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Oxidized Low-Density Lipoprotein (Oxidized LDL) and the Risk of Preeclampsia

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Summary

Oxidative stress plays an important role in the pathophysiology of preeclampsia. In a case-control study of 99 women with preeclampsia and 99 controls, we assessed maternal plasma oxidized low-density lipoprotein (oxidized LDL) in relation to preeclampsia risk. Logistic regression procedures were used to derive odds ratios (OR) and 95 % confidence intervals (CI). Plasma oxidized LDL was determined using enzyme immunoassay. Maternal plasma oxidized LDL was significantly positively correlated with lipids in both cases and controls. After adjusting for nulliparity, pre-pregnancy body mass index, physical inactivity, family history of chronic hypertension and plasma vitamin C concentrations, women who had elevated oxidized LDL concentrations (≥50 U/l) experienced a 2.9-fold increased risk of preeclampsia when compared with women having lower oxidized LDL concentrations (95 % CI 1.4-5.9). The risk of preeclampsia was markedly increased in women who had both elevated oxidized LDL and elevated triglyceride concentrations (OR=8.9, 95 % CI 3.1-26.2). Women with both elevated oxidized LDL and low vitamin C concentrations experienced a 9.8-fold increased risk of preeclampsia (95 % CI 3.0-32.2). Our results confirm the role of oxidative stress in the pathogenesis of preeclampsia. Prospective studies are needed to determine if elevated oxidized LDL concentrations can predict the occurrence of preeclampsia.

Key words

Oxidized LDL • Preeclampsia • Risk factors • Lipids • Vitamin C

Introduction

The oxidative conversion of low-density lipoproteins (LDL) to oxidized LDL is considered to be a key event in the biological process that initiates and accelerates the development of the early atherosclerotic lesion – the fatty streak (Steinberg 1997a, Berliner *et al.* 1995). Experimental studies have shown that LDL becomes atherogenic when it is converted to oxidized LDL (Steinberg 1997b, Witztum and Horkko 1997). Oxidized LDL is found in monocyte-derived macrophages in atherosclerotic lesions, but not in normal arteries (Yla-Herttuala 1998). LDL must first be converted to oxidized LDL so that it can be recognized

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by its receptors on macrophages. The binding of oxidized LDL to macrophages is a necessary step by which oxidized LDL induces cholesterol accumulation in macrophages, and thus transforms the macrophages into lipid-laden foam cells (Chisolm *et al.* 1999). Holvoet *et al.* (1998) were the first who clearly demonstrated that patients with coronary artery disease had significantly elevated plasma levels of oxidized LDL. Hulthe and Fagerberg (2002) demonstrated the relationship between subclinical atherosclerosis and circulating oxidized LDL levels by showing that oxidized LDL levels were related to intima-media thickness and plaque occurrence in the carotid and femoral arteries. Other investigators also found elevated levels of oxidized LDL in patients with metabolic syndrome (Sigurdardottir *et al.* 2002).

Preeclampsia, a vascular disorder of pregnancy, is a leading cause of maternal morbidity as well as perinatal morbidity and mortality. Accumulating evidence from clinical and epidemiologic studies suggests that diffuse endothelial dysfunction, resulting from oxidative stress, plays an important role in the pathogenesis of preeclampsia (Walsh 1998). Women with preeclampsia are more likely than normotensive pregnant women to experience metabolic disturbances that are similar to those seen in non-pregnant patients with coronary heart disease. For instance, metabolic disturbances consistently noted in preeclampsia include hypertriglyceridemia (Kaaja *et al.* 1995), hyperleptinemia (Muy-Rivera *et al.* 2005), oxidative stress (Walsh 1998), insulin resistance (Kaaja *et al.* 1995) and systemic chronic inflammation (Williams *et al.* 1999, Qiu *et al.* 2004). Moreover, histological studies of placental arteries delivered from preeclamptic women showed fibrin and complement deposition and the involvement of foam cells in atheromatous lesions (Robertson *et al.* 1986). Experimental models indicated that increased oxidized LDL is capable of inducing many endothelial changes of potential relevance to preeclampsia (Simon *et al.* 1990, Galle *et al.* 1994). However, it remains to ascertain whether circulating oxidized LDL is increased in preeclampsia. Increased autoantibodies to an epitope of oxidized LDL, which is considered an indirect marker of oxidized LDL, have been described in women with preeclampsia relative to normal pregnancy (Branch *et al.* 1994, Uotila *et al.* 1998), although negative reports also exist (Kurki *et al.* 1996, Bowen *et al.* 2002).

