

Lipoprotein lipase *PvuII* polymorphism is associated with variations in serum lipid levels in non-diabetic pregnant women

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Abstract

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Publication supported by FAPESP.
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Received August 18, 2006
Accepted March 2, 2007
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The aim of the present study was to determine if there is an association between the single nucleotide polymorphisms (SNPs) of the lipoprotein lipase (LPL) and apolipoprotein E (apo E) genes and the serum lipid profile in pregnancy and puerperium. Non-diabetic women of European descent in the third semester of pregnancy (N = 120) were selected. Those with diseases or other condition that could modify their lipid profile were excluded from the study (N = 32). Serum lipids were measured by routine laboratory procedures and genomic DNA was extracted by a salting out method. LPL (*PvuII* and *HindIII*) and apo E (*HhaI*) SNPs were detected by the polymerase chain reaction and restriction fragment length polymorphism. Categorical and continuous variables were compared by the chi-square test and Student *t*-test or ANOVA, respectively. Women carrying the LPL P1P1 genotype had higher serum LDL cholesterol (N = 21; 155 ± 45 mg/dL) than women carrying the P1P2/P2P2 genotypes (N = 67; 133 ± 45 mg/dL; P = 0.032). During the puerperium period, serum levels of triglycerides and VLDL cholesterol were significantly reduced in women carrying the P1P1 (73%, P = 0.006) and P1P2 (51%, P = 0.002) genotypes but not in women carrying the P2P2 genotype (23%, P > 0.05). On the other hand, serum concentrations of lipids did not differ between the LPL *HindIII* and apo E genotypes during pregnancy and after delivery. We conclude that LPL *PvuII* SNP is associated with variations in serum lipids during pregnancy and the puerperal period in non-diabetic women.

Key words

- Lipoprotein lipase
 - LPL *PvuII* polymorphism
 - Apolipoprotein E
 - Single nucleotide polymorphism
 - Serum lipids
 - Non-diabetic pregnancy
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Introduction

Lipoprotein lipase (LPL) is a glycoprotein synthesized by several tissues and cell types, including adipose, cardiac and skeletal tissues (1). LPL hydrolyzes triglycerides (TG) of circulating very low-density lipoproteins (VLDL) and chylomicrons, and generates fatty acids for storage in adipose tissue (1). During this process, these TG-rich lipoproteins are converted to remnant particles which are removed from the circulation by interaction of apolipoproteins E (apo E) and B with specific receptors in the liver.

Structural and functional alterations of LPL and apo E can cause reduction of TG hydrolysis and removal of TG-rich lipoproteins from plasma, leading to hypertriglyceridemia (HTG) (1). Two common variants (*PvuII* and *HindIII*) of the LPL gene have been associated with variations of TG and cholesterol levels in plasma, cardiovascular diseases and acute myocardial infarction in Caucasian populations (2-7). In addition, the apo E gene has an *HhaI* single nucleotide polymorphism (SNP) that encodes three apo E isoforms named E2, E3 and E4 (8). These apo E isoforms interact with B/E and E receptors with different affinities that affect the removal of apo E-rich lipoproteins, VLDL and chylomicron remnants, and contribute to TG and cholesterol elevation in plasma (8).

HTG is the most remarkable lipid alteration found in the third trimester of pregnancy. During this period, hepatic production of TG is increased and the clearance of VLDL is reduced probably due to the lower activity of LPL in adipose tissue (9,10). Severe HTG can be developed in the late stages of pregnancy as a consequence of several factors such as diabetes, alcohol consumption or weight gain (10).

LPL mutations have been associated with extremely high TG levels and may be a common cause of pancreatitis associated with

pregnancy (11,12). It has been suggested that this abnormality in serum lipid levels is caused by LPL mutations in combination with apo E2 (13,14). However, the contribution of common variants of the LPL and apo E genes to the variations in serum lipids during pregnancy remains to be elucidated.

The aim of the present study was to investigate the effects of LPL and apo E SNPs on serum lipids of non-diabetic women in the third trimester of pregnancy and during the puerperium period.

Material and Methods

Subjects and study protocol

A group of 120 unrelated non-diabetic women (18 to 36 years old) of European descent who were assisted during pregnancy by public services of the Metropolitan Health Department of São Paulo city were selected from September 2002 to March 2003. The women were evaluated during the third trimester of pregnancy and the puerperal period (up to 6 weeks after delivery). Pregnant women with heart, liver, kidney, thyroid or vascular diseases, hypertension, or taking medication that could influence their lipid profile (N = 32) were excluded from the study. All women reported a normal glucose tolerance test.

