Lipoprotein metabolism of pregnant women is associated with both their genetic polymorphisms and those of their newborn children[1]

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Abstract **To explore whether the placenta contributes to the lipoprotein metabolism of pregnant women, we took advantage of the fact that placental proteins are encoded from the fetal genome and examined the associations between lipids of 525 pregnant women and the presence, in their newborns, of genetic polymorphisms of LPL and apolipoprotein E (APOE), two genes expressed in placenta. After adjustment for maternal polymorphisms, newborn LPL*S447X was associated with lower triglycerides (−21 ± 9 mg/dl), lower LDL-cholesterol (LDL-C; −12 ± 5 mg/dl), lower apoB (−14 ± 4 mg/dl), higher HDL-C (5 ± 2 mg/dl), and higher apoA-I (9 ± 4 mg/dl) in their mothers; newborn LPL*N291S was associated with higher maternal triglycerides (114 ± 31 mg/dl); and newborn APOE*E2 (compared to E3E3) was associated with higher maternal LDL-C (14 ± 6 mg/dl) and higher maternal apoB (14 ± 5 mg/dl). These associations (all $P < 0.05$) were independent of polymorphisms carried by the mothers and of lipid concentrations in newborns and were similar in amplitude to the associations between maternal polymorphisms and maternal lipids. Such findings support the active role of placental LPL and APOE in the metabolism of maternal lipoproteins and suggest that fetal genes may modulate the risk for problems related to maternal dyslipidemia (preeclampsia, pancreatitis, and future cardiovascular disease).**—Descamps, O. S., M. Bruniaux, P-F. Guilmot, R. Tonglet, and F. R. Heller. **Lipoprotein metabolism of pregnant women is associated with both their genetic polymorphisms and those of their newborn children.** ***J. Lipid Res.*** **2005.** 46: **2405–2414.**

Supplementary key words low density lipoprotein • high density lipoprotein • fetus • pregnancy • placenta

Whether human placenta plays an important role in the metabolism of lipoproteins in the pregnant woman still remains unclear (1). Although this idea is consistent with the assumption that maternal lipoproteins supply lipids to the fetus via the placenta, at present it is only supported by in vitro arguments: immunochemistry and RNA hybridization in placental tissues demonstrated the presence of some key players of lipoprotein metabolism (lipoprotein lipases, receptors, and apolipoproteins) (2–9), whereas tissue cultures of placental fragments indicated the capture and hydrolysis (10–13) as well as the synthesis and secretion (14) of lipoproteins. However, in vivo evidence is still lacking in humans. Furthermore, the simple idea of a substantial lipid transfer between mother and fetus remains challenged by the absence of a strong correlation between the concentrations of lipoproteins in pregnant women and in their newborns at birth [see (15) for references].

To explore the hypothesis that placental proteins contribute effectively to the lipoprotein metabolism of pregnant women, we took advantage of the fact that placental proteins are encoded by genes belonging to the fetal genome. Under these conditions, the demonstration of an association between genetic polymorphisms present in newborns and variations of lipoprotein concentrations in their mothers may argue in favor of the hypothesis.

For several reasons, we focused our attention on the common polymorphisms of the genes of lipoprotein lipase [LPL*S447X and LPL*N291S (16)], apolipoprotein E [APOE*E2 and APOE*E4 (17)], and apolipoprotein C-III [APOC3*S2 (18)]. First, these genes are known to be involved in the metabolism of triglyceride-rich lipoproteins, which dramatically increase in concentration during pregnancy (19). Second, two of these genes, LPL and APOE, are abundantly expressed in the placenta (2, 3, 11), whereas APOC3 is less expressed in placenta and in

Manuscript received 17 November 2004 and in revised form 3 June 2005 and in re-revised form 28 July 2005.

Published, JLR Papers in Press, August 16, 2005.
DOI 10.1194/jlr.M500223-JLR200

[1] The authors acknowledge the help and encouragement of Dr. René Tonglet, who died during the course of these investigations.

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fetus (20), offering the possibility to compare the influence of genes variably expressed by the placenta. Third, their polymorphisms are well known for their associations with lipoprotein concentrations in adults (16–18), and the mechanisms by which these associations occur have been linked directly to quantitative or qualitative alterations of the proteins encoded by these genes. In adults, the S447X allele of LPL, which enhances the lipolytic activity of the enzyme, is associated with lower triglycerides and higher HDL-cholesterol (HDL-C), whereas the N291S allele of LPL, which reduces the activity of the enzyme, is associated with increased triglycerides and reduced HDL-C (16). The S2 allele of APOC3 is also associated with higher triglycerides in adults, because of the hepatic overproduction of apolipoprotein C-III, an inhibitor of lipoprotein lipase (18). The E2 allele of APOE is associated with lower LDL-C, because of the lower affinity of the isoform apoE2 for the apoE receptor (→ delayed clearance of apoE-rich chylomicron remnants → depletion of hepatocyte pool of cholesterol → overexpression of LDL receptor → increased clearance of LDL particles) (17, 21). Finally, a previous study in pregnant women has shown that polymorphisms (APOE*E2, LPL*S447X, and LPL*N291S) present in the genome of these women were associated with variations of their own lipoprotein concentrations (22).

Here, we describe, for the first time, associations between genetic polymorphisms carried by newborns and variations of lipoprotein concentrations in their mothers.

METHODS

Subjects

The present study was part of a project (15) aimed at characterizing the reciprocal influences between the lipoprotein metabolisms of pregnant women and of their children at the end of gestation. As described previously (15), 525 mothers and their newborns were consecutively recruited in the maternity ward of our hospital based on the following criteria: Caucasian origin, eutocic delivery with cephalic presentation after a programmed labor induction (to allow the standardization of blood sampling) between the beginning of the 37th week and the end of the 41st week, singleton live birth, no gestational complication, no diabetes, no congenital malformation or perinatal problem, body weight between 2,500 and 3,999 g, Apgar score of ⩾7 during the first minute and ⩾9 by the 5th minute. All mothers gave informed consent, and the ethical committee of the hospital approved the study protocol.