Hence, we used data from a cross-sectional case-control study to examine the relation between maternal plasma oxidized LDL concentrations and preeclampsia risk. We also assessed the joint and independent effect of maternal oxidized LDL and other biological markers related to increased preeclampsia risk such as elevated triglyceride or low plasma vitamin C (ascorbic acid) concentrations.

Methods

Study design and population

We analyzed data from a case-control study conducted from April 1998 to June 2002 at Swedish Medical Center and Tacoma General Hospital in Seattle and Tacoma, Washington, respectively. This study has been described in detail elsewhere (Sorensen *et al.* 2003). We identified women with preeclampsia according to the then-current American College of Obstetricians and Gynecologists guidelines (American College of Obstetricians and Gynecologists 1996), which defined preeclampsia as sustained pregnancy-induced hypertension with proteinuria. Women without a history of pregnancy-induced hypertension or proteinuria during the study pregnancy, delivering on the same date as the studied cases, were eligible as controls. About 82 % (310 out of 376) eligible preeclampsia cases and 53 % (502 out of 952) eligible controls participated in the study. Reasons for non-participation included lack of time, lack of interest, or missed appointments.

From that study population, we identified 155 women with preeclampsia according to the more stringent and currently advocated diagnostic criteria (National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy 2000). Hence, for the purposes of this analysis, preeclampsia was defined as persistent (6 or more hours) blood pressure of at least 140/90 mm Hg arising after 20 weeks gestation. Proteinuria was defined as urine protein concentration ≥30 mg/dl or more (or 1+ on a urine dipstick) in at least two random specimens collected at least 4 hours apart. Then we randomly selected 100 preeclampsia subjects for the present analysis.

From the 502 available normotensive controls, we randomly selected 100 subjects to serve as controls in the current analysis. All cases and controls were normotensive and non-diabetic prior to the index pregnancy.

Data collection

During participants' postpartum hospital stay, we administrated structured interview questionnaires to collect

information on maternal socio-demographic, medical, reproductive and life style characteristics. All interviews were performed in English. We reviewed maternal and infant medical records to collect detailed information concerning antepartum, labor and delivery characteristics, and conditions of the newborn. Pre-pregnancy body mass index (BMI) and the measure of adiposity was calculated as pre-pregnancy weight in kilograms divided by height in meters squared. Detailed information on habitual dietary intake during the 12 months prior to delivery of the index pregnancy was provided by study participants who completed a 121-item semi-quantitative validated food frequency questionnaire (FFQ) used for the Women's Health Initiative Clinical Trial (Patterson *et al.* 1999). The FFQ included fruits, vegetables and other food items.

Non-fasting blood samples were collected in EDTA 10 ml Vacutainer tubes during the intrapartum period. These were protected from ultraviolet light, kept on wet ice and processed within 30 min of phlebotomy. The median time between participants' last meal and phlebotomy was 2 hours both for cases and controls. Plasma decanted into cryovials was preserved with metaphosphoric acid/dithiothreitol solution and frozen at –70 °C until analysis. Blood samples were available for 99 % (99 of 100) of cases and 99 % of controls (99 of 100). Plasma oxidized LDL concentrations were measured using a mAb-4E6-based ELISA (Mercodia, Uppsala, Sweden). Maternal plasma cholesterol and triglyceride concentrations were measured enzymatically employing assays standardized by the Lipid Standardization Program of the Centers for Disease Control and Prevention, Atlanta, GA. Plasma vitamin C concentrations were analyzed on Cobas Mira Plus (Roche, Branchburg, NJ) Chemistry Analyzer using a colorimetric procedure (Lee *et al.* 1997). The intra- and inter-assay coefficients of variation for the assays used were all <10 %. All assays were performed without knowledge of the pregnancy outcome.

