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Atherogenic lipoprotein subfraction profile in preeclamptic women with and without high triglycerides: different pathophysiologic subsets in preeclampsia☆

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Abstract

Abnormal lipid metabolism has been proposed as a pathogenic factor of preeclampsia, although whether it is a constant feature in all preeclamptic patients is unclear. We assessed whether plasma triglyceride (TG) levels can distinguish a subgroup of preeclamptic women with alterations in lipoprotein profile from those with normal lipid metabolism and can be used to identify 2 distinct pathogenic groups in preeclampsia. This prospective study included 34 women with preeclampsia and 23 healthy pregnant women. Preeclamptic women were further subclassified into normal-TG (<250 mg/dL) and high-TG (≥250 mg/dL) groups on the basis of the 90th percentile in our population. Low-density lipoproteins (LDLs) were ultracentrifuged and were separated into 4 subfractions, and lipid distribution in the subfractions was analyzed in all study groups. Vascular cell adhesion molecule–1 was also measured as a marker of endothelial dysfunction. Sixteen women with preeclampsia had high TGs (47% vs 13% in control subjects, $P < .001$). This subgroup showed a significant shift in lipid distribution, mainly, TGs, toward the small, dense LDL subfractions. However, preeclamptic patients in the normal-TG subgroup showed LDL subfraction lipid distribution similar to that of healthy pregnancies. Vascular cell adhesion molecule–1 levels were significantly elevated in preeclamptic patients in comparison with those in control subjects regardless of TG levels. The presence of a proatherogenic lipoprotein profile, previously described in preeclampsia, is characterized by increased small dense LDL and is exclusive to a subset of preeclamptic patients with high TG levels. These findings support the concept of *heterogeneous pathogenic lines* in preeclampsia and the use of subclassifications in pathophysiologic research on this condition.

1. Introduction

Preeclampsia is a pregnancy-specific disorder characterized by a generalized inflammatory state and endothelial dysfunction, resulting in disseminated microangiopathic disease with vasospasm and hypercoagulation. The pathogenesis of preeclampsia continues to be a challenge. Several lines of evidence suggest that preeclampsia is a multietiologic syndrome with heterogeneous biologic pathways [1]. This fact has been cited to explain the variability in its clinical presentation and the relatively common inconsistency of pathophysiologic studies. It is becoming widely accepted that different pathophysiologic lines may be grouped under the common clinical syndrome. Therefore, any attempt to analyze preeclampsia as a whole can be strongly influenced by the composition of the population under study. Thus, it is being increasingly proposed to subclassify preeclampsia for research purposes to identify subsets that are more likely to share pathogenic similarities. Recent studies have attempted to establish subgroups on the basis of main clinical features, such as the time of onset, that is, preterm vs term [2], or the presence or absence of fetal growth restriction [3]. However, no attempts have been made to distinguish subgroups in preeclampsia on the basis of biochemical features.

☆ Dyslipoproteinemic changes are exclusive to a subset of preeclamptic patients with hypertriglyceridemia.

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Lipid-mediated endothelial damage has long been proposed as a major pathogenic pathway of preeclampsia. Substantial evidence supports the idea that preeclampsia and chronic vascular disease have remarkable epidemiologic [4-6] and biochemical similarities [7,8]. The lipid profile observed during the symptomatic phase of preeclampsia is characterized by hypertriglyceridemia and a predominance of small, dense low-density lipoproteins (LDLs) [9,10], and the generation of this lipoprotein subspecies is one component of the dyslipidemic syndrome known as the atherogenic lipoprotein phenotype [11]. However, this lipid profile seems to be present only in a certain percentage of preeclamptic patients [10], which is in agreement with the hypothesis of a multietiologic model to explain preeclampsia. The ability to distinguish the subgroup of cases associated with dyslipidemia from those that are not could be of help in the interpretation of pathophysiologic studies, and eventually, in the design of interventional measures for the prevention of preeclampsia.

In this study, we investigated whether preeclamptic patients with an abnormal lipid profile can be identified by a single parameter that could subsequently be used to subclassify preeclampsia for research purposes. Serum triglyceride (TG) levels, as a relatively simple and reproducible parameter, were used to subdivide preeclampsia into cases with high or normal TG levels. Because hypertriglyceridemia is a feature of altered lipid metabolism that correlates strongly with the predominance of small dense lipoproteins, lipoprotein composition and subclass distribution were analyzed. A further aim was to assess whether this classification is associated with different clinical features of preeclampsia and whether it distinguishes preeclamptic patients with dyslipoproteinemia from those with a normal lipid profile.