Clinical, biological, and genetic data

We analyzed the concentrations of cholesterol, triglycerides, HDL-C, LDL-C, apoB, and apoA-I (referred in the text as the "maternal lipids" and "newborn lipids") in the peripheral blood of mothers taken at the start of the labor induction and in the cord of newborns at birth. We also recorded "maternal nonlipid factors," such as age, smoking status, weight and height of the mothers before delivery, and "newborn nonlipid factors," such as sex, gestational age, weight, height, body mass index, head circumference, and Apgar score of the newborns. Because the women were recruited upon entering the delivery room in the obstetrical unit and their weight before pregnancy was recorded in only 142 (27%) obstetrical files, we collected these data retrospectively in 279 (53%) other women, whereas they remained unknown for 104 women (19%). The weight gain during pregnancy was calculated in these 142 and 279 women.

Newborn and maternal DNA were analyzed for the following polymorphisms: the C-to-G transversion at nucleotide 1,595 in exon 9 of the LPL gene, converting serine to a premature termination (the LPL*S447X allele) (16); the A-to-G transition at nucleotide 1,127 in exon 6 of the LPL gene, converting asparagine to serine (the LPL*N291S allele) (16); the variants E2 and E4 (the APOE*E2 or APOE*E4 allele) of the APOE gene (17); and the G-to-C transversion at nucleotide 3,238 in the 3′ untranslated region (called APOC3*S2 because it is detectable by *Sst*I restriction fragment-length polymorphism; the wild type is S1) of the APOC3 gene (18). Because of the very low frequencies of homozygous genotypes for the less common alleles of these polymorphisms, we defined dichotomically the "genetic factor" as "carriage" and "noncarriage" of the less common allele(s) rather than on the basis of the genotypes: LPL*S447X$^+$ (Ser/Stop or Stop/Stop at codon 447) and LPL*S447X$^-$ (Ser/Ser at codon 447); LPL*N291S$^+$ (Asn/Ser or Ser/Ser at codon 291) and LPL*N291S$^-$ (Asn/Asn at codon 291); APOC3*S$^+$ (S2/S2 or S1/S2) and APOC3*S2$^-$ (S1/S1); APOE*E2$^+$ (E2/E2 or E3/E2), APOE*E4$^+$ (E4/E3 or E4/E4), and APOE*E3E3 (E3/E3). Because alleles E2 and E4 are known to determine opposite biological effects, we excluded the E2$^+$ mothers with an E4$^+$ newborn and E4$^+$ mothers with an E2$^+$ newborn.

Sample size

Our hypothesis was that lipids of pregnant women are associated with the genetic factors of their newborns, independently of their own genetic factors. Our strategy consisted primarily of comparing, two by two, lipids of different groups of mothers classified according to their genetic status and the genetic status of their newborns (seven groups for the APOE gene and four for the other loci). After having collected the first 100 mothers, we estimated the sample size from the data on the two major blood lipids (LDL-C ∼ 165 ± 45 mg/dl and triglyceride ∼ 260 ± 70 mg/dl): to detect a 15% difference between two groups requires at least 52 mothers in each group for a two-sided significance level of 0.05 and a statistical power of 80%. Based on the allelic frequencies of these 100 pairs of mothers and newborns, and given Mendel's laws of genetic transmission (assuming random mating), we estimated the expected proportion of each group of mothers for each polymorphism (LPL*N291S was not considered because it was very rare in this first sample). From the proportion of the smallest group (∼10%), we set our target sample size to 520.

Statistics

Before testing our hypothesis, we examined possible correlations between maternal lipids and all nonlipid and nongenetic (maternal and newborn) factors to identify potential confounders in our subsequent analysis. As previously done by McGladdery and Frohlich (22), we examined how maternal genetic factors alone associated with maternal lipids (as well as other nonlipid parameters) and, by multivariate analysis [analysis of covariance (ANCOVA)], how they interacted with nonlipid factors to determine maternal lipids.

Our hypothesis was tested first using a subgroup analysis and then a multivariate analysis. *1*) The subgroup analysis was performed by comparing two by two (unpaired two-tailed Student's *t*-test) the maternal lipids in different groups of mothers classified according to their genetic status and the genetic status of their newborns for each polymorphism separately. *2*) ANCOVA was then used to estimate the independent effects of each of the significant (maternal and newborn) genetic factors on maternal

lipids. To limit the number of variables, we proceeded first by looking separately at models relating one maternal lipid variable with the polymorphism at one locus in the mothers and the newborns: these models thus included two dummy variables for the presence of the rare allele in the mothers or in the newborns, a product term of these two dummy variables (to assess possible interactions between maternal and newborn genes). We extended these models by adding variables for nongenetic factors that were associated with maternal lipids in our preliminary correlation analysis, and product terms of these last factors with the genetic dummy variables (to assess interactions between nongenetic and genetic factors). Thereafter, we built more general models relating one lipid variable with all of the terms associated ($P < 0.10$) in these first models, including also product terms to assess the gene-gene interactions between different polymorphisms carried in one or another of the two genomes (mother and newborn) as well as product terms to assess the gene-environment interactions between genetic factors and some nongenetic factors (even if not correlated with maternal lipids in the preliminary correlation analysis) that are commonly described in the general population (e.g., the interaction between weight and LPL*S447X or APOC3*S2 for triglycerides). Because there was no statistically significant interaction (see Results), all final models simplified to additive models as presented in **Table 1**, where the β-coefficients represented the quantitative estimates of independent lipid changes associated with each factor (genetic and nongenetic) and the changes of R^2 estimated the contribution of each factor to the variance of the maternal lipids. These final multivariate models can be easily deduced from Table 1 (column 2); example for maternal LDL-C: $LDLm = \beta_0 + \beta_1.Em + \beta_2.Cm + \beta_4.Xm + \beta_5.En + \beta_4.Xn$. [X indicates the presence of the X allele of LPL*S447X, C indicates the presence of APOC3*S2, and E indicates the presence of APOE*E2 in mother (m) or in newborn (n).]

Finally, to exclude the hypothesis that the variations of maternal lipids associated with newborn polymorphisms were associated with these newborn polymorphisms, we examined whether the addition of a variable for the newborn lipid best correlated with the maternal lipid modified the β-coefficients of the final models (Table 1, column 3). All multilinear regression analyses were performed using SPSS for Windows, version 12.0.

