

# Serum lipid, lipoprotein and apolipoprotein changes in gestational diabetes mellitus: a cross-sectional and prospective study

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## Abstract

**Aims**—To compare serum lipid, lipoprotein and apolipoprotein concentrations during and six to 12 months after pregnancy in control and diabetic women.

**Methods**—The serum lipid, lipoprotein and apolipoprotein concentrations were measured in 20 women with gestational diabetes mellitus (GDM) and 22 women with normal glucose tolerance (controls) during the third trimester of pregnancy and six to 12 months after delivery.

**Results**—During pregnancy the women with GDM had higher serum triglyceride (mean (95% confidence interval (CI)), 2.91 (2.22–3.51) v 2.1 (1.75–2.52)) but lower low density lipoprotein (LDL) cholesterol concentrations compared with controls (mean (SD), 3.08 (1.2) v 4.01 (1.1)). Total cholesterol, high density lipoprotein (HDL) cholesterol and apolipoprotein concentrations were not significantly different between the two groups. After pregnancy, total cholesterol, HDL cholesterol, triglyceride, and apolipoprotein A1 and B decreased in a parallel manner, resulting in lower concentrations, comparable between the two groups. LDL cholesterol concentrations decreased after pregnancy in the controls (mean (SD), 4.01 (1.1) v 2.69 (0.6)) but not in those with GDM (3.08 (1.2) v 2.72 (0.7)). The change in lipid concentrations was not related to change in weight.

**Conclusion**—Development of diabetes during pregnancy induces a state of dyslipidaemia characterised by elevated triglyceride concentrations, as seen in other insulin resistance states. However, GDM seems to blunt the increase in LDL cholesterol during pregnancy and this requires further investigation. Whether the changes in lipoprotein metabolism in GDM are significant for the health status of the mother and the foetus requires further study.

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Change in lipoprotein metabolism during pregnancy is well established and is reflected by increased plasma concentrations of triglycerides, cholesterol and apolipoproteins.<sup>1-3</sup> Diabetes mellitus is known to induce dyslipo-

proteinaemia<sup>4,5</sup> and, therefore, it may be hypothesised that the emergence of diabetes during pregnancy may further alter lipoprotein metabolism. Although previous studies have shown that fasting hypertriglyceridaemia is accentuated and high density lipoprotein (HDL) cholesterol concentrations lower in diabetic pregnancies,<sup>6-8</sup> other investigators were recently unable to demonstrate differences in serum lipid, lipoprotein and apolipoprotein concentrations between normal and diabetic women.<sup>9</sup> Moreover, there are still very few prospective studies and relatively little is known about the changes in lipid metabolism during pregnancy complicated by gestational diabetes mellitus (GDM).<sup>10</sup>

In this study we examined the changes in lipid, lipoprotein and apolipoprotein concentrations during and six to 12 months after pregnancy in women with GDM and in controls with normal glucose tolerance.

## Methods

Twenty women in the third trimester of pregnancy, who presented for an oral glucose tolerance test and in whom GDM was diagnosed, were studied. The control group consisted of 22 women who also presented for screening for GDM and who had a normal glucose tolerance test. Both groups were studied contemporaneously. Because of the known differences in lipid, lipoprotein and apolipoprotein concentrations between different ethnic groups<sup>11</sup> and because our patients were racially heterogeneous (Caucasians, Asians and Africans/Afrocaribbeans), we matched the two groups for ethnicity. All subjects were on an ad libitum diet prior to testing. Fasting venous blood was drawn with minimal stasis for measurement of serum total cholesterol, HDL cholesterol, triglycerides, and apolipoproteins A1 and B (Apo A1 and B, respectively), followed by the 75 g oral glucose tolerance test. Venous blood samples for plasma glucose were drawn after 60 and 120 minutes. Gestational diabetes was diagnosed according to the European Association for the Study of Diabetes (EASD) criteria,<sup>12</sup> namely a plasma glucose concentration  $\geq 9$  mmol/l 120 minutes after the 75 g oral glucose load. Maternal age, gestational age, parity, height, and weight were recorded on the day of the test. Body mass index (BMI = kg/m<sup>2</sup>) was derived for each subject. All women attended for a follow up visit 6 to 12 months after delivery and a fasting blood sample was drawn for

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**Table 1** Clinical characteristics of women with GDM and with normal glucose tolerance (controls) during pregnancy. Results are expressed as mean (SD)