Statistical analysis

We examined the frequency distributions of maternal socio-demographic characteristics and medical and reproductive histories according to case-control status. We also examined the distribution of continuous variables (e.g. oxidized LDL) and found them to be approximately normal, hence we used Student's t-test to evaluate unadjusted mean differences according to the case and control status. When making case and control comparisons for categorical variables, we used the Chi-square test or the Fisher's exact test where appropriate. Associations of maternal plasma oxidized LDL concentrations with maternal plasma lipid profiles (total cholesterol, LDL, HDL and triglyceride), as well as pre-pregnancy BMI and maternal age was estimated using Spearman's correlation coefficients.

To estimate the relative association between preeclampsia and levels of maternal plasma oxidized LDL, we categorized each subject according to quartiles determined by the distribution of concentrations among controls. We used the lowest quartile as the reference group, and estimated odds ratios (OR) and 95 % confidence intervals (95 % CI) for each of the remaining three quartiles. To assess confounding, we entered covariates into a logistic regression model one at a time, then compared the adjusted and unadjusted odds ratios (Rothman and Greenland 1998). Final logistic regression models included covariates that altered unadjusted odds ratios by at least 10 %, as well as those covariates of *a priori* interest (*e.g*, maternal parity and pre-pregnancy BMI).

We also assessed the joint and independent effect of maternal oxidized LDL and two biological markers of preeclampsia risk (i.e, elevated plasma triglyceride and low plasma vitamin C concentrations). We categorized women into four groups based on combinations of whether or not their plasma concentration for each biological marker was elevated. For these analyses elevated oxidized LDL was defined as concentrations ≥50 U/l (determined using the median value observed among controls). Elevated triglyceride was defined as concentrations ≥181 mg/dl (defined as being above the median value observed in normotensive controls). For the oxidized LDL and triglyceride analysis, the four resulting categories were as follows: no elevations of either biological marker, elevated oxidized LDL only, elevated triglyceride only, and both elevated oxidized LDL and triglyceride. Women with no elevations comprised the reference group, against which women in the other three categories were compared. A similar analysis was conducted to assess the joint and independent effect of maternal oxidized LDL and low plasma vitamin C levels. Vitamin C is the primary anti-oxidative (Halliwell and Gutteridge 1990) and several lines of evidence indicate that low plasma concentrations are associated with an increased risk of preeclampsia (Zhang *et al.* 2002). For this study we defined low vitamin C *a priori* on the basis of the 25th percentile observed among controls (<42.9 μmol/l). Women with no

Table 1. Distribution of preeclampsia cases and controls according to selected characteristics (Seattle and Tacoma, Washington, 1998-2002).

Characteristics	Preeclampsia cases (n=99)		Control subjects (n=99)	
	n	%	n	%
Maternal Age (years)				
<20	4	4.1	4	4.1
20-34	61	61.6	63	63.6
≥35	30	30.3	30	30.3
missing	4	4.0	2	2.0
Maternal age (years)	30.9±0.7[1]		31.6±0.6	
Maternal race/ethnicity				
White Non-Hispanic	71	71.7	67	67.7
African American	7	7.1	3	3.0
Other	17	17.2	27	27.3
Missing	4	4.0	2	2.0
Unmarried	20	20.2	18	18.2
<12 Years of education	21	21.2	16	16.2
Nulliparous	75	75.8	54	54.6
Family history of chronic hypertension	60	60.6	40	40.4
Smoked during pregnancy	16	16.2	13	13.1
Physical inactivity during pregnancy	42	42.4	37	40.4
<5 serving of fruits and vegetables/day	62	62.6	49	49.5
Daily vitamin C intake < 70 mg	44	44.4	29	29.3
Maternal plasma vitamin C (μmol/l)	45.1±1.9[1]		53.2±1.5	
Pre-pregnancy body mass index (kg/m^2)				
<19.9	6	6.1	30	30.3
20-24.9	44	44.4	61	61.5
25-29.9	24	24.2	9	9.1
≥30	20	20.2	7	7.1
Missing	5	5.1	2	2.0
Pre-pregnancy body mass index (kg/m^2)	25.7±0.6[1]		22.5±0.4	
Maternal plasma oxidized LDL (U/L)[2]	57.4±2.5[1]		52.3±1.9	

