Changes in plasma lipids and markers of oxidative stress in normal pregnancy and pregnancies complicated by diabetes

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ABSTRACT

The purpose of the present study was to determine changes in plasma lipids and markers of oxidative stress longitudinally in pregnancy complicated by diabetes compared with non-diabetic pregnancy. This was carried out by following a group of normal pregnant women (n = 17) and groups of pregnant women with Type I diabetes (n = 19), Type II diabetes (n = 12) and gestational diabetes mellitus (n = 12) throughout pregnancy, with sampling carried out at the end of each trimester. Serum total cholesterol and triacylglycerols (triglycerides) were determined using standard colorimetric techniques and low-density lipoprotein (LDL) subfraction profile by disc PAGE. Total antioxidant capacity (TAC) was determined by enhanced chemiluminescence and lipid hydroperoxides by the ferrous oxidation of Xylenol Orange method. Total cholesterol and triacylglycerols increased significantly throughout pregnancy in all groups, but there were no significant differences between normal and diabetic women with respect to either. The LDL score was significantly higher (P < 0.001) in diabetic women compared with normal women at each point throughout pregnancy, although there were no significant differences between the diabetic groups. There was evidence of greater oxidative stress in diabetic compared with normal women throughout. Corrected TAC was significantly lower (P < 0.001) in all diabetic women throughout pregnancy. In addition, lipid hydroperoxides were higher in all diabetic compared with normal women, particularly so in those with Type II diabetes (P < 0.05). These changes may have important implications for diabetic women during pregnancy, as an elevated risk of pre-eclampsia is thought to reflect an oxidative stress-related mechanism. In addition, these changes may have important implications for the development of atherosclerosis and the long-term cardiovascular health of women with diabetes.

INTRODUCTION

In recent years, a number of authors have focused attention on the risk of cardiovascular events in women [1–3]. In addition, epidemiological studies have demonstrated a relationship between parity and an increased risk of cardiovascular disease in later life [4,5]. Oxidative stress is being increasingly recognized as an important cardiovascular risk factor in high-risk patient groups [6,7], and is defined as the imbalance between free radical damage (e.g. the oxidation of lipids) and antioxidant protection. The oxidative modification of low-density lipoprotein (LDL) is an important step in the development of atherosclerosis. Smaller dense LDL particles are considered to be more atherogenic, because they have reduced affinity for the native LDL receptor and are,
therefore, retained in the circulation for longer, and they are more susceptible to oxidation, possibly because they contain less intrinsic antioxidants [8,9]. When LDLs are oxidized, they are readily taken up into macrophages, via the scavenger receptor, promoting foam cell formation and the development of atherosclerotic lesions, thereby initiating vascular occlusion and endothelial dysfunction [10].

Lipid metabolism changes during pregnancy [11,12]. The anabolic phase of early pregnancy encourages lipogenesis and fat storage in preparation for rapid fetal growth in late pregnancy [13]. Lipolysis is increased as a result of insulin resistance, leading to increased flux of fatty acids to the liver promoting the synthesis of very-LDLs and increased triacylglycerol concentrations. Because of a decrease in the activity of lipoprotein lipase, very-LDL remains in the plasma for longer and leads to the accumulation of LDL. An increase in LDL is associated with the development of atherosclerosis [14].

We have demonstrated previously [15] that normal pregnancy is associated with the formation of susceptible oxidizable particles (high LDL score) and an increase in oxidative damage. Diabetes is an independent risk factor for the development of coronary artery disease and is also associated with the development of an atherogenic lipid profile and increased oxidative stress. Pregnancy in women with diabetes mellitus may further exaggerate these changes.

To date, no studies have examined the correlation between changes in lipid metabolism found in uncomplicated singleton pregnancies in women with diabetes with any potentially harmful markers of oxidative stress. The present study was designed to examine sequential changes in lipid profile, based on the measurements of total cholesterol, high-density lipoprotein (HDL)-cholesterol and triacylglycerols, and also to quantify the atherogenic small dense LDL subfractions in women with pre-gestational and gestational diabetes mellitus throughout the three trimesters (T1, T2 and T3) of pregnancy. These changes were correlated with markers of oxidative stress, including total antioxidant capacity (TAC), uric acid (an important contributor to TAC) and lipid hydroperoxides (LPHs), which result from the free radical oxidation of polyunsaturated fatty acids [16].

