IMPAIRED OGTT AND HIGH INSULIN LEVEL ARE ASSOCIATED WITH ELEVATED RATIO OF OXIDIZED LDL LIPIDS TO HDL-CHOLESTEROL AND TRIGLYCERIDES DURING EARLY PREGNANCY

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Joulukuu 2013

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ASTA PUUMALAINEN-NYKÄNEN: HEIKENTYNYT GLUKOOSINSIETO JA KORKEAT INSULIINIPITOISUUDET OVAT YHTEYDESSÄ SUURENTUNEESEEN HAPETTUNEIDEN LDL-LIPIDIEN JA HDL-KOLESTEROLIN SUHTEESEEN SEKÄ SUURENTUNEESEEN TRIGLYSERIDIPITOISUUTEEN ALKURASKAUDESSA

Kirjallinen työ: 15 s
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Joulukuukuu 2013

Avainsanat: raskausajan diabetes, seerumin rasva-aineet, sydän- ja verisuonisairaudet

Raskausajan diabetes kehittyy 3-5 % raskaana olevista naisista. Se altistaa erityisesti tyypin 2 diabetekselle ja metaboliselle oireyhtymälle sekä sydän- ja verisuonisairauksille myöhemmin elämässä. Lisäksi hapettuneet LDL-lipidit ovat riskitekijä sydän- ja verisuonisairauksille ja niiden pitoisuus verenkierrossa on suurentunut diabeteksessa.

Vertasimme seerumin rasva-aineiden ja hapettuneiden LDL-lipidien tasoja raskausajan diabeetikoilla raskaana oleviin naisiin, joiden sokeriaineenvaihdunta on normaali. Raskausajan diabetes määritettiin oraalisella glukoosirasituskokeella raskausviikoilla 8-12. Verinäytteistä tutkimme seerumin kokonaiskolesterolin, triglyseridit, HDL- ja LDL-kolesterolit, hapettuneet LDL-lipidit sekä paraoksonaasi- ja insuliinitasot.

619 osallistujasta 28 % oli raskausajan diabetes. Raskausajan diabeetikoilla oli suurempi hapettuneiden LDL-lipidien suhde HDL-kolesteroliin ($p=0,012$) ja suurempi seerumin triglyseridipitoisuus ($p=0,049$) sekä pienempi HDL-kolesterolipitoisuus ($p=0,016$). Lisäksi jaoimme osallistujat kolmeen ryhmään insuliinipitoisuuden mukaan. Henkilöillä, joiden insuliinipitoisuus oli korkein, oli suurempi triglyseridipitoisuus ($p=0,005$) ja suurempi hapettuneiden LDL-lipidien suhde HDL-kolesteroliin ($p=0,049$). Tuloksissa huomioitiin tupakointi, painoindeksi ja ikä.

SISÄLLYS

Impaired OGTT and high insulin level are associated with elevated ratio of oxidized LDL lipids to HDL-cholesterol and triglycerides during early pregnancy

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Running title: Early pregnancy glucose tolerance and ratio of Ox-LDL to HDL

Key words: insulin, oxidized LDL to HDL ratio, oral glucose tolerance test, pregnancy, serum lipids

Abstract

We investigated the effect of altered glucose metabolism and high insulin concentration on oxidized LDL lipids and serum lipids during early pregnancy. During early pregnancy (gestational weeks 8-12), 691 women took part in measurements for oral glucose tolerance test (OGTT), serum lipids and oxidized LDL lipids (ox-LDL). Based on the OGTT, subjects were divided to pathological OGTT and normal OGTT. Further, subjects were stratified into tertiles according to their insulin levels (low, average and high insulin level). Ox-LDL was determined by baseline level of conjugated dienes in LDL lipids. Women with pathological OGTT had significantly higher serum triglycerides and ratio of ox-LDL to HDL-cholesterol and lower HDL-cholesterol than women with normal OGTT after controlling BMI, age and smoking status ($p=0.049$, $p=0.012$ and $p=0.016$, respectively). In insulin tertiles, the women with low insulin level had significantly lower serum triglycerides and the ratio of ox-LDL to HDL-cholesterol than the women with high insulin level ($p=0.005$ and $p=0.049$, respectively) after adjusting for BMI, age and smoking. To conclude, pathological OGTT and high concentration of serum insulin were associated with elevated level of serum triglyceride and high ratio of oxidized LDL lipids to HDL-cholesterol after adjusting the results for BMI, age and smoking during early pregnancy. The present study showed that pathological glucose metabolism during early pregnancy may be associated with atherogenic lipid profile.