ApoE phenotyping of maternal plasma

At the end of our study, we tried to verify the hypothesis that placental apoE (encoded from the fetal genome) was secreted in the maternal circulation by performing apoE phenotyping [isoelectric focusing electrophoresis (IEF) followed by immunoblotting (23, 24)] in EDTA plasma of eight APOE*E3E3 mothers bearing APOE*E3E2 or APOE*E3E4 newborns. In such cases, the presence of apoE2 or apoE4 in maternal plasma would support the hypothesis. Plasma was analyzed with (23) and without (24) neuraminidase treatment. IEF was performed under the classical conditions for optimal apoE isoform separation (23). Immunoblotting was assayed using two different human apoE antibodies [mouse monoclonal and goat polyclonal antibodies from Calbiochem (La Jolla, CA)] followed by anti-mouse or anti-goat IgG conjugated with peroxidase. The detection limit of our method was examined by testing a mix of pooled plasma of E3E3 mothers with E3E3 newborns (diluted at 1:2) in which we added different dilutions (final dilutions of 1:2, 1:4, 1:8, 1:10, 1:20, and 1:40) of cord plasma of newborns with E3/E4 or E2/E3. In these mixes, we found that the E2 or E4 bands of newborn plasma were still visible at the 1:8 final dilution but not at higher dilutions.

TABLE 1. Multivariate analysis for lipoprotein and apolipoprotein concentrations in the mothers

		Maternal Genetic Factors Only			Maternal and Newborn Genetic Factors Only			Maternal and Newborn Genetic Factors and Newborn Lipids		
Lipids	Factors in the Models	β ± SEM	R^2	P	β ± SEM	R^2	P	β ± SEM	R^2	P
LDL-C	Mother APOE*E2	−29 ± 6	4.5%	<0.001	−33 ± 6	4.5%	<0.001	−29 ± 6	2.9%	<0.001
	Mother APOC3*S2	9 ± 6	0.5%	0.09	9 ± 6	0.5%	0.08	14 ± 5	1.2%	0.007
	Mother LPL*S447X			NS	9 ± 5	0.5%	0.09	11 ± 5	0.5%	0.03
	Newborn APOE*E2				14 ± 6	1.0%	0.02	21 ± 6	1.9%	<0.001
	Newborn LPL*S447X				−12 ± 5	0.7%	0.02	−9.7 ± 5	0.6%	0.05
	Newborn LDL-C							0.99 ± 0.16	6.6%	<0.001
		Total R^2	5.0%	<0.001	Total R^2	7.2%	<0.001	Total R^2	13.7%	<0.001
ApoB	Mother APOE*E2	−23 ± 5	4.9%	<0.001	−27 ± 5	4.9%	<0.001	−24 ± 5	4.9%	<0.001
	Mother APOC3*S2	10 ± 4	1.1%	0.01	11 ± 4	1.2%	0.009	14 ± 4	1.9%	0.001
	Mother LPL*S447X			NS	8 ± 4	0.8%	0.04	10 ± 4	1.1%	0.01
	Newborn APOE*E2				14 ± 5	1.5%	<0.001	17 ± 5	2.2%	0.001
	Newborn LPL*S447X				−14 ± 4	1.8%	<0.001	−13 ± 4	1.5%	0.001
	Newborn LDL-C							0.56 ± 0.13	2.0%	0.001
		Total R^2	6.1%	<0.001	Total R^2	10.2%	<0.001	Total R^2	13.6%	<0.001
HDL-C	Newborn weight (kg)	−7 ± 2	2.1%	0.001	−7 ± 2	2.1%	0.01	−7 ± 2	2.1%	<0.001
	Mother ApoE*E2	6 ± 2	1.1%	0.02	5 ± 2	0.9%	0.03	4 ± 1	0.6%	0.05
	Newborn LPL*S447X				5 ± 2	1.4%	0.006	5 ± 2	1.4%	0.02
	Newborn HDL-C							0.18 ± 0.09	1.1%	0.01
		Total R^2	3.2%	<0.001	Total R^2	4.4%	<0.001	Total R^2	5.3%	<0.001
ApoA-I	Newborn weight (kg)	−9 ± 4	0.8%	0.04	−9 ± 4	0.8%	0.04	−9 ± 4	0.9%	0.04
	Mother ApoE*E2	13 ± 5	1.2%	0.02	13 ± 5	1.2%	0.01	12 ± 5	1.2%	0.02
	Newborn LPL*S447X				9 ± 4	0.9%	0.03	9 ± 4	0.9%	0.04
	Newborn apoA-I							0.26 ± 0.1	0.8%	0.04
		Total R^2	2.0%	0.007	Total R^2	2.9%	0.002	Total R^2	3.8%	0.001
Triglycerides	Mother LPL*S447X	−20 ± 9.0	0.9%	0.02	Not significant (P = 0,19)			Not significant (P = 0,28)		
	Newborn LPL*S447X				−21 ± 9	1.1%	0.02	−20 ± 9	1.1%	0.02
	Newborn LPL*N291S				114 ± 31	2.8%	<0.001	108 ± 31	2.8%	<0.001
	Newborn triglyceride							0.35 ± 0.2	0.008	0.04
		Total R^2	0.9%	0.02	Total R^2	3.9%	<0.001	Total R^2	4.7%	<0.001

ApoE, apolipoprotein E; LDL-C, LDL-cholesterol.

RESULTS

The characteristics of our cohort of 525 mothers and newborns (261 girls and 264 boys) have been described previously (15). Briefly, maternal characteristics were as follows: age, 28.8 ± 5.3 years; height, 162.8 ± 6.1 cm; weight at the end of pregnancy, 78.9 ± 15.4 kg; 19% smoked regularly during the whole pregnancy. In 421 women, prepregnancy weight was known (279 self-reported retrospectively and 142 recorded in file: 65.9 ± 16.3 kg) and weight gain was calculated: 12.8 ± 5.1 kg. Lipid concentrations in mothers (in mg/dl) were as follows: total cholesterol, 297 ± 52; LDL-C, 162 ± 47; HDL-C, 83 ± 18; triglycerides, 258 ± 82; apoA-I, 225 ± 39; and apoB, 151 ± 35. Newborn characteristics were as follows: gestational age, 39.3 ± 1.1 weeks; newborn weight, 3,230 ± 412 g; newborn height, 49.0 ± 2.6 cm; and head circumference, 34.0 ± 1.4 cm. Lipid concentrations in newborns (in mg/dl) were as follows: total cholesterol, 72 ± 16; LDL-C, 29 ± 13; HDL-C, 34 ± 9; triglycerides, 43 ± 19; apoA-I, 77 ± 13; and apoB, 28 ± 9.