The Ethics Committees of the Medical School of the University of São Paulo, Dante Pazzanese Institute of Cardiology, and Metropolitan Health Department (São Paulo, SP, Brazil) approved the protocol and written informed consent was obtained from the 88 women included in the study.

All women filled out a questionnaire with information about personal clinical history and family antecedents. Age, height, weight gain (currently and prior to pregnancy), previous number of pregnancies, diets, alcohol consumption, smoking, and any relevant information on current or previous medical history were also recorded. Gestational age

was determined by ultrasonography.

Laboratory analyses

Blood samples were collected after a overnight 12-h fast for analysis of the serum lipid profile and other laboratory tests. Triglycerides, total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by standard enzymatic methods using a Hitachi 912 auto-analyzer (Hitachi Ltd., Tokyo, Japan). VLDL cholesterol and low-density lipoprotein (LDL) cholesterol were calculated by the Friedewald formula. Plasma glucose and serum creatinine, urea, alanine and aspartate aminotransferases, gamma-glutamyl transpeptidase, and thyroid-stimulating hormone were also determined.

Genomic DNA analysis

Genomic DNA was extracted from blood leukocytes by a salting out method (15). *PvuII* (intron 6) and *HindIII* (intron 8) SNPs of the LPL gene and the *HhaI* (exon 4) polymorphic region of the apo E gene were analyzed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism as previously described (16,17).

PCR assays were performed with 200 ng DNA, 200 μ M deoxynucleotides (Amersham Pharmacia Biotech of Brazil, São Paulo, SP, Brazil), 10 pmol primers (Life Technologies Inc., Gaithersburg, MD, USA), 1.0 U DNA polymerase (Biotools B&M Labs, Madrid, Spain), and amplification buffer (50 mM KCl, 20 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgCl_2 , and 75 mM Tris-HCl, pH 9.0). The thermal cycler protocol consisted of an initial cycle at 98°C for 3 min, followed by 30 cycles at 96°C for 1 min, 60°C for 1 min, and 72°C for 1 min, and a final cycle at 72°C for 10 min. Amplification was carried out in a thermal cycler, PTC-200 (MJ Research Inc., Waltham, MA, USA). PCR products were analyzed by 1.5% agarose gel electrophoresis after ethidium bromide staining.

PCR products were digested with 10 U *PvuII*, *HindIII* or *HhaI* (Amersham Pharmacia Biotech of Brazil) at 37°C for 4 h. Restriction fragments were identified by electrophoresis on 2% agarose gels (*PvuII* and *HindIII*) and 8% polyacrylamide gels (*HhaI*).

Genotypes for LPL and apo E polymorphisms were determined using a homozygous sample for the restriction site as positive control. In addition, 10% of the analyses were repeated at random.

Statistical analysis

Data were initially analyzed by descriptive statistics. Categorical variables were compared by the chi-square test and continuous variables were compared by the Student *t*-test for two independent variables. One-way ANOVA was used for comparison between more than two independent variables, that were further evaluated by the Tukey-HSD test for multiple comparisons. One-way repeated measures ANOVA was used to compare the mean serum concentration of lipids in the third trimester of pregnancy and during the puerperium. The level of significance was set at 5% in all analyses.

Results

The demographic characteristics and biochemical data of the pregnant women studied are shown in Table 1. Mean gestational age was 34 ± 3 weeks and the number of previous pregnancies ranged from 1 to 6, with a mean of 2 ± 1 . Most women (53%) were in their first pregnancy, 22.7% in the second pregnancy, 18.2% in the third, and the others had had three or more gestations. The reported weight gain during pregnancy was 10 ± 5 kg. All women consumed their habitual diet according to Brazilian patterns and none mentioned being a vegetarian. None admitted consuming alcohol during pregnancy but 8% were smokers. Family history indicated that 40 to 60% of their parents had

Table 1. Demographic characteristics and biochemical data of the pregnant women studied.