Introduction

It is estimated that around 3-5 % of pregnant women develop gestational diabetes mellitus (GDM) (1,2). In Finland the prevalence of GDM varies between 7 to 25 % (3). During pregnancy insulin sensitivity decreases which may lead to glucose intolerance and GDM (4). In a majority of women with GDM glucose metabolism normalizes after delivery but they still have an increased risk of diabetes mellitus or impaired glucose tolerance (IGT) and metabolic syndrome later in their life. GDM also increases the risk of obesity among women and their children (5-8).

Major risk factors of GDM are increasing maternal age, family history of type 2 diabetes, overweight before pregnancy, high pregnancy weight gain, lack of physical activity before pregnancy (9,10) and GDM or IGT during earlier pregnancies (6). There are some evidence that high intake of saturated fat and low intake of polyunsaturated fat increase the risk of developing GDM (2,11-13). In earlier studies, prevention of gestational weight gain by dietary and physical activity counseling have found to be favorable and structured aerobic exercise training has been shown to decrease birth weight of the newborns (14,15).

LDL molecules may be oxidatively modified under certain conditions (16). Oxidized LDL lipids (Ox-LDL) are a known risk factor of atherosclerotic diseases, like coronary artery disease (17,18). Diabetes mellitus is associated with increased oxidative stress, increased ratio of the oxidized LDL to LDL cholesterol (reviewed in (19)) and increased levels of oxidized LDL (20). Although Kathir et al. found no increase in amount of oxidized fatty acids or cholesterol in diabetic subjects (21).

In this study, we investigated the effect of altered glucose metabolism and high insulin concentration on oxidized LDL lipids and serum lipids during early pregnancy. We hypothesized that gestational diabetes and elevated concentration of insulin are associated with high concentration of oxidized LDL and unfavorable serum lipids in a large sample of pregnant women.

Materials and Methods

The cohort study was collected from primary health care maternity clinics in Pirkanmaa region, situated south-western part of Finland. The nurses recruited 2071 pregnant women when they contacted the maternity clinic for the first time by telephone (up to 12 weeks' gestation) (22). Pregnant women were eligible for the study if they had at least one of the following risk factors: body mass index (BMI) ≥ 25 kg/m², GDM or any signs of glucose intolerance or macrosomic newborn (≥ 4500g) in any earlier pregnancy, type 1 or 2 diabetes in first or second grade relatives or age ≥ 40 years. Exclusion criteria were pre-pregnant type 1 or 2 diabetes, inability to speak Finnish, age < 18 years, twin pregnancy, physical restriction preventing from physical activity, substance abuse, treatment or clinical history of psychiatric illness. 1016 women were eligible and from 619 women, the fasting blood sample was taken and oral glucose tolerance test was conducted. Ethical approval for this study was obtained from the Ethical board of Pirkanmaa Hospital District.

Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was taken at 8-12 weeks' gestation. Analyses were performed at the UKK Institute. The standard OGTT was performed by giving GlukodynR, including 75 g glucose in 330 ml water after overnight fasting (8 to 14 hours). The Finnish GDM criteria were used to identify participants with GDM: abnormal fasting blood glucose ≥5.3 mmol/l, >10.0 mmol/l at 1-hour and >8.6 mmol/l at 2-hour (23).

Laboratory tests

Analyses of blood samples included determination of serum lipids (cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol), oxidized LDL lipids and other biological markers as paraoxonase and insulin. Medical laboratory technologists from the UKK Institute or from the Centre Laboratory of Medicine in Pirkanmaa hospital district took the blood samples (4 × 10 ml) and performed the OGTT for all participating pregnant women. For lipid analyses venous blood was drawn into EDTA tubes. Plasma samples were stored frozen at -80°C until analyzed. Samples were analyzed in MCA research laboratory, Turku (paraoxonase, insulin and oxidized LDL lipids) and at the UKK Institute (serum lipids and glucose). For assessment of LDL-cholesterol and baseline diene conjugation (ox-

