Lipid profile, plasma apolipoproteins, and pre-eclampsia risk in the GenPE case-control study

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Short title: Apolipoproteins and Pre-eclampsia

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Abstract

Background and aims. Pre-eclampsia constitutes a leading cause of maternal and perinatal morbidity and mortality. Pre-eclampsia susceptibility is believed to be associated with altered lipid profiles and abnormal lipid metabolism via lipid peroxidation that leads to endothelial dysfunction. The goal of this study was to evaluate the association of maternal blood lipid and apolipoprotein levels with pre-eclampsia in a large-scale study.

Methods. Using data from a large case-control study (1,366 cases of pre-eclampsia and 1,741 normotensive controls), the association between the distributions of eight lipid fractions and pre-eclampsia risk was evaluated using adjusted logistic regression models. Pre-eclampsia was defined as blood pressure ≥140/90mmHg and proteinuria ≥300mg/24hours (>1+ dipstick). Sub-group analyses were conducted for early (<34 weeks) and late (≥37 weeks) pre-eclampsia, estimating the effect of 1 standard deviation increase in log-transformed lipid fraction levels in adjusted multinomial regression models.

Results. After adjustment for potential confounders, concentrations of triglycerides, apolipoprotein E (ApoE) and the relationship between apolipoprotein B and A1 (ApoB/ApoA1) showed the strongest associations with pre-eclampsia, particularly for those cases with an early onset.

Conclusions. Higher levels of triglycerides, ApoE and the ApoB/ApoA1 ratio are associated with an increased risk of pre-eclampsia. Further studies that allow for a causal inference are needed to confirm or refute the aetiological role of blood lipids in pre-eclampsia.

Keywords: Pre-eclampsia, apolipoprotein, aetiology, dose response, hypertensive disorders of pregnancy.

Introduction

Pre-eclampsia is a complex multisystemic disorder of pregnancy that constitutes a worldwide leading cause of maternal and perinatal morbidity and mortality [1]. The pathogenic mechanisms of pre-eclampsia remain still unclear despite considerable research have been conducted. Nevertheless, epidemiological reports confirm a key role of genetics factors in the risk of pre-eclampsia. Twin studies have examined the contribution of genetic and environmental factors to this disorder. Indeed, they have showed a 55% correlation of presenting pre-eclampsia [2]. Also, pre-eclampsia risk have been reported to be 2-fold to 5-fold higher in women with close relatives that have been diagnosed with pre-eclampsia [3]. That said, maternal genetics, immunologic and environmental factors have been considered to be essential players in the development of pre-eclampsia [3-4].

In an effort to understand the aetiology of pre-eclampsia, normal pregnancy processes have been analyzed to deconvolute the interactions that may occur between the maternal and fetus genotypes. These approaches have increased insight into the role of specific processes within the pathophysiology of this disorder. For instance, various studies have reported a fail in the angiogenesis step [2, 4]. Specifically, during the development of the placental vasculature, different factors are secreted by natural killer cells such as vascular endothelial growth factor and placental growth factor; however, secretion of antagonists of these factors have been found in women with pre-eclampsia [3]. These processes may be an early defect that leads to the clinical symptoms of pre-eclampsia, which usually appear after the 20th week of gestation [2, 4].

In candidate gene approaches, numerous genes that participate in several pathways implicated in the pathophysiology of pre-eclampsia have been investigated. These pathways include events such as thrombophilia, endothelial function, vasoactive proteins, oxidative stress and lipid metabolism, and immunogenetics [5]. Nevertheless, results have been inconsistent, and no single susceptibility gene to pre-eclampsia has been identified due to the complex interactions that cause the clinical manifestations. In particular, various studies have reported the synergy that may exist among a number of factors that affect the pathophysiology of pre-eclampsia including

maternal allele variants (for instance single nucleotide polymorphisms), environmental factors and fetus genotype (genes inherited from father and mother) [5].