[1]Data are mean ± S.E.M. [2]p value for Student t test of maternal plasma oxidized LDL = 0.10.

elevations of oxidized LDL and high vitamin C comprised the reference group, against which women in the other three categories (elevated oxidized LDL only, decreased vitamin C only, and both elevated oxidized LDL with decreased vitamin C) were compared. All analyses were performed using Stata 7.0 statistical software (Stata, College Station, TX). All continuous variables are presented as mean ± S.E.M. All reported confidence intervals were calculated at the 95 % level.

The procedures used in this study were in agreement with the protocols approved by the Institutional Review Boards of Swedish Medical Center and Tacoma General Hospital, respectively. All participants provided written informed consent.

Results

The socio-demographic, medical and reproductive characteristics of cases and controls are shown in Table 1. Most cases tended to be nulliparous and had a higher BMI. Although maternal age and reports of physical inactivity during pregnancy were similar for

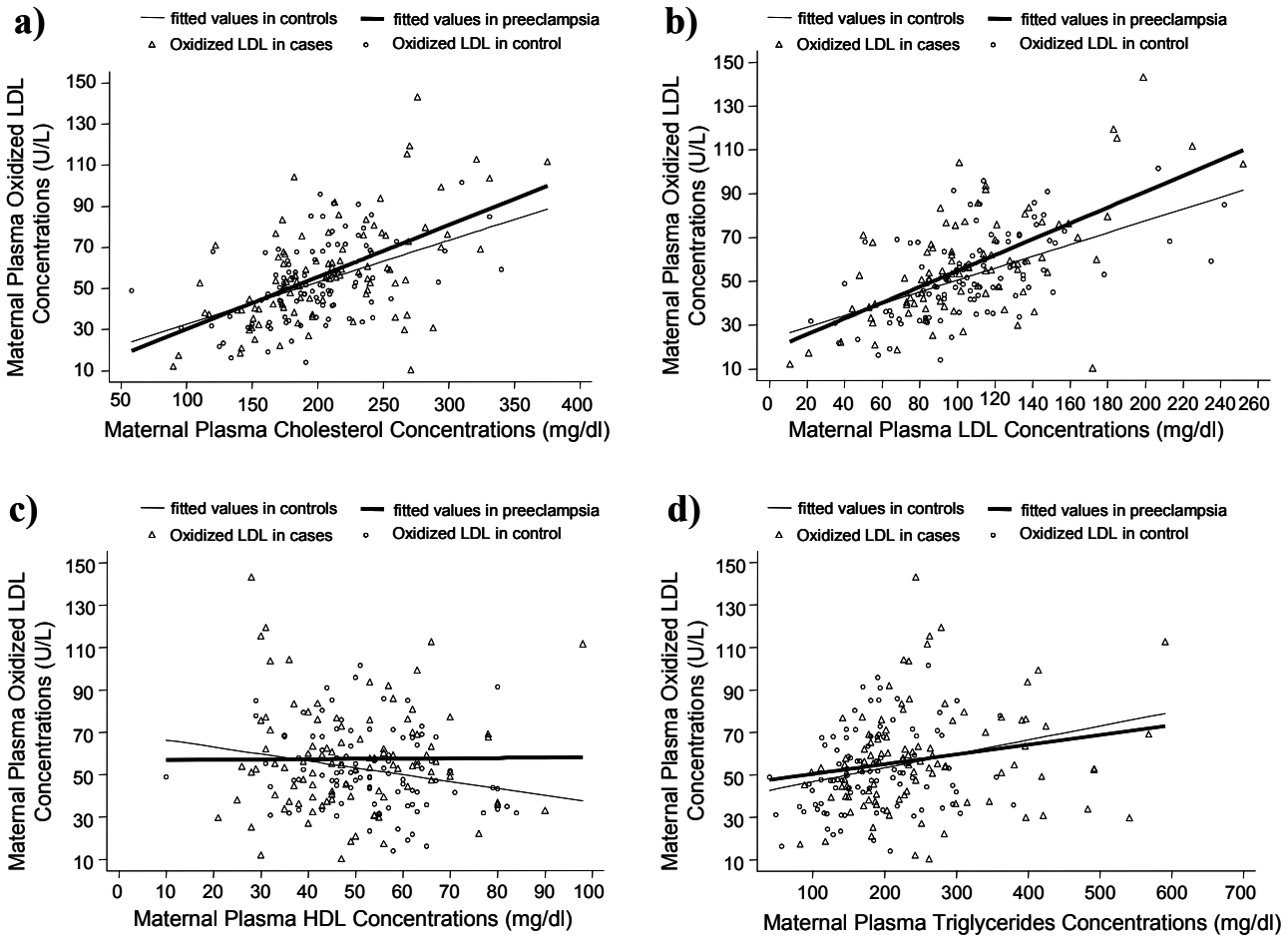

Fig. 1. The linear regression between maternal plasma oxidized LDL concentrations and maternal plasma lipid profile according to preeclampsia cases and controls, (a) with total cholesterol, (b) with LDL cholesterol, (c) with HDL cholesterol, (d) with triglycerides (Seattle and Tacoma, Washington, 1998-2002).

cases and the controls, cases were more likely to report consumption of less than 70 mg of vitamin C daily. Cases also reported less than five servings of fruits and vegetables per day as compared with the controls. Mean plasma oxidized LDL concentrations were 9.8 % higher among the followed cases than in the controls, although this difference was not statistically significant (57.4±2.5 vs. 52.3±1.9 U/l, p=0.10).