LDL) (24), LDLc was first isolated by precipitation (24,25). The coefficient of variation (CV) for the within-assay precision (12 determinations of the same serum) of the precipitation phase was 3.6%. After resuspension of the LDLc, the concentration of cholesterol was measured using a kit (CHOD-PAP method, Boehringer Mannheim, Mannheim, Germany). Thereafter, lipids were extracted from the LDLc samples (100 μl) by chloroform – methanol (2:1), dried under nitrogen, then redissolved in cyclohexane, and analyzed spectrophotometrically at 234 nm (26). Oxidation during the sample preparation was prevented by EDTA. The CV for the within-assay precision was 4.4 % (20 determinations of the same sample); the CV for the between-assay precision was 4.5 % (over the period of 3 months). Total cholesterol, HDL cholesterol and triglyceride concentrations were measured with enzymatic assays using Roche Cobas Mira Plus analyzer. Paraoxonase activity was determined using paraoxon (O,O-diethyl-O-*p*-nitrophenylphosphate) as the substrate (27). Serum insulin was measured with time-resolved fluoroimmunoassay method using Wallac AutoDELFIA analyzer (Wallac, Turku, Finland). All analyses were made in duplicate.

Statistical analysis

First we divided women into two groups based on their oral glucose tolerance test results: women with pathological OGTT and women with normal OGTT. Further, we divided women into tertiles based on their serum insulin levels: the low insulin group (serum insulin level less than 9.40 mU/L), the average insulin group (serum insulin level 9.40 – 13.30 mU/L) and the high insulin group (serum insulin level greater than 13.30 mU/L).

Univariable and multivariable linear regression analyses were used to test the differences between groups. We adjusted our multivariable models for body mass index, age and smoking. Based on Kolmogorov-Smirnov test the residuals of triglyceride, paraoxonase, ox-LDL and ratios of ox-LDL to HDL-cholesterol and ox-LDL to LDL-cholesterol were not normally distributed and therefore these dependent variables were examined after logarithmic transformation. Spearman's rank correlation coefficient was used to determine the association between lipids and the concentration of insulin and between lipids and the concentration of 2 h glucose from OGTT.

A P value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were two-tailed and performed using STATA version 12.0 for Windows (StataCorp LP, College Station, Texas, USA).

Results

From the 619 women tested, 173 women (28 %) had pathological OGTT.

Pathological OGTT vs. normal OGTT

Women with pathological OGTT had significantly higher serum triglycerides than women with normal OGTT (1.33 vs. 1.17, p<0.001; regression coefficient 0.14 and 95% confidence interval (95% CI) 0.06-0.23). Also, they had significantly higher, concentration of ox-LDL (26.8 vs. 25.6, p=0.026; coefficient 1.15 and 95% CI 0.13-2.25). Women with pathological OGTT had significantly lower HDL-cholesterol than women with normal OGTT (1.58 vs. 1.69, p<0.001, coefficient -0.11 and 95% CI -0.17 to -0.06). After adjusting the results for BMI, age and smoking the ratio of ox-LDL to HDL-cholesterol, concentration of HDL-cholesterol and serum triglycerides remain significant between women with pathological and normal OGTT (p=0.012, p=0.016 and p=0.049, respectively). There were no significant difference between women with pathological and normal OGTT in concentrations of serum total cholesterol, LDL-cholesterol, paraoxonase and the ratio of ox-LDL to LDL-cholesterol (Table 1).

Insulin tertiles

We divided women into three groups based on their serum insulin levels: insulin < 9.40 mU/L (low insulin group), 9.40 – 13.30 mU/L (average insulin group) and >13.30 mU/L (high insulin group) (Table 2). Concentration of serum triglycerides was lower in the low insulin group compared to the high insulin group (coefficient 0.23 and 95% CI 0.14-0.32). The women with low insulin level had significantly higher HDL-cholesterol than the women with high insulin level (coefficient -0.13 and 95% CI -0.19 to -0.07). Concentration of ox-LDL was significantly lower in the low insulin group compared to the high insulin group (coefficient 0.05 and 95% CI 0.01-0.10). The ratio of ox-LDL to HDL-cholesterol was significantly lower in the low insulin group compared to the high insulin group (coefficient 0.14 and 95% CI 0.08-0.19). There were no significant difference between groups in concentrations of serum total cholesterol, LDL-cholesterol, paraoxonase and the ratio of ox-LDL to LDL-cholesterol. After adjusting the results for BMI, age and smoking the difference remained significant between the low insulin and high insulin groups in serum triglycerides and the ratio of ox-LDL to HDL-cholesterol (coefficient 0.14 and 95% CI 0.04-0.25 and coefficient 0.07 and 95% CI 0.00-0.13, respectively), while no other differences were seen in other variables between the insulin groups.