Pre-eclampsia has two major components: a primary placental involvement and a secondary maternal syndrome that develops in susceptible women, commonly those with prevalent risk factors like type-2 diabetes, obesity, or chronic high blood pressure [6-8]. Pre-eclampsia, as a maternal syndrome, is characterized by systemic endothelial dysfunction associated with a hypertensive state, systemic vascular disturbances, and reduced blood flow to multiple organs [7]. Accumulating evidence suggests that causes of the endothelial dysfunction in pre-eclampsia may involve altered lipid profiles and abnormal lipid metabolism associated with oxidative stress [9-13].

Pregnancy induces metabolic changes associated with hyperlipidemia that are not believed to be atherogenic [14-15], but supraphysiological elevations in cholesterol and triglyceride levels during gestation appear to be associated with offspring macrosomia [16] and atherosclerosis [17], respectively. Compared to healthy pregnancy, women with pre-eclampsia have an abnormal lipid profile characterized by elevated concentrations of triglyceride-rich lipoproteins [18]. However, there is no consensus regarding the aetiological involvement of these changes in lipid concentrations in pre-eclampsia. Results from observational studies have been largely inconsistent. The largest meta-analysis evaluating non-adjusted differences in lipid levels between pre-eclampsia cases and healthy pregnant women reported statistically significant associations with elevated total cholesterol, non-high density lipoprotein cholesterol (non-HDL-C), triglyceride levels, and HDL-C; nevertheless, all associations showed significant heterogeneity [19].

The potential association between maternal levels of apolipoproteins and pre-eclampsia has also been investigated. Apolipoproteins are proteins involved in the mediation of inflammatory responses and removal of excess cholesterol from the circulation [20]. In particular, the ApoE isoforms and gene variants have been postulated and highlighted as potential predictors of pre-

eclampsia development [21-22]. However, the specific role of these metabolites in pre-eclampsia aetiology remains unclear. The present study investigates the relationship between plasma lipids (cholesterol, triglyceride, LDL-C and HDL-C) and lipoproteins (apolipoproteins A1, B, and E: ApoA1, ApoB and ApoE), with both pre-eclampsia and its sub-phenotypes in a large case-control study.

Material and Methods

Study design and participants

GenPE (Genetics and Preeclampsia) is a multicentre case-control study conducted across eight Colombian cities [23]. Young, primigravid women (8407) were recruited at the time of delivery between December 2000 and February 2012. Pre-eclampsia was defined as the presence of blood pressure ≥140/90 mmHg and proteinuria ≥300mg in 24 hours or ≥2+ dipstick reading in a random urine sample with no evidence of urinary tract infection after the 20th week of gestation. At least one control was recruited from the same centre that provided the case within 1 week (aiming for 24 hours) of the identification of the case. A control was defined as a woman with an uneventful pregnancy presenting in labour at term delivery (37–42 weeks). In order to improve the homogeneity of the phenotype under evaluation, women with a prior history of autoimmune, metabolic (including diabetes or gestational diabetes), renal, or cardiac (including chronic hypertension) diseases were excluded from the study. In addition, all cases and controls were validated by an outcome committee composed by epidemiologists and consultant obstetricians to further minimize outcome misclassification.

Pre-eclampsia sub-phenotypes were defined according to time of onset. Early and late pre-eclampsia were defined as presentation before 34 weeks gestation and after 34 weeks, respectively.

Data collection

A verbal interview was conducted by trained personnel in order to ascertain demographic and clinical data such as maternal age, race, socioeconomic status, smoking and infection during the pregnancy (e.g. vaginosis, urinary tract infections, sexually transmitted diseases and others), family history of pre-eclampsia, and gestational age at recruitment. All participants signed a written informed consent form at recruitment, and this project was approved by the Research Ethics Committee of Universidad Autónoma de Bucaramanga – UNAB.