Among all of the maternal (age, weight before and at the end of pregnancy, weight gain, and smoking status) and newborn (sex, gestational age, weight, height, head circumference, and body mass index) nonlipid variables, only newborn weight was correlated with maternal HDL-C ($r = -0.15$, $P = 0.001$) and less with maternal apoA-I ($r = -0.09$, $P = 0.07$). This absence of correlation was not surprising given the very weak level (or the absence) of such correlations in the literature (22, 25–27) and given the narrow range of variations of maternal age, gestational age, and newborn weight attributable to our selection process. As described previously (15), maternal lipids correlated slightly with newborn lipids, the best correlations being between newborn and maternal LDL ($R = 0.26$, $R^2 = 6.7\%$, $P < 0.001$), between newborn and maternal HDL-C ($R = 0.11$, $R^2 = 1.2\%$, $P = 0.009$), between newborn and maternal triglyceride ($R = 0.11$, $R^2 = 1.2\%$, $P = 0.02$), between newborn and maternal apoA-I ($R = 0.10$, $R^2 = 1.0\%$, $P = 0.02$), and between newborn LDL-C and maternal apoB ($R = 0.18$, $R^2 = 3.2\%$, $P < 0.001$). The factors affecting newborn lipid levels were examined in our previous paper (15).

Maternal lipid concentrations according to maternal polymorphisms

Tables 2–4 describe the effects of polymorphisms of mothers on their own concentrations of lipids. The LPL*S447X$^+$ mothers had significantly lower triglycerides (−8%; $P = 0.03$) compared with LPL*S447X$^-$ mothers (Table 2). By contrast, there was no difference between carriers and noncarriers of the LPL*N291S allele, but the number of LPL*N291S$^+$ mothers was very small (n = 7). The APOC3*S2$^+$ mothers had significantly higher total cholesterol (+4%; $P = 0.04$), higher LDL-C (+6%; $P = 0.05$), and higher apoB (+10%; $P = 0.005$) compared with the APOC3*S2$^-$ mothers (Table 3). The APOE*E2$^+$ mothers had significantly lower LDL-C (−17%; $P < 0.001$) and apoB (−15%; $P < 0.001$) compared with the APOE*E3E3 mothers, whereas the APOE*E4$^+$ mothers showed no difference compared with APOE*E3E3 mothers (Table 4).

Between these genotypic groups, there was no difference in maternal nonlipid factors such as age, smoking status, weight before and at the end of pregnancy, weight gain, and height before delivery, or in newborn nonlipid factors such as sex, gestational age, weight, height, body mass index, head circumference, and Apgar score. Table 1 (first column) summarizes the independent effects and

TABLE 2. Characteristics of the mothers in association with LPL polymorphisms

Characteristic	Rare Allele Absent	Rare Allele Present	*P*
	*LPL*S447X*$^-$	*LPL*S447X*$^+$	
Number	422	103	
Maternal age (years)	28.8 ± 5.4	28.8 ± 4.9	NS
Prepregnancy weight (kg)[a]	65.4 ± 16.6	68.1 ± 14.7	NS
Weight before delivery (kg)	78.9 ± 14.9	79.1 ± 14.9	NS
Weight gain (kg)[a]	12.6 ± 13.6	13.6 ± 5.2	NS
Maternal height (cm)	162.7 ± 6.0	163.1 ± 6.6	NS
Newborn weight (g)	3,275 ± 400	3,219 ± 415	NS
Gestational age (weeks)	39.36 ± 1.09	39.20 ± 1.05	NS
Total cholesterol (mg/dl)	297 ± 50	298 ± 56	NS
HDL-C (mg/dl)	83 ± 18	83 ± 18	NS
Triglycerides (mg/dl)	262 ± 84	243 ± 69	0.04
LDL-C (mg/dl)	162 ± 46	167 ± 50	NS
ApoA-I (mg/dl)	225 ± 39	224 ± 36	NS
ApoB (mg/dl)	150 ± 34	154 ± 38	NS
	*LPL*N292S*$^-$	*LPL*N292S*$^+$	
Number	517	8	
Maternal age (years)	29.6 ± 4.5	28.8 ± 5.1	NS
Prepregnancy weight (kg)[a]	65.8 ± 16.1	73.8 ± 25.8	NS
Weight before delivery (kg)	78.9 ± 15.2	80.2 ± 23.1	NS
Weight gain (kg)[a]	12.9 ± 5.1	8.9 ± 4.7	NS
Maternal height (cm)	162.8 ± 6.1	163.5 ± 4.3	NS
Newborn weight (g)	3,389 ± 369	3,227 ± 414	NS
Gestational age (weeks)	39.21 ± 1.03	39.33 ± 1.09	NS
Total cholesterol (mg/dl)	297 ± 52	290 ± 63	NS
HDL-C (mg/dl)	83 ± 18	87 ± 24	NS
Triglycerides (mg/dl)	258 ± 82	280 ± 76	NS
LDL-C (mg/dl)	164 ± 47	145 ± 49	0.19
ApoA-I (mg/dl)	225 ± 38	233 ± 37	NS
ApoB (mg/dl)	151 ± 36	149 ± 35	NS

[a] These data were available for 339 LPL*S447X$^-$ mothers, 82 LPL*S447X$^+$ mothers, 415 LPL* N292S$^-$ mothers, and 6 LPL*N292S$^+$ mothers.

TABLE 3. Characteristics of the mothers in association with the APOC3 polymorphism

Characteristic	*APOC3*S2*$^-$	*APOC3*S2*$^+$	*P*
Number	432	93	
Maternal age (years)	28.3 ± 5.1	29.0 ± 5.0	NS
Prepregnancy weight (kg)[a]	66.3 ± 15.9	64.1 ± 18.1	NS
Weight before delivery (kg)	79.2 ± 15.1	77.6 ± 16.6	NS
Weight gain (kg)[a]	12.9 ± 5.1	12.3 ± 4.9	NS
Maternal height (cm)	162.8 ± 6.1	162.7 ± 6.1	NS
Newborn weight (g)	3,279 ± 395	3,219 ± 416	NS
Gestational age (weeks)	39.26 ± 1.07	39.34 ± 1.08	NS
Total cholesterol (mg/dl)	295 ± 51	307 ± 53	0.04
HDL-C (mg/dl)	83 ± 18	85 ± 19	NS
Triglycerides (mg/dl)	256 ± 81	266 ± 80	NS
LDL-C (mg/dl)	161 ± 45	171 ± 46	0.05
ApoA-I (mg/dl)	224 ± 38	228 ± 43	NS
ApoB (mg/dl)	149 ± 34	159 ± 40	0.02

[a] These data were available for 346 APOC3*S2$^-$ mothers and 75 APOC3*S2$^+$ mothers.