Clinical variables	GDM (n = 20)	Controls (n = 22)	p value
Age (years)	30.5 (4.4)	29.9 (5.1)	0.7
BMI (kg/m <sup>2</sup> )	32.2 (5.2)	29.7 (5.4)	0.1
Gestational age (weeks)	31.3 (3.4)	30.0 (3.4)	0.24
Parity	3.0 (1.4)	3.4 (2.2)	0.48
HbA1 (%)	6.6 (0.7)	6.0 (0.7)	< 0.01

measurement of blood glucose, total cholesterol, HDL cholesterol, triglyceride, and Apo A1 and B concentrations. Total serum cholesterol and triglyceride concentrations were measured by enzymatic analysis on a DAX-96 analyser (Bayer Diagnostics, Basingstoke, UK): interassay coefficient of variation (CV) for cholesterol and triglycerides < 1.9% and < 2.8%, respectively. HDL cholesterol was measured after dextran sulphate/magnesium chloride precipitation of Apo B containing lipoproteins (Bayer Diagnostics): interassay CV < 6.4%. Apolipoproteins A1 and B were assayed by immunoturbidimetry using antiserum from Immuno (Sevenoaks, UK): interassay CVs < 2.5% and < 1.5%, respectively. These analyses were performed on Cobas Fara or Cobas Bio analysers (Roche Diagnostic Systems, Welwyn Garden City, UK). LDL cholesterol was calculated by the Friedewald equation, excluding specimens with triglyceride concentrations higher than 4.5 mmol/l (three women with GDM and one control).<sup>13</sup> The ratios of LDL:HDL cholesterol and LDL cholesterol:Apo B were calculated. The change in the lipid concentrations ( $\delta$ ) was calculated as the difference between the pregnant minus non-pregnant value. Triglyceride concentrations were log-transformed owing to their positively skewed distributions. Glycosylated haemoglobin (HbA1) was measured using an automatic high pressure liquid chromatography method (interassay CV 2.8%, normal range 5.4–7.4%).

**Table 2** Serum lipid, lipoprotein and apolipoprotein concentrations in women with GDM and normal glucose tolerance (controls) during pregnancy. Results are expressed as mean (SD)

	GDM (n = 20)	Controls (n = 22)	p value
Total cholesterol (mmol/l)	6.23 (1.6)	6.71 (1.0)	0.25
HDL cholesterol (mmol/l)	1.71 (0.4)	1.72 (0.3)	0.97
LDL cholesterol (mmol/l)	3.08 (1.2)	4.01 (1.1)	< 0.01
Triglycerides* (mmol/l)	2.92 (2.22–3.51)	2.10 (1.75–2.52)	< 0.05
Apolipoprotein A1 (g/l)	1.93 (0.2)	1.88 (0.3)	0.61
Apolipoprotein B (g/l)	1.30 (0.4)	1.14 (0.4)	0.22
LDL:HDL cholesterol	1.77 (0.6)	2.35 (0.8)	0.017
LDL cholesterol:Apo B	0.94 (0.25)	1.19 (0.22)	< 0.01

\*Geometric mean (95% CI).

**Table 3** Serum lipid, lipoprotein and apolipoprotein concentrations in women with GDM and normal glucose tolerance (controls) at follow up. Results are expressed as mean (SD)

	GDM (n = 20)	Controls (n = 22)	p value
Total cholesterol (mmol/l)	4.64 (0.7)	4.43 (0.8)	0.38
HDL cholesterol (mmol/l)	1.30 (0.3)	1.22 (0.3)	0.86
LDL cholesterol (mmol/l)	2.72 (0.7)	2.69 (0.6)	0.22
Triglycerides* (mmol/l)	1.16 (0.9–1.5)	0.94 (0.7–1.2)	0.2
Apolipoprotein A1 (g/l)	1.07 (0.3)	0.94 (0.1)	0.09
Apolipoprotein B (g/l)	0.81 (0.2)	0.73 (0.1)	0.14
LDL:HDL cholesterol	2.23 (1.0)	2.36 (0.8)	0.67
LDL cholesterol:Apo B	1.30 (0.16)	1.46 (0.16)	< 0.01

\*Geometric mean (95% CI).

#### STATISTICAL ANALYSIS

Comparisons were carried out using the paired and unpaired *t* tests and values were expressed as mean (1 SD) or mean with 95% confidence intervals (CI) for log transformed data. Analysis of covariance was used to test the correlation between triglyceride concentrations and body weight and the correlation between lipid, lipoprotein and apolipoprotein concentrations during and after pregnancy.