Sampling and Biochemical analysis

Blood samples were taken at the time of recruitment from the antecubital vein under non-fasting conditions, and blood components were separated within 1 hour. Serum was extracted and stored at –80 °C until assays were performed. Lipid profiles were measured in 1,366 cases of pre-eclampsia and 1,741 normotensive controls.

Total cholesterol was measured by the enzymatic endpoint method (cholesterol oxidase). Direct assays were used for the measurement of HDL and LDL cholesterol. Triglycerides were quantified by the enzymatic colorimetric test (GPO-PAP). Apolipoprotein levels were determined by an automated immunoturbidimetric assay (RX imola – Randox). Each assay was performed according to manufacturers' specifications. Laboratory technicians were blinded to the case-control status of participants.

Statistical analyses

Data were described using means and standard deviations (SD) for continuous variables and counts (percentages) for categorical variables. Differences between cases and controls were evaluated using the t-test and the Kruskal-Wallis test for normally and non-normally distributed continuous variables, respectively. For discrete variables, the Chi-squared test or the Fisher exact test were used to contrast proportions. For the observations with complete lipid profiles, the

percentage of missing values for the variables included in regression models ranged from 0.0% to 2.1% (Supplementary table 1).

Multiple logistic regression was used to evaluate the association between quintiles of lipid fractions and lipoproteins, and pre-eclampsia. Crude odds ratios (OR), 95% confidence intervals (95%CI) and in some cases standard deviation were estimated for each blood marker in addition to estimates adjusted for maternal age, ethnicity, socioeconomic status, multiple pregnancy, smoking, infections during pregnancy, recruitment centre, and recruitment year. In order to evaluate possible changes of the effect of the biomarkers over time, interactions between the biomarkers and the year of recruitment (*i.e.* year of sampling) were tested in the multivariable models by means of the likelihood ratio test (LRT) (comparing nested models with and without the interaction term). For sub-phenotype analyses, all biomarkers were log-transformed, standardized, and introduced as continuous variables into adjusted multinomial logistic models that simultaneously estimated the association of each metabolite with the presence of early- and late-onset pre-eclampsia.

All estimates and the 95% confidence intervals (95% CI) were calculated incorporating the complex design of the sample into the Stata software, version 14 (Stata Corporation, College Station, TX).

Results

After the exclusion of 592 observations without lipid measurements and 60 observations without complete information on the covariates to be used in multivariable regression models, data from 1,552 patients with a diagnosis of pre-eclampsia and 1,986 controls were available for analysis. 1,366 cases (88.0%) and 1,741 controls (87.7%) had complete lipid profiles. By definition, cases gave birth earlier and had higher blood pressure than controls. Table 1 presents the baseline characteristics of the participants with complete lipid profiles. In this sample of women, over 90% belonged to a low socioeconomic group, and the proportion of smokers was lower than 3%.

Patients with pre-eclampsia were more likely to be multiple pregnancy ($p < 0.001$) and from Afro-Caribbean ethnicity ($p < 0.001$) compared to controls.

On average, median total cholesterol and HDL cholesterol concentrations were lower among cases than controls; but LDL cholesterol concentration was not different between groups (supplementary table 2). In addition, the non-HDL Cholesterol is considered near ideal according to the cut point (130 – 159 mg/dL) (Supplementary table 2). As previously reported, pre-eclampsia cases had higher triglyceride levels as controls. In unadjusted models, there was no difference in lipoprotein level of ApoB; however, both the ApoB/ApoA1 ratio and ApoE concentrations were significantly higher in cases than controls (supplementary table 2).

After restricting the analysis to participants with complete lipid profiles, there was evidence of a dose-response relationship between HDL cholesterol, triglycerides, ApoA1, ApoE, and the ApoB/ApoA1 ratio and pre-eclampsia after adjustment for several covariates. HDL cholesterol and ApoA1 showed inverse associations with odds of disease (Figure 1). Triglycerides and ApoE concentrations had the strongest associations with preeclampsia.