TABLE 4. Characteristics of the mothers in association with the presence or absence of the alleles APOE*E2 (E2) and APOE*E4 (E4) of the apoE gene

Characteristic	E2 and E4 Absent	E2 Present	*P*	E4 Present	*P*
Number	334	73		109	
Maternal age (years)	28.8 ± 5.0	28.3 ± 5.4	NS	29.2 ± 4.9	NS
Prepregnancy weight (kg)[a]	65.6 ± 15.9	64.8 ± 19.2	NS	67.6 ± 15.3	NS
Weight before delivery (kg)	78.7 ± 14.7	79.1 ± 17.9	NS	79.1 ± 16.5	NS
Weight gain (kg)[a]	12.7 ± 5.1	12.1 ± 5.16	NS	13.6 ± 5.0	NS
Maternal height (cm)	1.63 ± 5.9	162.1 ± 6.0	NS	162.9 ± 6.7	NS
Newborn weight (g)	3,230 ± 399	3,314 ± 429	NS	3,205 ± 431	NS
Gestational age (weeks)	39.3 ± 1.09	39.5 ± 1.07	NS	39.3 ± 1.09	NS
Total cholesterol (mg/dl)	301 ± 52	278 ± 41	<0.001	299 ± 49	NS
HDL-C (mg/dl)	83 ± 18	87 ± 17	0.06	81 ± 19	NS
Triglycerides (mg/dl)	256 ± 78	272 ± 83	0.12	259 ± 90	NS
LDL-C (mg/dl)	167 ± 45	138 ± 37	<0.001	167 ± 46	NS
ApoA-I (mg/dl)	226 ± 39	236 ± 33	0.05	215 ± 40	NS
ApoB (mg/dl)	155 ± 33	132 ± 33	<0.001	152 ± 35	NS

Data for the nine mothers carrying APOE*E2E4 are not displayed. Their lipoprotein concentrations were not statistically different from those of APOE*E3E3 mothers. Mothers with E2 and E4 absent represent the APOE*E3E3 carriers.

[a] These data were available for 268 mothers without E2 or E4, 59 mothers with E2, and 94 mothers with E4.

the percentages of the variances in lipids explained by the maternal genotypes alone, estimated by ANCOVA.

Maternal lipid concentrations according to both maternal and newborn polymorphisms

When we examined the lipid concentrations in the groups of mothers classified according to their genotypes as well as the genotypes of their newborns, we found several associations between maternal lipids and newborn polymorphisms independent of the polymorphisms carried by the mothers (**Figs. 1**, **2**).

The presence of LPL*S447X in newborns affected the concentrations of triglycerides, HDL-C, apoB, and LDL-C in their mothers with similar patterns whether or not the mothers carried the LPL*S447X allele (Fig. 1). Among LPL*S447X$^-$ mothers, those bearing a LPL*S447X$^+$ newborn had lower apoB (−7%; $P = 0.02$), lower triglycerides (−7%; $P = 0.09$), higher HDL-C (+9%; $P = 0.003$), and higher apoA-I (+5%; $P = 0.04$) compared with those bearing a LPL*S447X$^-$ newborn. Among LPL*S447X$^+$ mothers, those bearing a LPL*S447X$^-$ newborn also had lower LDL-C (−20%; $P < 0.001$), lower apoB (−9%; $P = 0.06$), and lower triglycerides (−9%; $P = 0.10$) but no significant change of maternal HDL-C and apoA-I (although a slight increase is visible in Fig. 1). The reduction of maternal triglycerides produced by the presence of LPL*S447X in newborns was additive with the reduction associated with the presence of LPL*S447X in mothers (difference between group A and group D: −13%; $P = 0.005$), whereas, for the other maternal lipid fractions, the effects of the newborn LPL*S447X were counterbalanced by the inverse effects of maternal LPL*S447X (as is particularly visible for the apoB concentration in Fig. 1).

The presence of the LPL*N291S allele in newborns was also associated with maternal lipids, although these associations should be regarded with more caution because of the low frequency of this allele (Fig. 2). Among LPL*N291S$^-$ mothers (n = 518), those bearing a LPL*N291S$^+$ newborn (n = 5) had higher triglycerides (+65%; $P < 0.001$), higher total cholesterol (+18%; $P = 0.02$), higher apoB (+20%; $P = 0.05$), and trends toward higher LDL-C (not shown in Fig. 2; 193 ± 11 vs. 162 ± 47 mg/dl; +18%; $P = 0.13$) and lower HDL-C (not shown in Fig. 2; 75 ± 9 vs. 83 ± 18 mg/dl; −10%; $P = 0.10$) compared with those bearing a LPL*N291S$^-$ newborn (n = 513). There were too few LPL*N291S$^+$ mothers (n = 7, with six bearing a LPL*N291S$^-$ newborn and one bearing a LPL*N291S$^+$ newborn) to examine the association between maternal lipids and newborn LPL*N291S.

For the apoE gene, the APOE*E2 allele of newborns, but not the APOE*E4 allele (data not shown), was associated with changes in maternal LDL-C and apoB compared with the presence of APOE*E3E3 (**Fig. 3**). Among the APOE*E3E3 mothers, those bearing an APOE*E2$^+$ newborn had higher LDL-C (+9%; $P = 0.06$) and apoB (+11%; $P = 0,007$) compared with those bearing an APOE*E3E3 newborn. Among the APOE*E2$^+$ mothers, those bearing an APOE*E2$^+$ newborn had a tendency toward higher LDL-C (+11%; $P = 0.09$) compared with those bearing an APOE*E3E3 newborn.

Finally, we found no significant association of maternal lipids with the APOC3*S2 polymorphism of newborns (data not shown). There was also no difference in the maternal and newborn nonlipid variables between the various genotypic groups examined (data not shown).

