating VLDL levels as direct studies in the rat during late gestation have previously demonstrated an enhanced output of VLDL by the perfused liver (26). This effect seems to be mediated by the exaggerated increase in estrogen levels occurring during gestation as estrogen treatment has been shown to enhance liver VLDL production both in women (8, 27, 28) and experimental animals (29).

The specific enrichment of triglycerides by LDL and HDL during gestation and the increase in HDL-triglycerides as early as the first trimester of gestation and in LDL-triglycerides from the second trimester of gestation indicate that other factors besides those commented upon above also contribute to the development of maternal hypertriglyceridemia. The reduction in postheparin hepatic lipase activity during the first trimester of gestation could decrease the conversion of triglyceride-containing HDL_{2b} into the smaller HDL_{3a} or HDL_{3b}, and thus account for the specific relative increment of HDL_{2b}. This possibility is also supported by the highly significant lineal negative correlation found between postheparin hepatic lipase activity and the percentage of HDL_{2b} and the positive correlation found between the same enzyme activity and the percentage of HDL_{3a}. These changes are also probably triggered by the rising estrogen concentration which began at the first trimester of gestation. Estrogen is known to inhibit hepatic lipase activity (21, 30) and a highly significant negative correlation was found here when the logarithm of estradiol in plasma was plotted against both postheparin hepatic lipase activity (Table 8) and the percentage of HDL_{3a} (r = -0.3595, n = 105, $P < 0.001$), while the relation was positive when plotted against HDL_{2b} (r = 0.3784, n = 105, $P < 0.001$).

The increase in CETP found in pregnant women in the present study confirm previous findings from this (11) and other laboratories (10). It was found here that such change appears specifically at the second trimester of gestation and coincides with the greatest increase in the LDL- and HDL-triglyceride content as compared to the change in their cholesterol content. A similar relationship between HDL-triglyceride concentration and CETP activity has previously been found by others in the general normolipidemic population (31), and it is also known that CETP not only catalyzes the transfer of triglycerides from VLDL to HDL in exchange for cholesteryl esters but also from VLDL to LDL (32). No significant correlation could be found here between CETP activity and LDL- or HDL-triglycerides when all the individual values were plotted, indicating that other factors besides CETP would also determine the alteration in the lipoprotein composition during pregnancy. An additional factor that could contribute to the shift in HDL subclass distribution to large HDL_{2b} particles could be lecithin:cholesterol acyltransferase (LCAT) activity, which has been reported to increase during pregnancy (33, 34). The increase in apoA-I concentration seen here already at the second trimester of gestation could also facilitate increased LCAT activity, and through this mechanism enhanced conversion of HDL_{2a} into HDL_{2b} could enhance the availability of substrate for CETP involvement in the interchange of triglycerides and esterified cholesterol for the generation of triglyceride-enriched HDL_{2b}. The decrease in HDL_{2a} percentages and increase in HDL_{2b} found here during late pregnancy support such possibility.

The above major interactions occurring in lipoprotein metabolism during gestation may be schematized as shown in **Fig. 2.** Although the intense and progressive increase in estrogen levels occurring from early gestation could be responsible for some of the changes in key sites of the lipoprotein metabolism, such as the enhanced liver VLDL production or the decreased hepatic lipase activity, other changes may be an indirect consequence of the estrogenic condition or even be independent of it. Thus, from studies in the rat, it is known that adipose tissue is the main site for the decreased lipoprotein lipase activity seen during late gestation (35–37), and that this change is caused by the insulin-resistant condition (38–40) that normally takes place at

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this stage of pregnancy. The overall picture may be completed by the enhanced arrival of free fatty acids and glycerol to the liver as a result of the enhanced lipolytic activity in adipose tissue occurring during late pregnancy (37, 41–43), an effect that may well be related also to the insulin-resistant condition at this stage of gestation. This enhanced availability of substrates for triglyceride synthesis in the liver allows the estrogens to exert their stimulatory effect on the release of VLDL-triglycerides which, together with their action in decreasing hepatic lipase activity, are probably responsible for most of the changes found in the lipoprotein profile during pregnancy.

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