Comparing to the first quintile of triglyceride concentrations, the adjusted odds ratios for the second, third, fourth and fifth quintiles were 1.22 (95%CI: 0.97, 1.55), 1.56 (95%CI: 1.24, 1.98), 2.21(95%CI: 1.74, 2.79), and 3.17 (95%CI: 2.48, 4.06), respectively. In the case of ApoE, the corresponding odds ratios were 0.99 (95%CI: 0.76, 1.29), 1.44 (95%CI: 1.09, 1.89), 2.35(95%CI: 1.77, 3.14), and 3.76 (95%CI: 2.78, 5.10) for comparisons with the second, third, fourth and fifth quintiles, respectively. Although there were systematic differences in the concentration of lipid fractions and lipoproteins by year of recruitment (Supplementary figure 1), there was no statistical evidence of biomarker by year of recruitment interactions for any lipid fraction or lipoprotein (all LRT p-values for interaction > 0.05).

In the multivariate model that additionally adjusted for all the lipid fractions and lipoproteins, total cholesterol, triglycerides, ApoB/ApoA1 ratio, and ApoE concentrations were independently

associated with pre-eclampsia (Table 2). Odds ratios for pre-eclampsia comparing top and bottom quintiles were 0.28 (95%CI: 0.17, 0.47) for total cholesterol, 3.73 (95%CI: 2.67, 5.20) for triglycerides, 3.16 (95%CI: 2.26, 4.40) for the ApoB/ApoA1 ratio, and 2.04 (95%CI: 1.40, 2.96) for ApoE. When the association of the lipid fractions was evaluated as a continuous effect (with ORs for 1 SD increase in blood lipid levels), a trend for a stronger association with early pre-eclampsia compared to late pre-eclampsia was observed for triglycerides, ApoB and ApoE levels. Nevertheless, the separation of the two groups was only clear (as seen by the lack of overlapping of the confidence intervals) for ApoE (Figure 2). The association between HDL, ApoA1 and the ApoB/ApoA1 ratio and pre-eclampsia seemed to be attenuated for the early pre-eclampsia group compared to late pre-eclampsia group, although there was considerable overlapping of the confidence intervals. LDL cholesterol had a weak negative association with late pre-eclampsia but a positive association with early pre-eclampsia; this pattern was also stronger for the association between total cholesterol and pre-eclampsia.

Discussion

This study assessed the association between maternal lipid profiles and pre-eclampsia in a large study. The results from this analysis showed that elevated triglycerides and lower levels of HDL cholesterol measured at the end of pregnancy were associated with increased odds of pre-eclampsia, data consistent with previous reports [18-19, 24]. These associations showed a dose-response relationship that was present after adjustment for possible confounders. Furthermore, pre-eclampsia was positively associated with ApoE, and the ApoB/ApoA1 ratio, and negatively associated with ApoA1, in this sample of young Colombian women. Sub-phenotype analysis by pre-eclampsia onset suggested that the effect of HDL cholesterol might be more relevant in late-onset pre-eclampsia than early-onset pre-eclampsia, while the association of ApoE may be stronger for early-onset cases than late-onset cases.

One of the strengths of this report is the large sample size used allowed us to adjust the models for a set of potential confounders. Similarly, this sample size allowed to describe trends for the

associations between the different lipid fractions and apolipoproteins with pre-eclampsia risk, as well as to conduct sub-phenotype analyses retaining enough number of cases in each category analyzed. The comprehensive adjustment of our models suggests that the observed associations are independent from important potential confounders that are usually not controlled in studies of this nature. Furthermore, the consistency of our most important results with studies that measured the lipid fractions at the beginning of pregnancy suggests that reverse causation does not completely explain the observed associations. Nevertheless, we cannot rule out that residual confounding and reverse causation may be affecting the results presented in this study.