Correlations

The concentration of insulin correlated slightly positively with triglyceride ($r=0.21$, $p<0.0001$), ox-LDL ($r=0.094$, $p=0.020$), and with the ratios of ox-LDL to HDL-cholesterol ($r=0.20$, $p<0.0001$) and ox-LDL to LDL-cholesterol ($r=0.080$, $p=0.047$), and negatively with HDL-cholesterol ($r=-0.20$, $p<0.0001$), while no significant correlations were found with cholesterol, LDL-cholesterol and paraoxonase.

The concentration of 2 h glucose from OGTT correlated positively with triglyceride ($r=0.31$, $p<0.0001$), ox-LDL ($r=0.23$, $p<0.0001$), cholesterol ($r=0.14$, $p=0.004$), LDL-cholesterol ($r=0.16$, $p=0.001$) and with the ratio of ox-LDL to HDL-cholesterol ($r=0.29$, $p<0.0001$), and negatively with HDL-cholesterol ($r=-0.17$, $p<0.0001$), while no significant correlations were found with and paraoxonase and the ratio ox-LDL to LDL-cholesterol.

Discussion

We reported that pathological OGTT and high concentration of serum insulin (>13.3 mU/L) are both associated with elevated level of serum triglyceride and high ratio of oxidized LDL lipids to HDL-cholesterol after adjusting the results for BMI, age and smoking during early pregnancy. To our knowledge, this is the first study to demonstrate that pathological glucose metabolism during early pregnancy is associated with elevated ratio of ox-LDL to HDL-cholesterol. This finding may have clinical significance, because the ratio of ox-LDL to HDL-cholesterol is known to predict coronary artery disease and all-cause mortality (28,29).

Ox-LDL is known risk factor of atherosclerosis and other CVD. On the other hand, HDL-cholesterol and especially the beneficial functions of HDL are known to have cardioprotective effects. This protection includes anti-inflammation, LDL-specific antioxidant activity and upregulation of nitric oxide (30). Further, we recently reported that HDL has an active role in body clearance of lipid peroxides during physiological oxidative stress like post-prandial state and during physical exercise (27). Therefore, the ratio of ox-LDL to HDL-cholesterol may be a suitable marker when measuring cardiometabolic risk associated with conditions like GDM. In the present study, both women with pathological OGTT compared to women with normal OGTT and the women with high insulin level compared to women with low insulin level had significantly higher the ratio of

ox-LDL to HDL-cholesterol. These differences were significant after adjusting BMI, age and smoking. Also, the concentration of HDL-cholesterol differed according to the OGTT. The women with normal OGTT had 7% higher concentration of HDL-cholesterol than women with pathological OGTT. Similarly, the women with low insulin level had 8% higher concentration of HDL-cholesterol than women with high insulin level, although this difference was no more significant after adjusting BMI, age and smoking. These results underline, that altered glucose metabolism already during early pregnancy is associated with atherogenic changes in serum HDL-cholesterol and the ratio of ox-LDL to HDL-cholesterol.

Serum concentration of triglyceride is known to increase during pregnancy (31). In the present study, women with pathological OGTT had 14% higher concentration of triglycerides compared to women with normal OGTT. Similarly, the women with high insulin level had 21% higher concentration of serum triglycerides compared to women with low insulin level. These differences persisted after controlling BMI, age and smoking. The concentration of ox-LDL was higher in women with pathological OGTT compared to the women normal OGTT and similarly in the women with high insulin level compared to women with low insulin level. However, these differences disappeared when BMI, age and smoking were controlled, which suggests that BMI as an important risk factor of abnormalities in glucose metabolism is also strongly associated with concentrations of ox-LDL as also shown in healthy girls and obese men (32,33). Interestingly, the concentration of LDL-cholesterol did not differ neither between women with normal and pathological OGTT nor between women with high and low insulin level. Therefore, in contrast to oxidized LDL lipids, the native LDL-cholesterol seems not be associated with altered glucose metabolism during early pregnancy. These results indicated that altered glucose metabolism (pathological OGTT or high insulin concentration) was associated with elevated level of serum triglycerides and oxidized LDL lipids already during early pregnancy, while serum cholesterol and LDL-cholesterol did not associate with altered glucose metabolism.