**METHODS**

**Subjects**

Four groups of women were recruited (Table 1). Seventeen healthy pregnant women were recruited at their first antenatal visit, whereas 19 women with Type I diabetes, 12 women with Type II diabetes and 12 women with gestational diabetes mellitus were recruited at their initial appointment at the joint Diabetic Antenatal Clinic. The study was approved by the Local Research Ethics Committee, and all women gave written informed consent. A full medical history was taken including family history of cardiovascular disease and diabetes mellitus, smoking status and alcohol intake.

Non-fasting (2 h post-prandial) venous blood was taken from all women and controls. Because of the tendency towards lower fasting blood glucose in pregnancy, we felt it was unethical to request fasting samples. Blood samples were placed on ice and centrifuged within 2 h of collection. The groups were matched for gestational age and blood was sampled in the last week of each trimester, i.e. weeks 12 (T1), 24 (T2) and 36 (T3).

**Measurement of TAC**

TAC (in μmol/l Trolox equivalent) was determined by enhanced chemiluminescence [17], uric acid (μmol/l) by standard colour spectrophotometry and plasma LHPs (μmol/l) by the ferrous oxidation of Xylenol Orange method [18]. Corrected TAC was calculated by subtracting uric acid values from TAC values (μmol/l).

**Measurement of lipids**

Serum total cholesterol (mmol/l), triacylglycerols (mmol/l) and HDL-cholesterol (mmol/l) were determined using standard colorimetric techniques. LDL.
subfraction profile was determined by disc PAGE [19–22]. This method assesses the relative mobility of each LDL fraction with respect to that of the HDL. Seven different fractions, denoted bands LDL-0 to LDL-6, may be determined by this method. The LDL score was assessed by determining the area under the curve for each of the peaks for each band present in the gel. The higher the score, the greater the number of small dense, potentially atherogenic, LDL particles. An LDL score of < 1.5 is considered to be normal in otherwise healthy adults [19]. Inter- and intra-assay coefficients of variation in this study were all within the accepted values for the stated analysis (i.e. < 5 % and < 10 % respectively).

Statistical analysis
A two-way ANOVA and post-hoc analysis using the least significant difference test were used to compare differences with time (trimester differences) and group differences. Pearson correlation coefficients were calculated for determination of the association between markers of lipid metabolism and oxidative stress. A $P$ value < 0.05 was taken as being statistically significant.

RESULTS

Plasma lipids
Pregnant women with gestational and Type II diabetes had a significantly higher pre-pregnancy body mass index (BMI) (kg/m²) than those in the pregnant control group, but there were no significant age differences (Table 1).

In T1, total cholesterol levels (mmol/l) were similar between diabetic and non-diabetic groups (Figure 1a). Cholesterol increased progressively throughout pregnancy in all groups. The changes in cholesterol in T3 compared with T1 were significant ($P < 0.001$) in normal women, in those with Type I diabetes and those with Type II diabetes. No significant differences were observed in total cholesterol concentrations between diabetic groups at each trimester. HDL-cholesterol concentrations were similar between diabetic and non-diabetic groups throughout pregnancy.

Triacylglycerol concentrations were similar in all groups in T1 and increased in all groups throughout pregnancy (Figure 1b). Triacylglycerol concentrations were significantly higher ($P < 0.001$) in T3 compared with T1 in all groups (Figure 1b). There were no significant differences between groups within each trimester.

The LDL score was significantly higher ($P < 0.001$) in T1 in diabetic women compared with normal women (Figure 1c) and in women with Type I diabetes. This pattern continued in T2 and in T3 (Figure 1c). The LDL scores increased significantly ($P < 0.001$) in all groups from T1 to T3. The LDL score for the diabetic subgroups were comparable throughout pregnancy, suggesting that the type of diabetes had no influence on LDL subfraction profile.

Oxidative stress
Values for TAC were transformed (i.e. log-transformed) before analysis. In all groups, TAC was lower than the normal range quoted for healthy adults (300–460 $\mu$mol/l Trolox equivalent) [17] at all time points of pregnancy. During T1, values were lower in diabetic groups compared with non-diabetic women (Table 2). This pattern continued in T2 and T3 for Type I and Type II diabetes.