Number of subjects	88
Age (years)	24.7±5.0
Weight (kg)	70.2±4.6
Height (cm)	160.1±6.5
Weight gain (kg)	10.5±4.6
Gestational age (weeks)	34±3
Number of pregnancies	2±1
Family history	
Hypertension	53 (60%)
Diabetes mellitus	43 (49%)
Dyslipidemia	35 (40%)
Cerebrovascular disease	24 (27%)
Coronary artery disease	15 (17%)
Smoking	7 (8%)
Plasma glucose (mg/dL)	75.0±8.4
Creatinine (mg/dL)	0.7±0.1
Urea (mg/dL)	15±4
Thyroid-stimulating hormone (mU/L)	2.3±1.2
Aspartate aminotransferase (U/L)	21±7
Alanine aminotransferase (U/L)	15±8
Gamma-glutamyl transpeptidase (U/L)	14±7
Total cholesterol (mg/dL)	244±44
Triglycerides (mg/dL)	196±56
HDL cholesterol (mg/dL)	68±14
LDL cholesterol (mg/dL)	138±41
VLDL cholesterol (mg/dL)	39±11

Data are reported as means ± SD or as number of individuals with percent in parentheses. HDL = high-density lipoprotein; LDL = low-density lipoprotein; VLDL = very low-density lipoprotein.

hypertension, diabetes mellitus, and dyslipidemia, and 27 and 17% of their relatives had cerebrovascular and coronary artery diseases, respectively.

Analysis of the lipid profile showed an increase in serum total and LDL cholesterol as well as TG compared to reference values (Table 1). Plasma glucose was in the normal range, as also were the biomarkers of thyroid, liver and kidney diseases. The demographic and biochemical results were similar when the pregnant women were divided into groups according to gestational month (data not shown).

Allele frequencies for LPL and apo E SNPs are shown in Table 2. The relative frequencies of the H1 and P1 alleles were 0.284 and 0.510, respectively. Alleles E2, E3 and E4 of the apo E polymorphism had relative frequencies of 0.057, 0.841 and 0.102, respectively. These allelic frequencies were similar to those found in other Caucasian populations ($P > 0.05$; Table 2).

In order to evaluate the serum lipid profile of women with different genotypes, we grouped the less common homozygous genotype with the heterozygous genotype for

Table 2. Relative allele frequencies for lipoprotein lipase (LPL) and apolipoprotein E (apo E) single nucleotide polymorphisms in pregnant women* and other Caucasian populations.

	N	LPL <i>HindIII</i>		LPL <i>PvuII</i>		Reference
		H1	H2	P1	P2	
Present study	88*	0.284	0.716	0.510	0.490	
Brazil	506	0.267	0.733	0.506	0.494	26
France	966	0.303	0.697	0.487	0.513	4
Germany	649	0.289	0.711	0.490	0.510	25
USA	923	0.243	0.757	0.455	0.545	2
USA	168	0.286	0.714	0.417	0.583	5
apoE <i>HhaI</i>						
		E2	E3	E4		
Present study	88*	0.057	0.841	0.102		
Brazil	100	0.080	0.810	0.110		17
Brazil	100	0.096	0.808	0.096		27
Belgium	525*	0.066	0.818	0.116		30
Italy	180	0.050	0.860	0.090		21
Portugal	144*	0.052	0.844	0.104		29
Canada	250*	0.076	0.814	0.110		28

LPL *PvuII* and *HindIII* SNPs. For apo E SNP analysis, the women carrying the E4/E4 genotype were grouped with the women carrying the E3/E4 genotype (E4 carriers). We excluded two E2/E4 genotype carriers because the E2 and E4 alleles have opposite effects. No relations were found between LPL and apo E SNPs and demographic characteristics or a family history of hypertension, diabetes, dyslipidemia, and cardiovascular and cerebrovascular diseases (data not shown).

Women carrying the P1P1 genotype had higher serum LDL cholesterol (155 ± 45 mg/

dL) than women carrying the P1P2/P2P2 genotype (133 ± 45 mg/dL; $P = 0.032$; Table 3). These differences were independent of the demographic variables. A similar serum lipid profile was found among carriers of the LPL-*HindIII* and apo E-*HhaI* genotypes ($P > 0.05$; Table 3).

During the puerperium period, serum TG and VLDL cholesterol levels were significantly reduced in women carrying the P1P1 (73%, $P = 0.006$) and P1P2 (51%, $P = 0.002$) genotypes but not in P2P2 carriers (23%, $P > 0.05$; Figure 1). Serum LDL cholesterol was also slightly reduced during the puerperium

Table 3. Serum lipid concentrations of pregnant women according to genotypes of the lipoprotein lipase (LPL) and apolipoprotein E (apo E) polymorphisms.