#### Results

The two study groups were of similar age, gestational age, parity, and BMI but as expected the GDM group had higher fasting blood glucose and mean HbA1 concentrations during pregnancy (table 1). The proportion of Caucasians, African/Afrocaribbeans and Asians in our groups was approximately 35, 40 and 25%, respectively. When retested, all women had normal fasting blood glucose concentrations (mean (SD) GDM, 5.4 (0.3); controls, 5.2 (0.3) mmol/l).

#### COMPARISON OF WOMEN WITH AND WITHOUT GDM DURING PREGNANCY

The serum lipid, lipoprotein and apolipoprotein concentrations and the ratios of these variables are given in table 2. Women with GDM had higher triglyceride, but lower LDL cholesterol concentrations compared with controls. No significant difference was found between the HDL cholesterol, and Apo A1 and B concentrations in the two groups.

Total cholesterol concentrations were lower in the women with GDM; however, this difference did not reach significance. The LDL cholesterol:Apo B and LDL:HDL cholesterol ratios were significantly different between diabetic women and controls.

#### COMPARISON OF WOMEN WITH AND WITHOUT GDM SIX TO 12 MONTHS AFTER DELIVERY

Table 3 shows the lipid, lipoprotein and apolipoprotein concentrations and the ratios of these variables. No difference was found in the BMI (mean (SD) 28.7 (4.8) *v* 27.6 (4.5)) and the lipid, lipoprotein and apolipoprotein concentrations between the women who previously had GDM and the women with normal glucose tolerance during pregnancy; triglyceride concentrations tended to be higher in the women who had had GDM. The LDL cholesterol:Apo B ratio remained significantly different between the two groups, whereas the LDL:HDL cholesterol ratio was similar.

#### COMPARISON OF THE LIPID PROFILES DURING AND AFTER PREGNANCY FOR EACH GROUP

There was a significant reduction in total cholesterol, HDL cholesterol, triglyceride, and Apo A1 and B concentrations after pregnancy in both groups (tables 2 and 3); the extent of the reduction for each variable was related to the pregnancy concentrations. The exception to this was LDL cholesterol concentrations in women with GDM, which were not significantly reduced after pregnancy (fig 1).

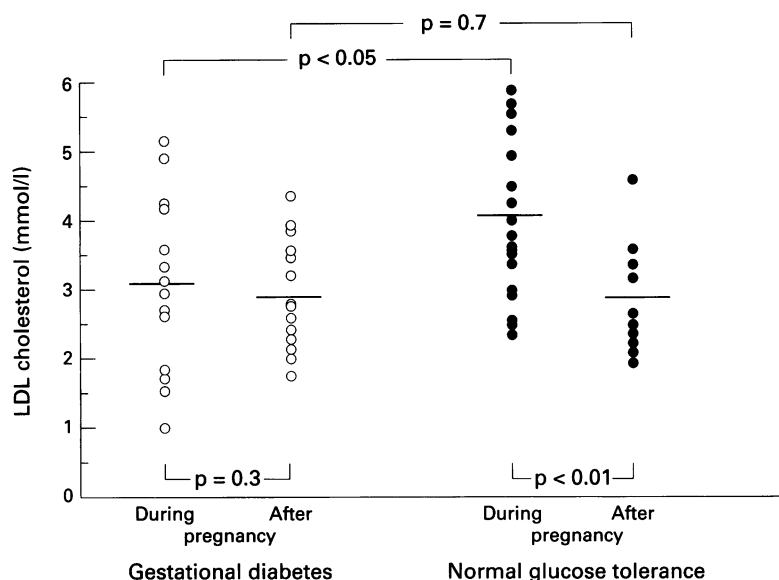


Figure 1 Change in plasma LDL cholesterol concentrations during and after pregnancy in women with GDM ( $\circ$ ) and women with normal glucose tolerance ( $\bullet$ ). Horizontal bars: mean values, *p* values from paired/unpaired *t* test.

### Discussion

This prospective study shows that in pregnancy, women with GDM have significantly higher serum triglyceride but lower LDL cholesterol concentrations than women with normal glucose tolerance, after adjusting for age, BMI, parity, and ethnic group. There was also a significant difference in the LDL cholesterol:Apo B ratio between the two groups. After pregnancy the difference in serum lipid and lipoprotein concentrations between the two groups only persisted for the LDL cholesterol:Apo B ratio, which remained significantly lower in women who had had GDM.