Multivariate analysis

All maternal lipids were associated with newborn genetic factors independently of the maternal genetic factors (Table 1, second column). The concentration of triglycerides was associated only with the polymorphisms (LPL*S447X and LPL*N291S) carried by the newborns. The association between maternal LPL*S447X and triglycerides that was significant in the statistical analysis ignoring the newborn polymorphisms (Table 1, first column; see also Table 2) was smaller and no more significant ($P = 0.19$) when we added newborn LPL*S447X in the multivariate model (Table 1, second column). Most likely, part

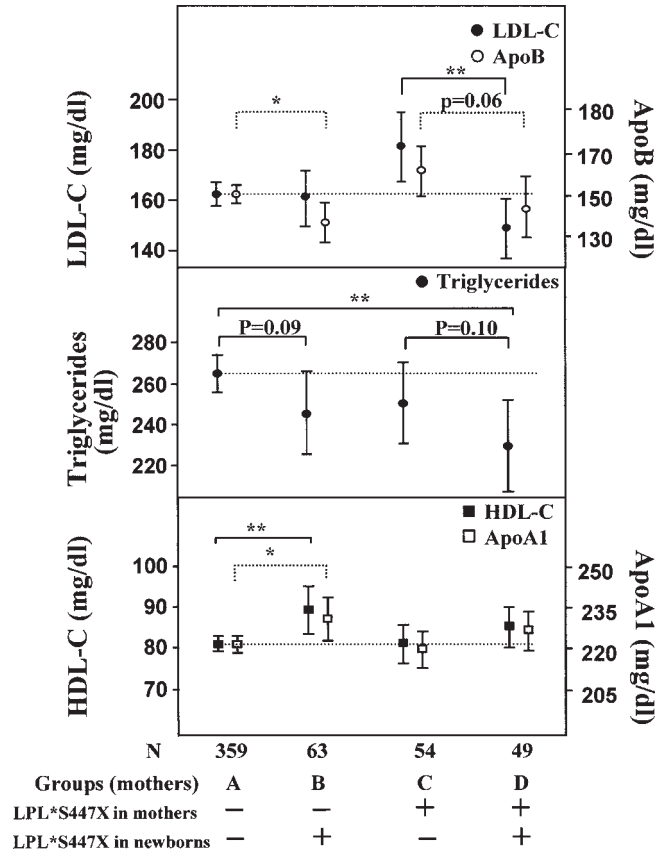

Fig. 1. Concentrations (means and 95% confidence intervals) of LDL-cholesterol (LDL-C), apolipoprotein B (apoB), triglycerides, HDL-C, and apoA-I in various groups of mothers classified according to the carriage of LPL*S447X by their newborns and by themselves. The Y axes were scaled to set the point representing the mean values of concentrations of the referent (group A) at the same graphic level. * $P < 0.05$ and ** $P < 0.01$ comparing the groups between brackets.

of the significant association between maternal triglycerides and maternal LPL*S447X seen in Table 2 was the result of the association between maternal triglycerides and newborn LPL*S447X, because half of LPL*S447X$^+$ mothers had LPL*S447X$^+$ newborns.

In contrast, maternal LDL-C and apoB were associated to a larger extent with maternal polymorphisms (mainly APOE*E2) and only to a small extent with newborn polymorphisms (APOE*E2 and LPL*S447X). It is interesting, however, that the association between maternal LPL*S447X and maternal LDL-C or apoB that was not significant ($P =$ 0.25 and 0.21) in analysis ignoring newborn LPL*S447X (Table 1, first column; see also Table 2) became almost significant when we added newborn LPL*S447X in the multivariate model. The effect remained very small, however (as can be seen also in Fig. 1). HDL-C and apoA-I were associated with the polymorphisms of the mothers (APOE*E2) and their newborns (LPL*S447X).

Overall, it was estimated that the maternal genetic factors contributed from 0.5% to 4.9% to the variability of the maternal lipids (the greatest for LDL-C and apoB), whereas the contributions of newborn genetic factors ranged between 0.6% and 2.8%.

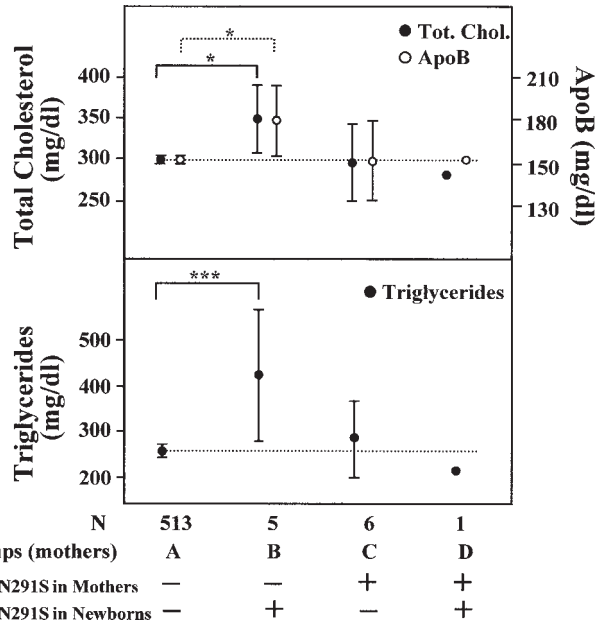

Fig. 2. Concentrations (means and 95% confidence intervals) of total cholesterol, apoB, and triglycerides in various groups of mothers classified according to the carriage of LPL*N291S by their newborns and by themselves. The Y axes were scaled to set the point representing the mean values of concentrations of the referent (group A) at the same graphic level. * $P < 0.05$ and *** $P < 0.001$ comparing the groups between brackets.