Paraoxonase is a HDL –associated enzyme, which is implicated in the lipid metabolism and suggested to play a role in the antioxidant function of HDL. Some earlier studies have measured elevated activities of paraoxonase during pregnancy (34,35). In the present study, the activity of paraoxonase did not differ between the women with pathological and normal OGTT. In the present study, the concentration of paraoxonase did not differ between the women with pathological and normal OGTT. Neither was there any difference in paraoxonase between the tertiles of insulin.

Therefore, paraoxonase related antioxidant activity of HDL is not the obvious mediator of lower ratio of ox-LDL to HDL-cholesterol observed in the present study.

The concentration of insulin correlated strongest with triglyceride (r=0.21), HDL-cholesterol (r=-0.20) and the ratio of ox-LDL to HDL-cholesterol (r=0.20). This is in line with earlier study, where insulin concentration was associated with ox-LDL (r=0.25, $p<0.0001$) in 766 Finnish women aged 24 to 39 years (36). We also investigated how the concentration of 2 h glucose from OGTT correlated with ox-LDL and serum lipids. The strongest correlations were shown against triglycerides (r=0.31), the ratio of ox-LDL to HDL-cholesterol (r=0.29) and interestingly with ox-LDL (r=0.23). These results suggested that concentrations of triglyceride, HDL-cholesterol, ox-LDL and also the ratio of ox-LDL to HDL-cholesterol were the lipid variables that strongest related with the measures of glucose metabolism, insulin and 2 h glucose from OGTT. Therefore, the above mentioned lipid variables might be the key measures when estimating lipid risk factors during early pregnancy.

The current study is based on the quite large sample of women who all underwent also OGTT during early pregnancy. This gives statistical power to the analysis. We were able to handle the known factors (like BMI, age and smoking) that could interfere with the lipid results. Therefore, we consider that the results are reliable.

In conclusion, pathological OGTT and high concentration of serum insulin were associated with elevated level of serum triglyceride and high ratio of oxidized LDL lipids to HDL-cholesterol after adjusting the results for BMI, age and smoking during early pregnancy. Also, strongest correlations between measures of glucose metabolism (concentration of insulin and 2 h glucose from OGTT) and lipids were shown in serum triglyceride, HDL-cholesterol, oxidized LDL and the ratio of oxidized LDL lipids to HDL-cholesterol. The present study showed that pathological glucose metabolism during early pregnancy may be associated with atherogenic lipid profile.

Acknowledgements

This study was funded by Competetive Research Funding of the Tampere University Hospital, Juho Vainio Foundation, Academy of Finland, Ministry of Education and Culture and Ministry of Social Affairs and Health. We thank Tiina Solakivi from University of Tampere for her help in serum lipid analysis.

Table 1. Serum oxidized LDL lipids and serum lipids in women with pathological OGTT and in women with normal OGTT. Means and standard deviation (SD). Regression coefficients (95% confidence intervals) from four regression models.

	OGTT pathological	OGTT normal	Model 1	Model 2	Model 3	Model 4
Cholesterol	4.70 (0.79)	4.65 (0.74)	0.05 (-0.08 to 0.18)	0.05 (-0.11 to 0.22)	0.06 (-0.11 to 0.22)	0.06 (-0.11 to 0.22)
HDL	1.58 (0.30)	1.69 (0.30)	**-0.11 (-0.17 to -0.06)**	**-0.07 (-0.14 to -0.01)**	**-0.07 (-0.14 to -0.01)**	**-0.08 (-0.15 to -0.02)**
LDL	2.52 (0.65)	2.43 (0.58)	0.09 (-0.02 to 0.19)	0.07 (-0.06 to 0.20)	0.08 (-0.05 to 0.21)	0.08 (-0.05 to 0.21)
Triglycerides	1.33 (0.54)	1.17 (0.42)	**0.14 (0.06 to 0.23)**	**0.05 (0.00 to 0.12)**	**0.05 (0.00 to 0.12)**	**0.05 (0.00 to 0.013)**
Paraoxonase	36.4 (21.3)	37.9 (23.2)	-0.81 (-3.70 to 2.80)	-0.90 (-3.22 to 3.92)	-0.57 (-2.46 to 4.76)	-0.39 (-2.33 to 5.16)
Ox-LDL	26.8 (6.1)	25.6 (5.8)	**1.15 (0.13 to 2.25)**	0.66 (-0.31 to 1.90)	0.59 (-0.32 to 1.89)	0.55 (-0.38 to 1.91)
Ox-LDL / LDL	12.4 (2.9)	12.4 (2.7)	-0.02 (-0.46 to 0.46)	-0.17 (-0.67 to 0.48)	-0.22 (-0.70 to 0.47)	-0.26 (-0.75 to 0.45)
Ox-LDL / HDL	17.7 (5.6)	15.7 (4.7)	**1.87 (0.99 to 2.85)**	**0.76 (0.15 to 1.59)**	**0.75 (0.13 to 1.70)**	**0.80 (0.14 to 1.81)**