If concentrations of uric acid were subtracted from TAC, this gave an indication of the potential change in other contributors to the overall antioxidant capacity (e.g. vitamins A, C, E and thiols), because uric acid is considered to be one of the major determinants of TAC [23]. The log-transformed corrected antioxidant activity ($\mu$mol) was lower in all diabetic groups during pregnancy compared with non-diabetic women (Figure 2a),
Table 2  TAC during each trimester in diabetic and normal pregnancy
Values are means ± S.D. The units for TAC are µmol/l Trolox equivalent.

<table>
<thead>
<tr>
<th>Type of diabetes</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pregnancy</td>
<td>265.5 ± 11.7</td>
<td>285.3 ± 8.6</td>
<td>319.9 ± 12.6</td>
</tr>
<tr>
<td>Type I</td>
<td>263.4 ± 16.9</td>
<td>248.6 ± 13.3</td>
<td>273.2 ± 12.5</td>
</tr>
<tr>
<td>Type II</td>
<td>245.6 ± 23.8</td>
<td>256.0 ± 18.2</td>
<td>264.6 ± 22.3</td>
</tr>
<tr>
<td>Gestational diabetes mellitus</td>
<td>Not applicable</td>
<td>275.8 ± 11.6</td>
<td>294.2 ± 19.5</td>
</tr>
</tbody>
</table>

Figure 2  Corrected antioxidant capacity (a) and LHP concentrations (b)
*P < 0.05 and **P < 0.001 compared with normal pregnancy.

indicating that the presence of a diabetic state has an impact on antioxidant defences.

LHP was transformed (i.e. log-transformed) before analysis. LHP was within the normal range (2–7 µmol/l) for normal women and those with Type I diabetes during T1 (5.4 and 7.0 µmol/l respectively) and T2 (6.1 and 7.2 µmol/l respectively). Those women with gestational diabetes mellitus also had a normal value in T2 (5.6 µmol/l). LHP increased throughout gestation (T1 to T3) in each group (Figure 2b). The values reached in T3 were above the upper limit of normal and comparable with values identified in high-risk people [24]. Absolute values were greater in women with Type II diabetes, and significantly higher (P < 0.05) compared with normal women in T1 and T2, increasing further in T3, a value almost twice the upper limit of normal (2–7 µmol/l; Figure 2b). In normal women, LHP was correlated significantly with LDL score (P < 0.01), triacylglycerols (P < 0.01) and total cholesterol (P < 0.01). There was no correlation between LHP and these parameters in women with diabetes.

**DISCUSSION**

The present study has confirmed that, during pregnancy, there are changes in serum cholesterol, triacylglycerols and LDL subfractions, which are normally associated with an increased risk for coronary artery disease. Furthermore, these changes were paralleled by evidence of increased oxidative stress, as demonstrated by a rise in LHPs and a decrease in TAC. The absolute values for LHP throughout pregnancy were greater in women with diabetes. Oxidative stress is associated with atheroma formation and coronary artery disease [7].

Collection of samples were taken in the non-fasting state as we felt it inappropriate to ask pregnant women to fast, because of the tendency towards morning hypoglycaemia. To minimize variability in plasma lipids, all samples were taken in the morning within 2 h of breakfast. This may have had an impact on triacylglycerol values in particular. However, both control and diabetic subjects encountered the same sampling conditions. Ideally, the groups should have had the same BMIs. BMIs will, however, vary in all individuals throughout pregnancy and may not be the same in all groups at each gestational age. In the present study, BMI was similar between controls and women with Type I diabetes, but was significantly greater in those with Type II diabetes and gestational disease. The greater BMI is a reflection of the disease process and may have influenced the lipid values, with one expecting statistically higher values in those with greater BMI. On the contrary, women with diabetes show a gradual increase in total cholesterol and triacylglycerol concentrations during pregnancy, but the changes were not significantly different from those observed in normal pregnancy, suggesting that they are independent of diabetic status and are simply a manifestation of pregnancy. This is in agreement with the findings of Hollingsworth and Grundy [25], who showed that normal pregnant women and those with Type I diabetes have no significant differences in cholesterol, triacylglycerols or any lipoprotein subfraction. Similarly, Montelongo et al. [26] demonstrated progressive increases in plasma triacylglycerol and cholesterol levels with gestational age, but failed to show differences between normal women and those with Type I diabetes.
Women with gestational diabetes mellitus had significantly increased triacylglycerols in T2 compared with normal pregnancy (2.27 ± 0.22 compared with 1.47 ± 0.12; \( P < 0.01 \)), suggesting that the expected hypertriglyceridaemia occurs earlier in pregnancy in this group. These observations are in contrast with findings by Koukkou et al. [27], who found the increase in LDL-cholesterol appears to be suppressed during pregnancy in gestational diabetes mellitus, leading to lower total cholesterol than in normal pregnancy.