None of the interactions (combining maternal genetic factors with newborn genetic factors or maternal/newborn nonlipid factors such as weight) was significant. It is possible, however, that our study lacked statistical power for interaction analysis. The absence of significant interaction between maternal and newborn genetic factors in our study suggests that the newborn genotypes might not

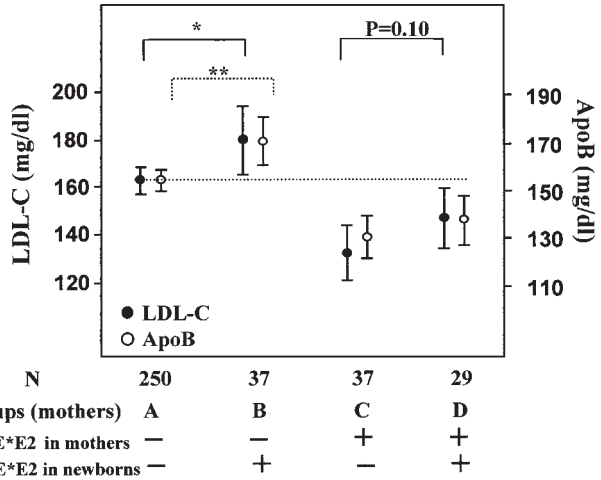

Fig. 3. Concentrations (means and 95% confidence intervals) of LDL-C and apoB in various groups of mothers classified according to the carriage of APOE*E2 by their newborns and by themselves. The Y axes were scaled to set the point representing the mean values of concentrations of the referent (group A) at the same graphic level. * $P < 0.05$ and ** $P < 0.01$ comparing the groups between brackets.

strongly affect the way in which the genotypes of the mothers determines maternal lipids but might simply add their effects to those of the mothers' genotypes.

As shown in Table 1 (third column), the addition of the newborn lipids that were best correlated with maternal lipids in bivariate correlation analysis did not reduce the amplitudes of associations between the maternal lipids and the newborn polymorphisms. This excluded the hypothesis that the variations in maternal lipids associated with newborn polymorphisms were the results of variations in newborn lipids associated with these newborn polymorphisms. Interestingly, the estimate of the increasing effect of newborn APOE*E2 on maternal LDL-C (or apoB) increased when the variable "newborn LDL-C" was added to the multivariate model. This can be explained by the fact that newborn APOE*E2 is associated with lower newborn LDL-C (or apoB), as demonstrated previously (15), and that newborn LDL-C is positively correlated with maternal LDL-C.

ApoE phenotyping of maternal plasma

By IEF and immunoblotting, we could not detect the presence of apoE2 or apoE4 in the plasma of APOE*E3E3 mothers bearing APOE*E3E2 or APOE*E3E4 newborns. It is possible, of course, that our method was not sensitive enough to detect trace amounts of such isoforms originating from the placenta.

DISCUSSION

The present study demonstrates for the first time that genetic polymorphisms present in newborns are associated with variations of lipid concentrations in their mothers. The presence of the S447X allele of LPL in newborns was associated with lower triglycerides, lower LDL-C, lower apoB, higher HDL-C, and higher apoA-I in their mothers, whereas the presence of the N291S allele of LPL in newborns was associated with higher triglycerides in their mothers. The presence of the E2 allele of APOE in newborns was associated with higher LDL-C and apoB in their mothers. In contrast to the polymorphisms of the LPL and APOE genes, the polymorphism of the APOC3 gene (APOC3*S2) was not associated with any significant variation of maternal lipoprotein concentrations. All of these associations were independent of the genotypes of the mothers and were independent of the variations of lipid concentrations in newborns associated with these newborn polymorphisms (15).

To explain these associations, we suggest the existence of an active and important contribution of the placental lipoprotein lipase and apoE to the metabolism of maternal lipoproteins. In this view, the absence of association of maternal lipoproteins with APOC3*S2 is consistent with what we know about the lower expression of the APOC3 genes by the placenta (20) as opposed to the LPL and APOE genes (2, 3, 8, 9, 11). Below, we present some possible mechanisms by which these proteins may play such a role.

Regarding the LPL polymorphisms (S447X and N291S), the associations of newborn genotypes with maternal triglycerides and HDL-C were similar to the associations classically observed in adults between their own genotypes and their own concentrations of these lipid fractions (16). This suggests that lipoprotein lipase of the placenta (encoded by the newborn genome) acts like the lipoprotein lipases of maternal adipose tissues and muscles (i.e., that its lipolytic activity is oriented toward the maternal circulation). In vitro cultures of tissue homogenates or cellular components of human placenta have previously demonstrated the ability of this tissue to lipolyze triglyceride-rich lipoproteins (3, 8, 9, 12, 13). However, it has not been possible in humans to show that this activity is directed toward the maternal compartment as in animals (28, 29). In our study, we present the first in vivo argument for such orientation of placental LPL activity. In addition, it allows a semiquantitative estimate of the contribution of placental lipoprotein lipase to this maternal lipoprotein metabolism. Indeed, because the variations of triglycerides associated with maternal LPL*S447X and newborn LPL*S447X had the same amplitude and these variations summed up when LPL*S447X was present on both genomes, it is likely that placental lipoprotein lipase and maternal lipoprotein lipase participate equally and complementarily to the metabolism of the maternal triglyceride-rich lipoproteins.

In contrast to LPL polymorphisms, the association between newborn APOE*E2 and higher maternal LDL-C presented an opposite picture of the association observed in adults, in which APOE*E2 is classically associated with lower LDL-C (a LDL-lowering effect was actually observed in our study for maternal APOE*E2). In our previous study (15), in which we analyzed the factors contributing to newborn blood lipids, we found that APOE*E2 in newborns was associated with lower newborn LDL-C. This finding, although similar to the adult's pattern, was difficult to explain by the classical mechanism (see introduction) because there is no food ingestion and, therefore, no significant chylomicron remnant metabolism in the fetus. As a whole, these associations of newborn APOE*E2 with lower LDL-C in the fetal compartment and higher LDL-C in the maternal compartment may be explained by a reduction of cholesterol traffic between the mother and her fetus in the presence of APOE*E2. This suggests the hypothesis that apoE, produced by the placenta, regulates the transplacental flux of cholesterol and that the E2 isoform is less effective for this role. Although this hypothesis needs to be examined carefully in the future, such a role is consistent with some of the known features of apoE. For instance, various cell types secrete apoE to promote their own uptake of lipoproteins (30, 31) or the efflux of their excess cholesterol to HDL particles (32, 33), with an efficiency dependent on the isoforms (17, 21, 34, 35). Therefore, we may speculate that the apoE is secreted on the maternal side of the placenta to facilitate the uptake of maternal lipoproteins and/or is secreted at the fetal side of the placenta to facilitate the efflux of cholesterol from the placenta to fetal lipoproteins. This idea is supported by early perfusion experiments of isolated lobules of hu-

man placenta that showed that apoE was secreted at both sides (fetal and maternal) of the placenta (11). The fact that we could not detect the presence of fetal apoE in mothers (the presence of apoE2 or apoE4 in plasma of the APOE*E3E3 mothers bearing APOE*E3E2 or APOE*E3E4 newborns) does not reject this hypothesis. Placental apoE may have a "paracrinal" role, in which, after its secretion at the surface of the placenta, it aggregates the maternal lipoproteins, which are rapidly taken up by the placenta. In these conditions, placental apoE would be almost absent in the peripheral circulation of mothers.