We next examined the correlations between maternal plasma oxidized LDL concentrations and maternal characteristics such as age, pre-pregnancy BMI as well as plasma lipid profile for preeclampsia cases and controls (Table 2). Oxidized LDL concentrations were all significantly positively correlated with pro-atherogenic lipids and lipoproteins in both preeclampsia cases and the controls (r=0.51 for total cholesterol, r=0.55 for LDL, r=0.22 for triglyceride in cases and r=0.50 for total cholesterol, r=0.59 for LDL, r=0.28 for triglyceride in the controls). Oxidized LDL concentrations were inversely correlated with maternal plasma HDL (r = –0.27, p<0.01), but this was only evident among the controls. Maternal plasma oxidized LDL concentrations were not significantly correlated with maternal age and pre-pregnancy BMI. Regression lines between maternal plasma oxidized LDL concentrations and maternal lipid profile according to preeclampsia cases and controls are displayed in Figure 1.

Unadjusted and adjusted odds ratios of preeclampsia risk based on quartiles of maternal plasma oxidized LDL concentrations are shown in Table 3. After adjusting for parity, maternal inactivity, parental history of chronic hypertension, pre-pregnancy BMI and plasma vitamin C concentrations, women with oxidized LDL in the third and fourth quartiles had increased preeclampsia risks (OR=4.6 or OR=2.5, respectively). There was no evidence of a clinically significant increased risk of preeclampsia associated with oxidized LDL concentrations in the range of values for the second

Table 2. Spearman's correlation coefficients (*r*) for maternal plasma oxidized LDL concentrations with selected maternal characteristics and lipid profile for preeclampsia cases and controls, respectively (Seattle and Tacoma, Washington, 1998-2002).