2. Material and methods

The study protocol was approved by the institutional ethics committee, and written informed consent was obtained in all cases. Subjects were selected among women attending the obstetrics department from June 2001 to June 2003. Eligible cases were singleton pregnancies with a diagnosis of preeclampsia. Women in labor and those with ruptured membranes, multiple pregnancies, or any concurrent medical complication before or developing during pregnancy, such as diabetes mellitus or inflammatory disease, were considered ineligible for the study. The control group comprised a consecutive sample of pregnant women followed up at our institution, undergoing a routine third-trimester blood analysis, and without any of the exclusion criteria. The criteria defining preeclampsia were those of the International Society for the Study of Hypertension in Pregnancy [12]. Preeclampsia was diagnosed if a previously normotensive woman had 2 repeat (4 hours apart) diastolic blood pressure measurements of 90 mm Hg or greater after the 20th week of gestation, together with proteinuria of more than 300 mg in a 24-hour urine specimen or 2+ or more protein in dipsticks in 2 repeat measurements (4 hours apart). For the purposes of the study, cases of preeclampsia were further subclassified according to TG levels and were referred to normal-TG (<250 mg/dL) or high-TG (≥ 250 mg/dL) groups. The cutoff level of 250 mg/dL was arbitrarily chosen because it roughly corresponds to the 90th percentile of TG levels in healthy pregnant women in our population (data not shown). The presence of intrauterine growth restriction (IUGR) was recorded in all cases. Intrauterine growth restriction was defined as either an estimated fetal weight of less than the 10th percentile for gestational age together with a Doppler pulsatility index in the umbilical artery more than the 95th percentile or an estimated fetal weight less than the third percentile for gestational age regardless of umbilical artery Doppler.

Venous blood samples were drawn after 4 to 10 hours of fasting and were processed within 1 hour. Blood was collected into EDTA tubes and was centrifuged at 1500*g* for 5 minutes at 4°C. Serum cholesterol and TG levels were measured by enzymatic methods (Trinder; Bayer Diagnostics, Tarrytown, NY) adapted to a Cobas Mira automated analyzer (Hoffmann Larroche, Basel, Switzerland). Low-density lipoprotein subfractions were isolated from the serum of each subject by sequential preparative ultracentrifugation under standard conditions. Briefly, the serum was adjusted to a density of 1.025 g/mL and was centrifuged at 40 000 rpm for 18 hours at 17°C in a Beckman 40.3 titanium rotor. The top 2 mL was decanted, and the infranatant fluid was adjusted to a density of 1.063 g/mL with sodium bromide solution. After centrifugation of this mixture at 17°C for 24 hours at 40 000 rpm, LDL in the top 1 mL was withdrawn and was dialyzed in sodium bromide solution of a density of 1.040 g/mL overnight, with 2 changes of dialyzing solution. The dialyzed LDL (2 mL) was layered carefully on an sodium bromide solution of a density of 1.054 g/mL (2.5 mL) in a Beckman cellulose nitrate centrifuge tube measuring 1/2 by 1/3 in (holds 7 mL), and 2.5 mL of sodium bromide solution of a density of 1.0275 g/mL was layered on the LDL. The tubes were then centrifuged to equilibrium at 40 000 rpm for 40 hours in a Beckman SW 45 rotor at room temperature (22°C-24°C). Low-density lipoprotein subfractions were removed sequentially according to the method of Shen et al [13]; LDL were separated into 4 subclasses according to their density: LDL-1, density of 1.025 to 1.035 g/mL; LDL-2, 1.035 to 1.040 g/mL; LDL-3, 1.040 to 1.050 g/mL; and LDL-4, 1.050 to 1.060 g/mL. Cholesterol and TG concentrations in the LDL subfractions were determined with the same methods as described previously but were adapted to lower values. Uric acid was measured by quantitative enzymatic assay (Sigma Chemical Co., St Louis, Mo) according to the manufacturer's protocol, and results were standardized using standard solutions for uric acid calibrators. Vascular cell adhesion molecule–1 (VCAM-1) circulating levels were determined with a commercially