In our study, we also observed associations between polymorphisms of pregnant women and their own lipoprotein concentrations, most of which (with the exception of the LPL*N291S polymorphism, which was too rare in our cohort to conclude) corroborated the previous observations of McGladdery and Frohlich (22). The APOE*E2 allele was associated with a significant reduction of LDL-C [-15%; $P < 0.05$ in (22); -17%; $P < 0.001$ in our study] compared with the APOE*E3E3 carriers. The LPL*S447X allele was associated with a significant reduction of triglycerides [-17%; $P < 0.003$ in (21); -8%; $P = 0.03$ in our study]. All of these effects resembled those classically found in the general population (16–18), except the absence of a lipid association with the APOE*E4 allele (usually associated with increased LDL-C in the general population) and the association (yet found only in mothers bearing LPL*S447X$^-$ newborns in our study; Fig. 1) between increased LDL-C and LPL*S447X (usually not associated with increased LDL-C in the general population). McGladdery and Frohlich (22) found the same absence of association with APOE*E4 and a tendency (+11 mg/dl; $P = 0.06$) toward greater LDL-C in the presence of the LPL*S447X allele. These differences with nonpregnant adults may reflect the changes of triglyceride-rich lipoprotein catabolism associated with pregnancy; for instance, lipoprotein lipase activity is strongly reduced during pregnancy. It may also simply result from the young age of the women; for instance, the LDL-C differences between APOE isoforms are usually smaller in premenopausal than in postmenopausal women (22).

A quantitative measure of how well the maternal and newborn genetic factors explain the maternal lipid concentration was given by the R^2 values or percentages of variance attributable to the genotypes. They were approximately in the same range (Table 1, second column) for maternal (0.5% and 4.9%) and newborn (0.8% and 2.8%) genotypes. Such values, although they may appear small, are commonly seen when a dependent variable, such as lipid concentration, is associated with many independent factors and when the independent factor of interest, such as a genetic polymorphism, occurs at low frequency. Furthermore, the contribution of an independent factor may be greatly decreased in circumstances in which another factor becomes predominant. For instance, the apoE polymorphism accounted for 8% of the total variance for LDL cholesterol (36) in the general population but for only 4% in familial hypercholesterolemia, in which the main factor that contributes to the increase of LDL-C is the presence of the LDL receptor mutation (37). In pregnant women, in whom hormones are mainly responsible for the lipid increase, it is not surprising that the genetic polymorphisms make only a small contribution to the variances of maternal lipids (e.g., only ~4.5% for APOE*E2).

Finally, we want to speculate on three possible clinical implications of this contribution of placenta and of fetal genetic makeup to maternal lipoprotein metabolism. First, because maternal dyslipidemia has been associated with preeclampsia (38), factors that influence blood lipids may be risk factors for preeclampsia. The maternal LPL*N291S allele has been associated with an increased risk of preeclampsia in one study (39) and with an increased risk of HELPP syndrome in another (40). The triglycerides/apoE ratio, which is partly determined by APOE polymorphism, was associated with mild preeclampsia in one study (41), whereas other studies found no association of APOE polymorphism with preeclampsia (42) or found an association limited only to the perinatal death (43). It is possible that fetal polymorphisms affect the risk associated with those maternal polymorphisms.

Another possible implication concerns the risk of future cardiovascular disease in women. Increased parity or gravidity has previously been associated with a higher cardiovascular risk (44, 45) at an older age, possibly because of the atherogenic changes of lipoprotein and glucose levels during pregnancy (19). Also, preeclampsia may increase the cardiovascular risk in young (46) and elderly (47) women. Not only maternal polymorphisms, but also fetal polymorphisms able to affect the maternal lipid profile, may thus contribute to this risk. Because the polymorphisms examined in the present study have been associated with protection from (APOE*E2 and LPL*S447X) or risk of (LPL*N291S) cardiovascular diseases (16–18, 48) in the general population, it would be interesting to examine in future epidemiological studies whether the future cardiovascular risk of women is partly determined by the polymorphisms of their children.

Finally, there may be potential implications of our findings regarding women with a severe inherited deficiency of lipoprotein lipase. In such cases, it is expected that pregnancy, which is usually associated with increased triglycerides, will severely exacerbate the preexisting hypertriglyceridemia and, consequently, the risk of pancreatitis. It is intriguing, however, to observe that not all women with familial lipoprotein lipase deficiency develop pancreatitis during pregnancy, as has been reported in the literature (49–53). In our lipid clinics, the three women known to be deficient in lipoprotein lipase had suffered from several episodes of pancreatitis, but never during their pregnancies (five pregnancies in total). It is possible that the placental lipoprotein lipase (expressed from the normal allele inherited from the father) may partly compensate for the deficit of the maternal lipoprotein lipase and thus may limit the increase of triglycerides. It is remarkable that in the gestational follow-up (when available) of our lipoprotein lipase-deficient women, triglycerides never reached concentrations higher than those observed during other periods in their lives.

In conclusion, fetal polymorphisms influence the concentrations of maternal lipoproteins. The demonstration of such associations supports the idea of an active contribution of the placenta to the metabolism of maternal lipoproteins during pregnancy. It generates a new hypothesis on the possible roles of apoE and lipoprotein lipase in the transplacental flux of cholesterol and suggests potential implications regarding the risk of severe dyslipidemia, preeclampsia, and pancreatitis during pregnancy or regarding the future risk of cardiovascular diseases. Future investigations of gene-lipoprotein associations in pregnant women should consider the newborn genotypes.

O.S.D. dedicates this work to his brother, Stéphane Descamps (1963–1997). The authors thank Ghislain Wesko and Yves Daumeries for their technical help as well as the dynamic nurses of the Department of Obstetrics and Gynecology. The authors also thank Dr. Rémy Couderc from the Biochemistry Department, Hôpital Armand Trousseau, Paris, for his help in confirming the apoE phenotyping.

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