Polymorphism	Genotype	Lipids (mg/dL)				
		TC	TG	HDL	LDL	VLDL
LPL- <i>PvuII</i>	P1P1 (N = 21)	258 ± 55	201 ± 62	67 ± 15	155 ± 45	40 ± 12
	P1P2/P2P2 (N = 67)	240 ± 40	195 ± 55	69 ± 14	133 ± 39	39 ± 11
	P^a	0.115	0.668	0.552	0.032	0.717
LPL- <i>HindIII</i>	H1H1/H1H2 (N = 48)	251 ± 42	189 ± 56	70 ± 15	143 ± 40	38 ± 11
	H2H2 (N = 40)	237 ± 47	206 ± 56	67 ± 13	133 ± 42	41 ± 11
	P^a	0.146	0.162	0.405	0.292	0.245
apoE- <i>HhaI</i>	E2E3 (N = 6)	237 ± 29	221 ± 79	71 ± 20	122 ± 29	44 ± 16
	E3E3 (N = 63)	245 ± 46	192 ± 57	70 ± 14	140 ± 42	38 ± 12
	E3E4 (N = 17)	244 ± 39	200 ± 39	62 ± 9	142 ± 40	40 ± 8
	P^b	0.648	0.704	0.146	0.605	0.677

Data are reported as mean ± SD and compared by the ^aStudent *t*-test or ^bone-way ANOVA. TC = total cholesterol; TG = triglycerides; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; VLDL = very low-density lipoprotein cholesterol.

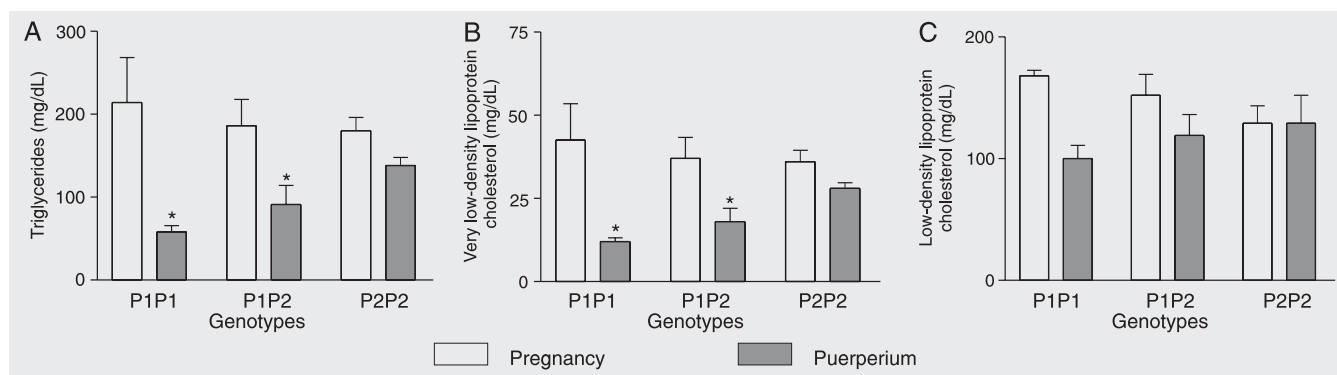


Figure 1. Serum concentration of triglycerides (panel A), very low-density lipoprotein cholesterol (panel B), and low-density lipoprotein cholesterol (panel C) in pregnant women classified according to the LPL-*PvuII* genotypes. Data are reported as means ± SEM. * $P < 0.01$ compared to the pregnancy period for the same genotype (one-way repeated measures ANOVA).

in women carrying the P1P1 (40%, $P = 0.051$) and P1P2 (22%, $P = 0.078$) genotypes. The effects of *PvuII* polymorphism on serum lipids appeared to be independent of age, weight gain, number of previous pregnancies, and serum lipid concentrations during pregnancy. LDL *HindIII* and apo E SNPs were not associated with variations in serum lipids during the puerperium period (data not shown).

Discussion

We selected a group of 88 non-diabetic women of European descent and investigated the relationship of LPL and apo E SNPs with the serum lipid profile during the third trimester of pregnancy. Environmental aspects such as weight gain and dietary habits did not show an effect on the lipid metabolism of pregnant women as reported by others (9,10). Although some longitudinal studies have shown that the serum lipid profile is modified during the last 3 months of pregnancy (18,19), the length of gestation was not associated with variation in serum lipid profile in our study.