Table 1
Clinical and biochemical features in the study groups

	Preeclampsia total (n = 34)	High-TG PE (n = 16)	Normal-TG PE (n = 18)	Control subjects (n = 23)
Age at delivery (y)	32.7 (5)	33.8 (5)	31.7 (5)	31 (5)
BMI, (weight [kg]/ height^2) at booking	23.3 (4)	24.6 (5)	22.4 (3)	23 (7)
GA at sampling (w)	33.2 (4)	34.3 (3)	32.2 (4)	34.2 (3)
GA at delivery (w)	33.8 (4)*	35.3 (3)*	32.6 (4)*	38.8 (1)
Systolic blood pressure (mm Hg)	168 (16)*	166 (11)*	169 (19)*	119 (10)
Diastolic blood pressure (mm Hg)	102 (6)*	102 (4)*	102 (7)*	65 (5)
MAP[a]	191 (12)*	190 (9)*	192 (14)*	127 (9)
Mean birth weight (g)	1847 (783)*	2100 (753)*	1622 (759)*	3374 (525)
Uric acid (μmol/L)	315 (95)**	333 (113)**	299 (75)**	238 (51)
VCAM (ng/mL)	751 (219)*	826 (226)*	682 (194)*	541 (160)

Values are given as mean (SD). PE indicates preeclampsia; BMI, body mass index; GA, gestational age; MAP, mean arterial pressure.

[a] MAP = diastolic blood pressure + 1/3 (systolic blood pressure + diastolic blood pressure).

* $P < .0001$ as compared with control subjects (analysis of variance–Tukey test).

** $P < .001$ as compared with controls (analysis of variance–Tukey test).

available enzyme immunoassay purchased from Roche Diagnostics (Mannheim, Germany).

Results were analyzed with the SPSS 11.0 statistical package (SPSS, Inc, Chicago, Ill). Statistical comparisons among groups were performed by Student unpaired *t* test or analysis of variance–Tukey, as appropriate. Data are given as mean (SD).

3. Results

A total of 34 women with preeclampsia and 23 healthy pregnant women were included in the study. Of 34 women with preeclampsia, 16 presented TG levels of more than 250 mg/dL (47% vs 13% of control subjects, $P < .001$) and were subsequently classified in the high-TG subgroup. Baseline clinical and biochemical features of the study groups are shown in Table 1. Women with preeclamptic pregnancies and healthy pregnant women had similar gestational age at blood sampling. However, women with preeclampsia delivered an average of 5 weeks earlier, and consequently, mean birth weight was lower than in control subjects. Overall, VCAM-1 levels were significantly increased in preeclamptic patients compared with those in control pregnant women, and the levels were similar in the high-TG and normal-TG groups. Likewise, plasma uric acid concentration was significantly increased in women with preeclampsia regardless of TG levels.

Table 2
Percentage of patients with early-onset preeclampsia (<34 weeks gestation) and associated IUGR, according to TG levels

	High-TG PE	Normal-TG PE	*P*
Early-onset preeclampsia (<34 w)	25 (4/16)	55.6 (10/18)	.073
Associated IUGR	16.8 (3/16)	44.4 (8/18)	.086

Values are presented as % (n) unless otherwise noted.

Table 3
Serum and lipoprotein cholesterol and TG concentrations in the study groups

	LP fractions	Preeclampsia total (n = 34)	High-TG PE (n = 16)	Normal-TG PE (n = 18)	Control subjects (n = 23)
Chol		269 (67)	300 (75)*	241 (46)	270 (54)
	LDL	171 (49)	191 (53)	154 (38)	180 (51)
	VLDL	23.8 (16)**	32 (20)***	16.5 (7)	18.2 (7)
	HDL	68 (17)	68.8 (20)	67.3 (15)	64 (13)
TG		251 (91)**	316 (82)****	194 (53)	207 (52)
	LDL	66.7 (32)	88.2 (35)****	47.7 (13)	58.2 (16)
	VLDL	64.4 (39)**	82.7 (42)****	48.1 (28)	42.5 (14)

Values are in milligrams per deciliter and are given as mean (SD). LP indicates lipoprotein; chol, cholesterol; HDL, high-density lipoprotein.

* $P < .05$ as compared with preeclamptic patients with normal TG.

** $P < .05$ as compared with control subjects (Student unpaired *t* test).

*** $P < .01$ as compared with normal-TG preeclampsia group and control subjects (analysis of variance–Tukey test).

**** $P < .001$ as compared with normal-TG preeclampsia group and control subjects (analysis of variance–Tukey test).

We analyzed the association of TG levels with the time of clinical onset of preeclampsia and the presence or absence of IUGR (Table 2). Women in the high-TG group were more commonly diagnosed later than 34 weeks gestation and had a lower incidence of associated IUGR. However, the differences did not reach statistical significance.

Total serum and lipoprotein lipid levels in the study groups are shown in Table 3. Total TGs, very low–density lipoprotein (VLDL) TGs, and VLDL cholesterol were significantly elevated in preeclamptic women analyzed as a whole. As expected, these differences were more pronounced in the high-TG group. Preeclamptic patients in the normal-TG group had no significant differences compared with the control subjects.