No studies have reported associations of apolipoproteins with pre-eclampsia in samples as large as the current study [20, 25-26]. As a result, the strong associations observed for ApoE and the ApoB/ApoA1 ratio deserve further mention. ApoA, the main component of HDL cholesterol, has two main forms: ApoA1, which constitutes 75% of ApoA, and ApoA2, which constitutes the remaining 25%. ApoB, on the other hand, is the main component of LDL cholesterol (constituting up to 80%) and very low density lipoproteins (VLDL, constituting up to 40%). ApoE is the main component of VLDL, and is responsible for modifying inflammatory responses and removing excess cholesterol from circulation by regulating the absorption of lipids in the liver [20]. Apolipoproteins are importantly involved in oxidative stress and lipid metabolism; in fact, imbalance in the levels of these metabolites are related to pro-atherosclerotic states with a link to endothelial dysfunction, which is a distinctive feature of pre-eclampsia [27].

Multi-organ endothelial dysfunction contributes to the clinical manifestations observed in pre-eclampsia, having a pivotal role the oxidative stress. In particular, the endothelial dysfunction has been associated to a decrease in the bioavailability of nitric oxide (NO) due to a number of factors including increased oxidative stress [28-30]. During pregnancy, hyperlipidemia has been reported to cause oxidative stress since lipid species are more easily oxidized (lipid peroxidation is higher) and stay longer periods in circulation by decreasing their ability to be recognized by their receptors [31-32]. Similarly, it has been reported upregulation of intercellular adhesion molecule (ICAM) in the endothelial cells, which increases the adhesion of leukocytes and may induce

inflammation [13]. Therefore, abnormal lipid profiles have a direct effect on the endothelial dysfunction, and their transport, in which apolipoproteins play a role, may impair their normal function, provoking the altered pathophysiology characteristic of pre-eclampsia [22, 33].

Elevated levels of ApoB, a constituent of atherogenic lipoproteins, and reduced levels of ApoA1, a component of anti-atherogenic HDL, cholesterol, are associated with increased risk of cardiac events [34-35]. Furthermore, ApoA1, ApoB, and the ApoB/ApoA1 ratio have been reported as important predictors of cardiovascular risk, outperforming the predictive ability of LDL cholesterol even in patients receiving lipid modifying therapy [35]. This is one of the reasons apolipoprotein levels are being investigated as predictors for pre-eclampsia [35]. However, the potential usefulness of apolipoproteins in pre-eclampsia research also relies on their well-studied genetics. For example, the ApoE gene, APOE, is located on chromosome 19, and has three common alleles that encode three isoforms: ApoE2, ApoE3, and ApoE4. The ApoE2 and ApoE4 isoforms have been reliably associated with high triglyceride and VLDL levels [27, 36]. Therefore, it has been postulated that APOE gene polymorphisms are associated with an increased risk of both cardiovascular disease and pre-eclampsia. This genetic evidence, in addition to the observed associations described here between ApoE and pre-eclampsia, provide support for a future Mendelian Randomization study that might help to determine the associated causality of certain lipid metabolites (including Apo-E and triglycerides) with pre-eclampsia.

Acknowledgment

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Financial support

This project was funded by COLCIENCIAS – Colombia (grant code No. 505 – 2009). The funders had no role either in the design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, and approval of the manuscript.

Author contributions

All authors contributed to the final version of the manuscript.