The frequency of overweight women in our sample (37.5%) was similar to that found in a large sample of pregnant women (25.0%) assisted by public health services in 6 Brazilian capital cities (20). Although weight gain during pregnancy has been associated with increased serum TG concentrations (20), no significant relationship was demonstrable between these variables in our sample.

The serum lipid concentrations detected in our study are similar to those found in another study (21) and are representative of what can be expected from a population of healthy pregnant women. The moderate HTG found in the third trimester of pregnancy may be due to the physiological response to the increase in plasma estrogen and progesterone levels (9,22). Increased plasma TG during the third trimester of pregnancy has been correlated with a significant reduction

of LPL mass (23), whose gene expression has been shown to be inhibited by estrogen in adipocytes (24).

The frequencies of the H1 and P2 alleles of the LPL polymorphisms were similar to those found in non-pregnant Caucasian American (2,5), German (25), and Brazilian women of European ancestry (26). Moreover, the apo E allelic frequencies in our sample were similar to those detected in non-pregnant Brazilian women of Caucasian descent (17,27) and in pregnant women from other Caucasian populations (21,28-30).

There was a significant association between the LPL P1P1 genotype and increased serum LDL cholesterol levels in pregnant women, possibly contributing to the variation in serum lipids found in women during the third trimester of pregnancy. The *PvuII* variant has been indicated as a possible marker for a functional mutation that alters LPL activity and interferes with TG and cholesterol metabolism (31,32). It has been shown that the S447X mutation of the LPL gene is associated with variation in serum LDL cholesterol and TG levels of pregnant women (28,30).

Despite the associations between *HindIII* polymorphism and variations in serum lipids found in other studies (3,6,26,33,34), we could not establish this association in pregnant women. The findings that the H2H2 genotype is associated with increased TG and reduced HDL cholesterol could be due to the lower activity of LPL in carriers of such genotype (2,25,34). The differences between our results and those obtained by other investigators may be due to the limited size of our sample since approximately 25% of the pregnant women were excluded. In addition, this caused the statistical power of the test performed to be below the desired level. Therefore, other studies involving larger samples (at least two to three times larger) are necessary to evaluate the relation between LPL polymorphisms and lipid pro-

file during pregnancy in our population.

Apolipoprotein E variants contribute to up to 10 and 50% variation in plasma concentrations of total cholesterol and LDL cholesterol, respectively (27,35,36). Belo et al. (29) reported that the apo E genotype is associated with changes in lipid and lipoprotein profiles in pregnant women. In our study, individuals carrying the E4 allele had a higher LDL/HDL cholesterol ratio than individuals carrying the E3 and E2 alleles, although the differences did not reach statistical significance. The lack of association between apo E SNP and serum TG concentrations was demonstrated in pregnant women with normal glucose tolerance (20).

Other studies have shown that pregnant women carrying the E2 allele have lower concentrations of total and LDL cholesterol than E3 and E4 allele carriers, suggesting that the E2 allele may have a protective cardiovascular effect in pregnancy (28-30). The differences in total cholesterol levels associated with apo E genotypes may have an effect on steroidogenesis and consequently determine the differential fertility that has been observed in Europeans (37).

During the puerperium, serum lipids were significantly reduced, as previously demonstrated in other studies (38-40). Significant

reductions in serum TG and VLDL cholesterol levels were found in women carrying the P1P1 and P1P2 genotypes, whereas in P2P2 genotype carriers the serum levels of these lipids remained increased up to six weeks after delivery. These differences may explain the variability of the physiological response of serum lipids found after delivery in non-diabetic pregnant women (38).

The effects of the *PvuII* variant on maternal serum lipids may be due to a possible interaction between *PvuII* and the S447X mutation or another functional mutation in the LPL gene, as previously shown in pregnant women (28). Moreover, immediately after delivery maternal TG was significantly lower in carriers of the S447X mutation of the LPL gene (30).

The results of our study suggest that *PvuII* SNP of the LPL gene contribute to the variability in serum lipids during the third trimester of pregnancy and in the puerperium period in non-diabetic women.

Acknowledgments

We thank the clinicians of the Metropolitan Health Department of São Paulo city for assistance during the selection of the pregnant women included in this study.

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