Characteristics/Lipids	Preeclampsia cases (n=99) r (P-value)	Control subjects (n=99) r (P-value)
Maternal age (years)	–0.08 (0.46)	–0.02 (0.82)
Pre-pregnancy body mass index (kg/m^2)	–0.13 (0.22)	–0.15 (0.15)
Plasma total cholesterol (mg/dl)	0.51 (<0.01)	0.50 (<0.01)
Plasma HDL cholesterol (mg/dl)	0.04 (0.66)	–0.27 (<0.01)
Plasma LDL cholesterol (mg/dl)	0.55 (<0.01)	0.59 (<0.01)
Plasma total triglycerides (mg/dl)	0.22 (0.03)	0.28 (<0.01)

Table 3. Odds ratios (OR) and 95 % confidence intervals (CI) of preeclampsia according to the quartile of oxidized LDL concentrations in control subjects (Seattle and Tacoma, Washington, 1998-2002).

Oxidized LDL (U/l)	Preeclampsia cases (n=99)	Control subjects (n=99)	Unadjusted OR (95 % CI)	Adjusted [1]OR (95 % CI)
Quartile 1 (<36.5)	18	25	1.0 (referent)	1.0 (referent)
Quartile 2 (36.5-49.9)	19	25	1.1 (0.5-2.6)	1.4 (0.5-4.0)
Quartile 3 (50-67.4)	34	25	1.9 (0.9-4.2)	4.6 (1.6-13.0)
Quartile 4 (≥67.5)	28	24	1.6 (0.7-3.7)	2.5 (0.9-7.1)
Quartile 1-2 (<50)	37	50	1.0 (referent)	1.0 (referent)
Quartile 3-4 (≥50)	62	49	1.7 (0.9-3.1)	2.9 (1.4-5.9)

[1]OR and 95 % CI adjusted for maternal nulliparity, pre-pregnancy body mass index (categorical), physical inactivity, family history of chronic hypertension and maternal plasma vitamin C (continuous).

quartile (36.5–49.9 U/l). Therefore, we performed *post hoc* analyses after creating a dichotomous variable. For the *post hoc* analysis women with oxidized LDL ≥50 U/l (i.e. the upper two quartiles) were classified as having elevated oxidized LDL. Women with concentrations below that value were considered as the reference group. From these analyses, we observed that elevated oxidized LDL was associated with an almost three-fold increase of preeclampsia risk (adjusted OR=2.9, 95 % CI=1.4-5.9).

We examined the independent and joint associations of maternal plasma oxidized LDL concentrations and high triglyceride status with risk of preeclampsia. As shown in Table 4, women with elevated oxidized LDL concentrations (≥50 U/l) only, as compared with women with no elevated oxidized LDL concentrations (≥50 U/l) and no elevated triglyceride concentrations (i.e. the reference group), experienced a 2.3-fold increased risk of preeclampsia (95 % CI 0.6-8.1). Women who had elevated triglyceride concentrations only experienced a 3.7-fold increased risk of preeclampsia (95 % CI 1.2-11.6). Also, the risk of preeclampsia was markedly increased in women who had both elevated oxidized LDL and elevated triglyceride concentrations (OR=8.9, 95 % CI 3.1-26.2). In this population, there was some evidence of a greater-than-additive effect between elevations of these two biomarkers on the risk of preeclampsia, although the interaction term in did not reach statistical significance (p=0.95).

We observed a similar pattern when we examined the independent and joint associations of elevated oxidized LDL concentrations and low vitamin C concentrations with the risk of preeclampsia. As is shown in Table 5, women with only elevated oxidized LDL concentrations experienced a 2.4-fold increased risk (95 % CI 0.9-5.9). The OR for women with only low vitamin C concentrations was 2.2 (95 % CI 0.7-6.6). Women with both elevated oxidized LDL and low

Table 4. Odds ratios (OR) and 95 % confidence intervals (CI) of preeclampsia according to maternal plasma oxidized LDL concentrations and maternal plasma triglyceride concentrations (Seattle and Tacoma, Washington, 1998-2002).