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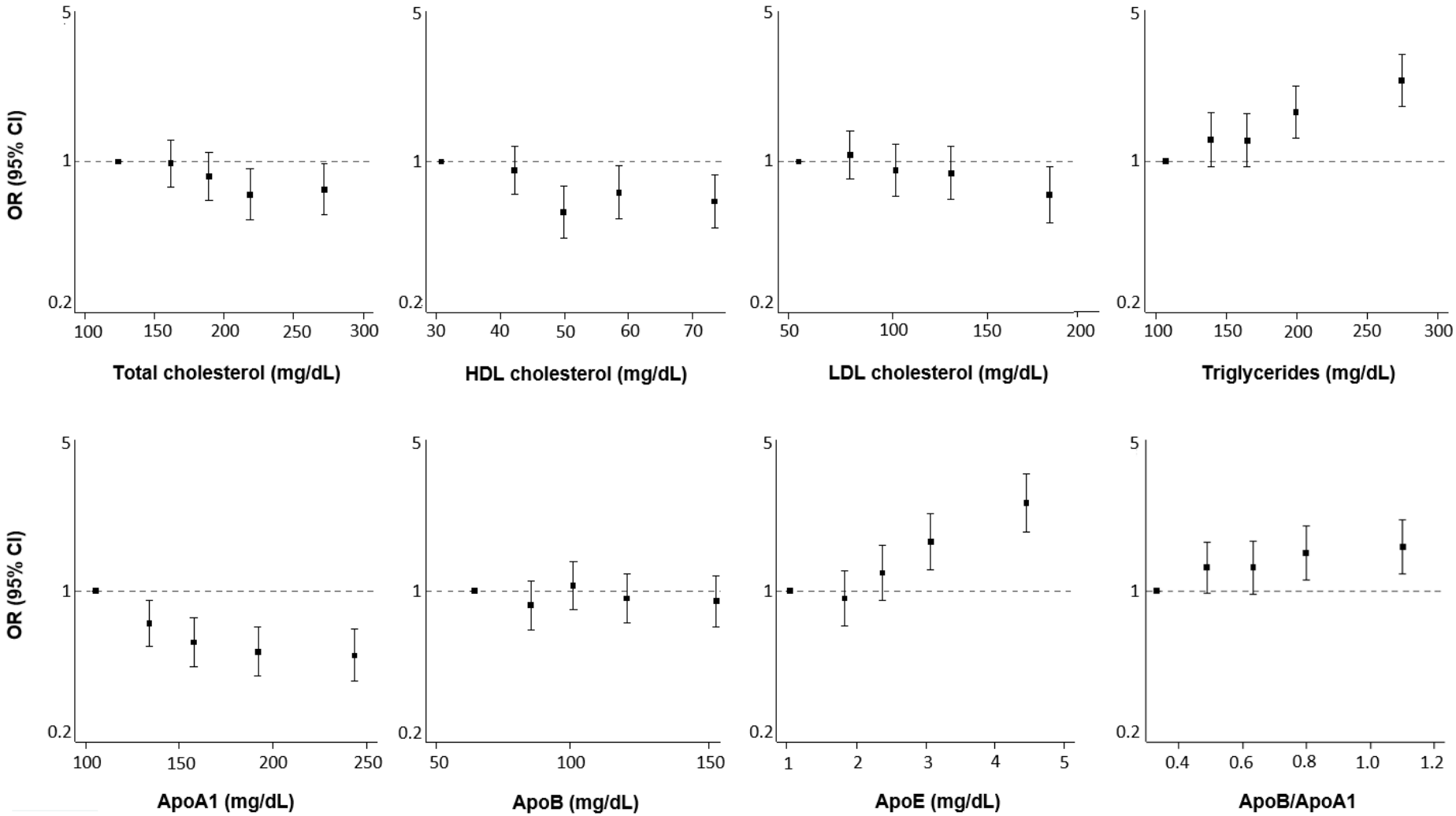
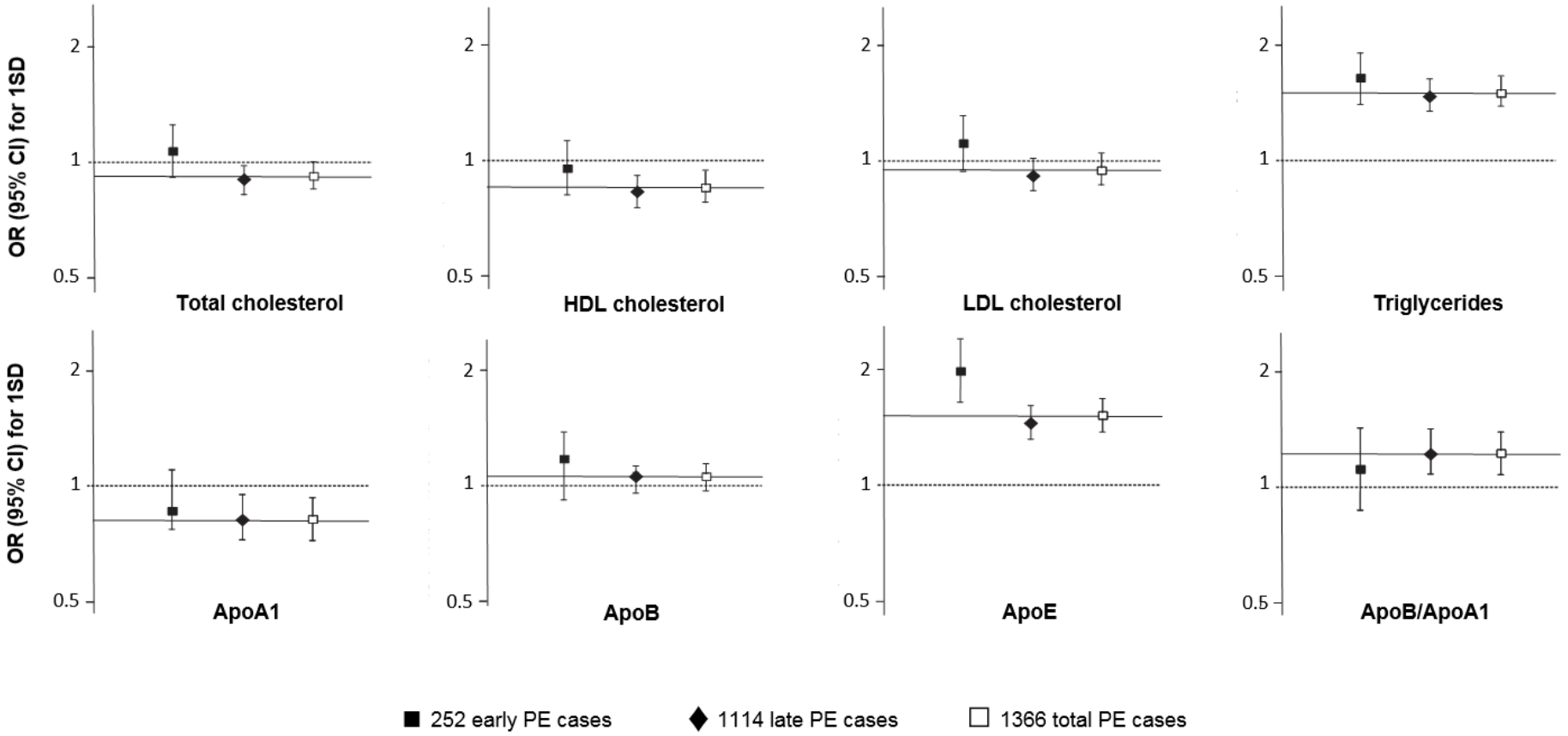
Figure 1. Adjusted odds ratios for pre-eclampsia by quintiles of lipid fraction and lipoprotein concentrations[a].



[a] Adjusted for maternal age, ethnicity, socioeconomic status, multiple pregnancy, smoking, infections during pregnancy, recruitment centre, and recruitment year.

Figure 2. Adjusted odds ratios for early- and late-onset pre-eclampsia by 1SD increase in log-transformed lipid fraction[a].



[a] Adjusted for maternal age, ethnicity, socioeconomic status, multiple pregnancy, smoking, infections during pregnancy, recruitment centre, and recruitment year.