Gestational diabetes has been shown to alter lipid metabolism. Knopp *et al*<sup>6</sup> studied 22 women with GDM and 38 controls in the third trimester of pregnancy. They found increased plasma concentrations of triglycerides and very low density lipoprotein (VLDL) in the former, but no difference in plasma cholesterol concentrations. Their results are in agreement with those of Metzger *et al*<sup>8</sup> and Hollinworth and Grundy,<sup>7</sup> who also reported lower serum LDL cholesterol concentrations in women with GDM compared with controls. More recently, however, Montelogo *et al*<sup>9</sup> did not find differences in serum lipid, lipoprotein or apolipoprotein concentrations between pregnant women with or without GDM. The discrepancies among studies may be the result of false negative tests of the null hypothesis in the studies with smaller sample sizes, as well as other methodological differences.

High serum oestrogen concentrations and increasing insulin resistance in late pregnancy are considered to be responsible for the hypertriglyceridaemia observed during "normal" pregnancy. In women with GDM increased insulin resistance may account for a further rise in triglyceride concentrations, as reported elsewhere.<sup>6-8</sup> We cannot, however, fully explain the lower serum LDL cholesterol concentrations in women with GDM. Insulin resistance is associated with decreased LDL catabolism

and a rise in plasma LDL cholesterol concentration, but the increased, direct removal of triglyceride enriched VLDL may lead to decreased production of LDL.<sup>4</sup> The total pool of LDL may be further decreased by the effect of hyperoestrogenaemia on its catabolism.<sup>14</sup> It has recently been pointed out<sup>15</sup> that the Friedewald equation should not be used in non-insulin dependent diabetes mellitus, because there are compositional changes in VLDL that result in overestimation of LDL cholesterol. If this also applies to GDM, we would expect that the women with GDM in the present study would have an even lower LDL cholesterol concentration. Plasma Apo B concentrations were comparable between women with and without GDM, but the LDL cholesterol:Apo B ratio was lower in those with GDM, suggesting a difference in particle composition, women with GDM having LDL which is cholesterol depleted and triglyceride enriched. Smaller and denser LDL particles with decreased numbers of cholesterol ester molecules per particle have been reported in patients with non-insulin dependent mellitus and impaired glucose tolerance,<sup>16</sup> as well as in women during the third trimester of pregnancy.<sup>17</sup> The precise mechanism by which small, dense LDL arises is not known; there is evidence that LDL size correlates negatively with plasma triglyceride content<sup>18,19</sup> and plasma concentrations of insulin.<sup>16</sup> After pregnancy, there was a trend for serum triglyceride concentrations to remain high and the LDL cholesterol:Apo B ratio remained significantly lower in the women who had had GDM, indicating that the underlying cause of these abnormalities, although less prominent, persisted.

Our study has potential limitations. Firstly, the relatively small sample size may have failed to detect significant differences in lipid concentrations after pregnancy. Secondly, our inference that changes in lipoprotein concentration are the result of compositional changes were not based on direct measurements of lipoprotein chemical composition. It would have been more appropriate to measure the chemical composition or size of lipoproteins using ultracentrifugation or electrophoresis. However, we consider that our findings regarding LDL cholesterol concentrations are valid, given that measurement of LDL cholesterol concentrations by ultracentrifugation, in a different group of women with and without GDM, yielded similar results (unpublished observations). We have also not provided evidence of the underlying mechanisms of abnormal lipoprotein metabolism and it would have been useful to measure oestrogen, insulin and free fatty acid concentrations. Stable isotope kinetic studies of lipid metabolism in GDM would be of considerable interest. Finally, we have not assessed dietary lipid intake, but as the patients in the present study received similar dietary advice we do not anticipate this to be a major confounding affect.

In conclusion, our findings indicate that GDM affects lipid and lipoprotein metabolism. The implications of our findings remain

to be established. The smaller and more dense LDL particles are associated with increased risk of cardiovascular disease<sup>17 19</sup> and are susceptible to oxidative modification which could in turn impair the physiology of nitric oxide<sup>20</sup> and potentially placental blood flow. Women with GDM are also at increased risk of developing frank diabetes later in life, which is known to eliminate or at least to attenuate the well known protection against ischaemic heart disease observed in women.<sup>21</sup> Finally, although the lipid and lipoprotein changes are quite subtle and of short duration they may increase the risk of cardiovascular disease, especially with multiple pregnancies.<sup>22</sup>

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