Lipid contents in each of the 4 LDL subfractions are displayed in Table 4. The concentration and relative distribution of cholesterol in each of the LDL subfractions showed no differences in preeclampsia, either as a whole or in the subgroups, as compared with control subjects.

Table 4
Cholesterol and TG concentrations in the LDL subfractions

	LDL subF	Total preeclampsia (n = 34)	High-TG PE (n = 16)	Normal-TG PE (n = 18)	Control subjects
Chol	1	78.7 (25)	85.8 (30)	72.4 (18)	87.7 (33)
	2	42 (19)	48.5 (22)	37.2 (15)	42 (15)
	3	26.3 (11)	29.4 (12)	23.6 (10)	25.1 (8)
	4	23.8 (11)	27.3 (13)	20.7 (9)	25 (11)
TG	1	25.6 (11)	32.7 (11)*	19.2 (5)	25.9 (6)
	2	12 (5)	15.7 (5)*	8.7 (3)	11 (3)
	3	12.9 (8)	17.3 (10)*	9 (3)	9.3 (5)
	4	16.1 (12)	22.4 (15)*	10.6 (4)	11.8 (6)

Values are in milligrams per deciliter and are given as mean (SD). SubF, subfraction.

* $P < .001$ as compared with normal-TG preeclampsia group and control subjects (analysis of variance–Tukey test).

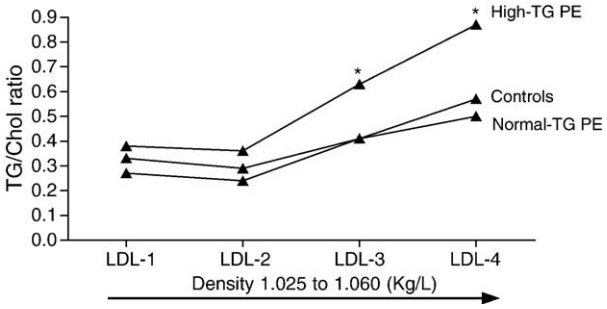

Fig. 1. Triglyceride-cholesterol ratio in LDL subfractions, in control subjects, and in preeclamptic women with high and normal TG levels. Chol indicates cholesterol; *$P < .001$ as compared with normal-TG preeclamptic group and control subjects (analysis of variance–Tukey test); PE, preeclampsia.

Triglyceride content was increased in the small dense subfractions in the overall group of preeclamptic patients, although the difference did not reach statistical significance. However, the data were markedly different in the preeclampsia subgroups. In the high-TG group, TG concentration was significantly increased in all LDL subclasses. The relative increment in TGs was much higher in the small dense subfractions (3 and 4) than in the larger subfractions (1 and 2) (88% vs 31%). In addition, in the larger subfractions, the increase of TGs was proportional to that of cholesterol, so that the TG-cholesterol ratio was similar to that in control subjects (Fig. 1). However, in the small dense subfractions, the TG-cholesterol ratio was increased by 2-fold and 3-fold, respectively ($P < .001$), in the subgroup of preeclamptic women with high TGs. Finally, lipid distribution and TG-cholesterol ratio in the normal-TG subgroup were similar to those in control subjects (Fig. 1).

4. Discussion

Research on the pathophysiology and prevention of preeclampsia has traditionally been hampered by poor understanding of the various pathological mechanisms that lead to preeclampsia and by the fact that it has mainly been studied as a single entity [14]. This has often led to conflicting results in a fair number of large trials trying to prevent preeclampsia on the basis of a single pathogenetic hypothesis. Therefore, delineation of major pathophysiologic groups should be one of the goals of preeclampsia research.

We speculated that TG levels could be used to identify 2 distinct pathogenic groups of preeclamptic patients. As with other features classically reported to be associated with preeclampsia, hypertriglyceridemia is only observed in a proportion of patients with this condition. In this study, a significant proportion of preeclamptic patients had TG levels more than the 90th percentile of our population of pregnant women, but more than half had values similar to those of control subjects. The lipid profiles of these 2 subgroups showed considerable biochemical differences. In the high-TG group, lipids were abnormally shifted toward the smaller and denser subfractions of LDL. In contrast to most previous studies [9,10], there was minimal overlap with control subjects. However, the subgroup with preeclampsia and normal TG levels had a lipid and lipoprotein subfraction profile similar to that of healthy pregnant women. These data suggest that lipid abnormalities are unlikely to be involved in the pathogenesis of the preeclamptic syndrome in this subgroup of patients.