The LDL subfraction profile follows different patterns in normal and diabetic pregnancy. In normal pregnancy, LDL subfractions are within the normal range in early pregnancy and rise throughout late pregnancy. By contrast, women with diabetes already have an established higher LDL profile in T1, which rises progressively throughout pregnancy and is greater than in the control group at all times. Diabetes type appears to have no impact on LDL score, as no differences were observed between the diabetes groups. This suggests that adverse modification of LDL is a characteristic of diabetes, since LDL score is elevated in the absence of high triacylglycerol concentrations.

Several differences in markers of oxidative stress were observed between normal and diabetic pregnancy. TAC is lower than the normal range for healthy female adults (381 ± 80) in both normal and diabetic pregnancies during T1 and is also significantly different between diabetic and normal women at this time (\( P < .001 \)). In normal pregnancy, TAC concentrations increase up to T3 (\( P < 0.05 \)). This increase in TAC is due mostly to an increase in uric acid concentrations, because the corrected antioxidant activity remained unchanged once this parameter was subtracted. In contrast, the corrected antioxidant activity in all diabetic groups was significantly reduced compared with controls (\( P < 0.001 \)). Thus, in diabetes, factors other than the changes in uric acid may be determining the apparent decrease in antioxidant defences as expressed by the reduced corrected antioxidant activity. Other major contributors to TAC are vitamins C and E. One can speculate that in diabetes these might be reduced, but without measuring these markers directly one cannot be certain. It has been demonstrated that non-pregnant patients with Type I diabetes have a lower total antioxidant activity and low levels of vitamin C [28]. Impaired antioxidant activity and low levels of vitamins C and E have been shown in women with severe pre-eclampsia [29,30].

The reduction of antioxidant levels may be linked to an increase in free-radical-mediated lipid peroxidation. Our present findings in normal pregnancy of elevated LHP concentrations allow us to confirm observations made by others [31] that oxidative damage is higher in healthy pregnant women than in non-pregnant women. In addition, differences in LHP concentrations between women with diabetes and those without were observed from T1. The difference was significantly greater in women with Type II diabetes (\( P < 0.05 \)). This suggests that these changes are perhaps not pregnancy related and oxidative stress is present in Type II diabetic subjects before pregnancy [30]. Nourooz-Zadeh et al. [32] observed that elevated LHP concentrations were independent of abnormalities in lipid metabolism and glycaemic control. In agreement, the present study has shown high LHP concentrations during T1 when triacylglycerol levels are within the normal range and no correlation of LHP with cholesterol or triacylglycerols in women with diabetes. Furthermore, glycaemic control, as assessed by glycated haemoglobin, was within the accepted ranges and did not alter significantly during pregnancy.

In conclusion, we have confirmed that diabetic pregnancy is associated with changes in lipid metabolism with a shift towards greater small dense LDL subfractions when compared with non-diabetic pregnancy. The observed changes are more severe in women with Type II diabetes. The present study has also demonstrated increased levels of LPHs and a reduction in antioxidant activity, again more marked in those with Type II diabetes. These associations may have important implications for diabetic women during pregnancy as an elevated risk of pre-eclampsia is thought to reflect an oxidative-stress-related mechanism [29]. Indeed, there are on-going clinical trials of antioxidants in women at high risk of pre-eclampsia, which includes diabetic women. In addition, these associations may have important implications for the development of atherosclerosis and the long-term cardiovascular health of women with diabetes, especially those with Type II disease. Epidemiological evidence already links the latter with a high risk of a cardiovascular event and a worse prognosis in women compared with men.

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