Oxidized LDL & triglycerides	Cases (n=99) n	Controls (n=99) n	Unadjusted OR (95 %CI)	Adjusted [1]OR (95 %CI)
Low:<50 U/l & Low:<181 mg/dl	13	30	1.0 (referent)	1.0 (referent)
High:≥50 U/l & Low:<181 mg/dl	10	19	1.2 (0.4-3.3)	2.3 (0.6-8.1)
Low:<50 U/l & High:≥181 mg/dl	24	20	2.8 (1.1-6.7)	3.7 (1.2-11.6)
High:≥50 U/l & High:≥181 mg/dl	52	30	4.0 (1.8-8.8)	8.9 (3.1-26.2)
P for the interaction			0.79	0.95

[1]OR and 95 % CI adjusted for maternal nulliparity, pre-pregnancy body mass index (categorical), physical inactivity, family history of chronic hypertension and maternal plasma vitamin C (continuous).

Table 5. Odds ratios (OR) and 95 % confidence intervals (CI) of preeclampsia/eclampsia according to maternal plasma oxidized LDL concentrations and maternal plasma vitamin C concentrations (Seattle and Tacoma, Washington, 1998-2002).

Oxidized LDL & vitamin C	Cases (n=99) n	Controls (n=99) n	OR (95 %CI)	OR (95 %CI) [1]
Low:<50 U/l & High:≥42.9 μmol/l	15	34	1.0 (referent)	1.0 (referent)
High:≥50 U/l & High:≥42.9 μmol/l	35	42	1.9 (0.9-4.0)	2.4 (0.9-5.9)
Low:<50 U/l & Low:<42.9 μmol/l	21	16	3.0 (1.2-7.2)	2.2 (0.7-6.6)
High:≥50 U/l & Low:<42.9 μmol/l	27	7	8.7 (3.1-24.5)	9.8 (3.0-32.2)
P for the interaction			0.50	0.42

[1]OR and 95 % CI adjusted for maternal nulliparity, pre-pregnancy body mass index (categorical), physical inactivity, and family history of chronic hypertension.

vitamin C concentrations experienced a 9.8-fold increased risk (95 % CI 3.0-32.2). There was some evidence of a greater-than-additive effect between the above-mentioned two risk factors on the risk of preeclampsia, although it did not reach statistical significance (p=0.42).

Discussion

Women, who had elevated oxidized LDL concentrations (≥50 U/l) had an almost threefold increased risk of preeclampsia, compared with women who had lower oxidized LDL concentrations. The risk of preeclampsia was markedly increased in women who had both elevated oxidized LDL concentrations and elevated triglyceride concentrations (OR=8.9, 95 % CI 3.1-26.2). Furthermore, women with both elevated oxidized LDL and low vitamin C concentrations experienced a 9.8-fold increased risk (95 % CI 3.0-32.2). These associations were independent of maternal physical inactivity, parental history of chronic hypertension and pre-pregnancy BMI.

There was only one published report of maternal plasma oxidized LDL concentrations in relation to preeclampsia risk (Raijmakers *et al*. 2004). In that study, women with preeclampsia had lower oxidized LDL concentrations (mean ± SEM: 181±12 vs. 219±14, p=0.027) than gestational-age-matched normotensive pregnant controls. The authors attributed this inverse association to the higher levels of autoantibodies to oxidized LDL that might be responsible for the rapid clearance of oxidized LDL. However, our results are consistent with some results of autoantibodies to oxidized LDL (indirect marker of *in vivo* oxidized LDL) from cross-sectional case-control studies (Branch *et al.* 1994, Uotila *et al.* 1998). For instance, Branch *et al.* (1994) assessed whether the titer of IgG autoantibody to an epitope of oxidized-LDL, malondialdehyde-conjugated LDL (MDA-mediated LDL), was increased in the serum