OGTT oral glucose tolerance test
Model 1: Unadjusted linear regression model
Model 2: Adjusted for body mass index
Model 3: Adjusted for body mass index and age
Model 4: Adjusted for body mass index, age and smoking

Table 2. Serum oxidized LDL lipids and serum lipids in insulin tertiles (low (<9.4 mU/L), average (9.4-13.3 mU/L) and high (>13.3 mU/L) insulin group). Means and standard deviation (SD). Regression coefficients (95% confidence intervals) from four regression models using low insulin group as a reference group.

	Insulin	Mean (SD)	Model 1	Model 2	Model 3
Cholesterol	Low	4.67 (0.79)			
	Average	4.71 (0.76)	0.04 (-0.10 to 0.19)	0.06 (-0.10 to 0.21)	0.07 (-0.09 to 0.22)
	High	4.63 (0.71)	-0.03 (-0.18 to 0.11)	-0.10 (-0.28 to 0.07)	-0.08 (-0.26 to 0.09)
HDL	Low	1.72 (0.31)			
	Average	1.66 (0.29)	**-0.06 (-0.12 to 0.00)**	-0.03 (-0.09 to 0.03)	-0.03 (-0.09 to 0.03)
	High	1.59 (0.30)	**-0.13 (-0.19 to -0.07)**	-0.06 (-0.13 to 0.01)	-0.06 (-0.13 to 0.01)
LDL	Low	2.44 (0.59)			
	Average	2.51 (0.62)	0.07 (-0.05 to 0.18)	0.07 (-0.05 to 0.19)	0.08 (-0.04 to 0.20)
	High	2.43 (0.58)	-0.01 (-0.13 to 0.11)	-0.11 (-0.24 to 0.03)	-0.10 (-0.23 to 0.04)
Triglycerides	Low	1.11 (0.40)			
	Average	1.19 (0.39)	**0.07 (-0.02 to 0.16)**	0.03 (-0.06 to 0.12)	0.04 (-0.05 to 0.13)
	High	1.34 (0.56)	**0.23 (0.14 to 0.32)**	**0.13 (0.03 to 0.23)**	**0.14 (0.04 to 0.25)**
Paraoxonase	Low	39.1 (23.8)			
	Average	36.3 (21.9)	-0.08 (-0.19 to 0.04)	-0.04 (-0.17 to 0.08)	-0.05 (-0.18 to 0.07)
	High	37.2 (22.2)	-0.04 (-0.16 to 0.07)	-0.04 (-0.17 to 0.10)	-0.03 (-0.17 to 0.11)
Ox-LDL	Low	25.3 (6.1)			
	Average	25.8 (5.6)	0.02 (-0.02 to 0.07)	0.02 (-0.03 to 0.06)	0.02 (-0.03 to 0.06)
	High	26.6 (6.0)	**0.05 (0.01 to 0.10)**	0.03 (-0.02 to 0.08)	0.03 (-0.02 to 0.08)
Ox-LDL / LDL	Low	12.3 (2.8)			
	Average	12.2 (2.8)	-0.00 (-0.05 to 0.04)	-0.01 (-0.06 to 0.03)	-0.01 (-0.06 to 0.03)
	High	12.7 (2.6)	0.04 (-0.00 to 0.08)	**0.05 (0.00 to 0.10)**	0.05 (-0.00 to 0.10)
Ox-LDL / HDL	Low	15.2 (4.5)			
	Average	16.1 (4.8)	0.06 (-0.00 to 0.11)	0.03 (-0.03 to 0.09)	0.03 (-0.03 to 0.09)
	High	17.5(5.5)	**0.14 (0.08 to 0.19)**	**0.06 (0.00 to 0.13)**	**0.07 (0.00 to 0.13)**

Model 1: Unadjusted linear regression model
Model 2: Adjusted for body mass index
Model 3: Adjusted for body mass index, age and smoking

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