This study found that a clearly abnormal pattern of lipoproteins is present in a proportion of patients with preeclampsia, which can be selected by means of plasma TG levels. This parameter was evaluated because alterations in plasma lipid concentrations encompass abnormalities in lipoprotein composition and in subclass distribution. The lipid profile in the high-TG subgroup was characterized by a shift in TG distribution and, therefore in total lipids, toward the small, dense LDL subfractions. Hypertriglyceridemia strongly correlates with a predominance of these subfractions in patients with atherosclerosis [15,16]. A proposed mechanism to explain this relationship is the interaction of LDL with TG-rich VLDL, as a result of which LDL lose cholesterol esters and gain TGs [17,18]. Triglyceride-enriched LDL is then modified by hepatic lipase, resulting in a shift in particle size into the small dense range [10,19]. Increased hepatic lipase activity has been reported in both atherosclerosis [20,21] and preeclampsia [9].

The distribution of LDL subfractions in preeclampsia has been the subject of recent investigations [9-11,22]. Sattar et al [9] measured LDL subfraction mass and reported a predominance of small dense lipoproteins in women with preeclampsia. Hubel et al [10] evaluated the distribution of LDL subfractions by measuring peak LDL diameter and demonstrated a predominance of small dense particles in a substantial proportion of women with preeclampsia. In a later study using equilibrium density gradient centrifugation, Winkler et al [22] did not confirm previous studies but rather suggested a dominance of the larger LDL subfractions. These apparently conflicting results might be explained by the heterogeneity of preeclampsia and the relatively small sample size used in this study. Thus, Hubel et al [10] reported considerable overlapping between cases and control subjects, and this led the authors to postulate that small, dense LDL predominance is not likely to be the only agent involved in vascular dysfunction in preeclampsia. This notion is in line with the results of the present study and further contributes to the idea that preeclampsia can be divided into patients with and without lipid abnormalities. Accumulating data suggest that a predominance of small dense LDL is causally related with endothelial injury and dysfunction in chronic vascular disease [23]. Small dense LDL are more atherogenic than larger LDL species because of a variety of mechanisms, including impaired endothelial clearance with longer stay in plasma [24], higher susceptibility to oxidation [25,26], and higher uptake by macrophages, resulting in the formation of foam cells [27].

Previously reported data [9], as well as findings from the present study, demonstrate the existence of remarkably increased VLDL TG levels in preeclampsia. Once again, these changes were only observed in the high-TG subgroup, whereas the normal-TG group had levels similar to those of control subjects. From the results of this study, we do not intend to suggest a greater risk for atherosclerotic disease in the group with hypertriglyceridemia. However, a considerable number of studies support preeclampsia as an independent risk factor for the development of dyslipoproteinemia, atherosclerosis, and cardiovascular disease later in life [14,28]. In a recent review summarizing available data, the additive risk for atherosclerotic disease in women with past preeclampsia was estimated to be 7-fold [28]. Therefore, preeclamptic patients with an abnormal lipoprotein profile are likely to have a higher risk for dyslipoproteinemia later in life than those with a lipoprotein profile similar to that of control subjects.

Gestational hyperlipidemia plays the physiological role of supplying both cholesterol and TGs to the rapidly developing fetus [29]. Maternal TGs are hydrolyzed on maternal lipoprotein receptors by lipoprotein lipase and are transported across the placenta via fatty acid–binding proteins. As observed in the present and other studies, in preeclampsia, TG levels are further raised in the third trimester, and this maternal hypertriglyceridemia has been proposed as a compensatory response to increase nutrient delivery to the placenta and fetus [30]. However, although the number of patients was too low for definite conclusions to be drawn, the results of this study do not support this concept because a trend toward a lower frequency of early-onset preeclampsia and IUGR was observed in the high-TG subgroup. It is possible that different pathogenic pathways are in turn associated with different clinical and epidemiologic patterns [1,2]. We considered that early-onset preeclampsia associated with IUGR might originate from placental underperfusion and that altered maternal metabolism such as dyslipidemic syndrome contributes to a greater degree to the pathogenesis of some preeclamptic features with less placental dysfunction and therefore normal fetal weight. It is noteworthy that VCAM-1 levels were similarly increased in both groups, suggesting similar degrees of endothelial dysfunction regardless of the potential existence of different pathogenic lines [31].

In summary, this study provides evidence that patients with preeclampsia can be subdivided into 2 different pathogenic groups according to serum TG levels, a simple reproducible parameter. Triglyceride levels distinguished a subgroup of these patients that presented an altered lipoprotein profile. The pathogenic implications of this finding can be of great help in the design and interpretation of future pathophysiologic studies or prevention strategies via delineation of a group of patients with a clear abnormal lipid profile. These findings support the idea that preeclampsia is a multietiologic syndrome with heterogeneous biologic pathways.

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