of preeclamptic patients. The investigators reported that 16 patients had significantly higher (27.6 %) mean titers than healthy pregnant women did (mean ± SEM: 3.98±0.32 vs. 3.12±0.17, p=0.03). Uotila *et al*. (1998) reported that antibody levels against copper-oxidized LDL were 25 % higher in women with preeclampsia (n=21) than controls (n=13), expressed as ratio of oxidized LDL/native LDL (mean ± SD: 2.39±0.43 vs. 1.91±0.54, p<0.01). However, our results are inconsistent with those reported by other investigators who showed no difference in autoantibodies against oxidized LDL concentrations for preeclamptic cases and controls (Kurki *et al.* 1996, Bowen *et al.* 2002). Fialová *et al.* (2002) reported that pregnancy-induced hypertension is associated with lower concentrations of autoantibodies against oxidized LDL (mean ± SD: 348±388 U/l in cases vs. 579±400 U/l in controls, p<0.01) using serum collected at the late 2nd trimester of pregnancy. Attention must be paid to the fact that results from the two studies may not be directly comparable, because women in our study had proteinuric pregnancy-induced hypertensions (i.e. preeclampsia). Variations in blood sample handling procedures, laboratory analytical techniques, limited statistical power, and uncontrolled confounding may have contributed to divergent results across these studies.

Hypertriglyceridemia has emerged as an important risk factor of preeclampsia (Enquobahrie *et al.* 2004). Elevated triglyceride values may compromise vascular function in several ways. For example, triglyceride-rich lipoprotein has a prothrombotic activity (Winkler *et al.* 2003). Elevated triglycerides might shift the pattern of LDL subclass towards proportional increases in smaller, denser, more atherogenic LDL particles (Krauss 1997). Low vitamin C concentrations have been shown to be related to increases in preeclampsia risk (Zhang *et al.* 2002). Vitamin C, a primary water-soluble antioxidant, readily scavenges reactive oxygen and nitrogen species and spare or recycle glutathione and vitamin E, two other important physiologic antioxidants (Halliwell and Gutteridge 1990). Our findings may serve as evidence suggestive of possible interactions of oxidized LDL with elevated triglycerides and low vitamin C, respectively, are consistent with the hypothesized role of oxidative stress/antioxidant in the pathogenesis of preeclampsia.

The association of oxidized LDL concentrations with preeclampsia is biologically plausible and is supported by the evidence from animal and cell culture studies. Experimental models showed that oxidized LDL increases artery sensitivity to pressor agonists and inhibits endothelial-dependent vasodilatation (Galle *et al.* 1994, Simon *et al.* 1990). Melatonin can inhibit vasospastic action of oxidized LDL in human umbilical arteries (Okatani *et al.* 2000). Longer incubations with oxidized LDL will inhibit endothelial PGI_2 production (Wang *et al.* 1989).

Several important limitations must be considered when interpreting our results. We cannot exclude the possibility of selection bias. In this study, the control participation rate was 53 % and 82 % in the cases. Although demographic and reproductive characteristics were largely similar for enrolled controls and members of the source population, from which they were drawn, we cannot exclude the possibility that the observed associations may be biased. However, our observations are largely consistent with findings reported from other clinical epidemiological studies (Branch *et al.* 1994, Uotila *et al.* 1998). The comparability of findings across these studies suggests that bias cannot completely account for our findings.

Because of the cross-sectional design of our study, we cannot determine whether the observed differences in plasma oxidized LDL concentrations preceded preeclampsia or whether the differences may be attributed to preeclampsia-related physiological alterations such as increased lipid peroxidation or systemic chronic inflammation. Prospective studies with serial measurements of plasma oxidized LDL concentrations in cohorts of pregnant women are required to confirm and expand our observations. Although we controlled for multiple confounding factors, we cannot with certainty conclude that the odds ratios reported are unaffected by residual confounding.

Maternal mortality and morbidity from hypertensive disorders of pregnancy, including preeclampsia, remain a high worldwide problem (Walker 2000) and represent a troublesome area of modern perinatology. The results from our study, when taken together with these observations, underscore the importance of developing clinical and epidemiological studies designed to identify maternal lifestyle characteristics that are determinants of elevated oxidized LDL and oxidative stress both before and during pregnancy. Information from such studies may contribute to the development and evaluation of behavioral and medical interventions aimed at reducing the occurrence of preeclampsia.

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