

# Serum lipids, lipoproteins and apolipoproteins in pregnant non-diabetic patients

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## Abstract

**Aims**—To investigate the effect of pregnancy on serum concentrations of lipids, lipoproteins, and apolipoproteins.

**Methods**—Fasting serum concentrations of total cholesterol, triglyceride, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), apolipoproteins AI, AII, and B, and lipoprotein (a) were measured in 178 women with normal glucose tolerance in the second and third trimesters of pregnancy and in a control group of 58 non-pregnant women of similar age. Data were analysed using the unpaired *t* test and by one-way analysis of variance.

**Results**—The pregnant women had significantly higher concentrations of total cholesterol, triglyceride, LDL cholesterol, HDL cholesterol, and apolipoproteins AI and B ( $p < 0.001$ ) and apolipoprotein AII ( $p = 0.003$ ) than the control women. The ratio of apolipoprotein B:apolipoprotein AI was significantly higher in the pregnant women than in the controls ( $p < 0.001$ ), but the total cholesterol:HDL cholesterol ratio was not significantly different. No significant difference was found in the concentration of lipoprotein (a).

**Conclusions**—Hyperlipidaemia is common in the second half of pregnancy. This may be a purely physiological response to pregnancy or it may be indicative of pathology in some women. These results warrant a follow up study to investigate whether the hyperlipidaemic response to pregnancy is variable and if so, whether it can predict future hyperlipidaemia in a manner analogous to that of impaired glucose tolerance during pregnancy, predicting non-insulin dependent diabetes in later life.

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Hyperlipidaemia is a well established risk factor for coronary heart disease (CHD) in both men and women.<sup>1-4</sup> Although pregnancy has been shown to increase serum total cholesterol and triglyceride,<sup>5-10</sup> its effect on apolipoproteins and lipoprotein (a) (Lp(a)) has not been adequately characterised. Moreover, the prevalence and extent of these alterations and the underlying mechanisms have not been fully documented. In this preliminary report we present the results of lipid, lipoprotein, and apolipoprotein measure-

ments performed on 178 healthy women in the second and third trimesters of pregnancy and compare these with those of non-pregnant women of similar age.

## Methods

Fasting venous blood was collected from 178 women presenting consecutively for oral glucose tolerance tests in the second and third trimesters of pregnancy. All women had a normal glucose tolerance (two hour capillary plasma glucose of  $< 9.0$  mmol/l following a 75 g oral glucose load).<sup>11</sup> The women attended for glucose tolerance tests for one or more of the following reasons: family history of diabetes ( $n = 80$ ), glycosuria ( $n = 60$ ), previous large baby ( $n = 31$ ), polyhydramnios ( $n = 22$ ), large for dates ( $n = 20$ ), obesity ( $n = 14$ ), previous gestational diabetes ( $n = 8$ ), previous stillbirth ( $n = 20$ ), recent miscarriage ( $n = 3$ ) and hypertension ( $n = 2$ ). No patient had clinical proteinuria or other evidence of secondary hyperlipidaemia.

The control group consisted of 58 non-pregnant female volunteers of similar age who were not taking oral contraceptives or receiving lipid-lowering treatment and who were not known to have diabetes. All subjects were in the sitting position for venesection which was carried out with minimal stasis. All women were consuming an unrestricted diet before the test.

Serum was analysed for cholesterol and triglyceride using enzymatic analysis on a DAX-96 analyser (Bayer Diagnostics, Basingstoke, Hants) (interassay CV of  $< 1.9\%$  and  $< 2.8\%$ , respectively). HDL cholesterol was analysed after dextran sulphate/magnesium chloride precipitation of apolipoprotein B containing lipoproteins (Bayer Diagnostics) (interassay CV of  $< 6.4\%$ ). Apolipoproteins AI, B and Lp(a) were assayed by immunoturbidimetry using antisera from Immuno (Immuno Ltd., Sevenoaks, Kent) (interassay CVs of  $< 2.5\%$ ,  $< 1.5\%$ , and  $6.1\%$ , respectively) and apolipoprotein AII by immunoturbidimetry using antisera from Boehringer (Boehringer Mannheim, Lewes, Sussex) (interassay CV of  $< 1.3\%$ ). These analyses were performed on Cobas Fara and Cobas Bio analysers (Roche Diagnostic Systems, Welwyn Garden City, Herts). LDL cholesterol was calculated using the Friedewald equation,<sup>13</sup> excluding specimens with triglyceride of  $> 4.5$  mmol/l.

Logarithms were taken of the data for

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triglyceride and Lp(a) to correct for positively skewed distributions. The mean concentrations obtained for the two groups were compared using the unpaired *t* test. One-way analysis of variance (ANOVA) was performed to detect differences in the concentrations of the lipids, lipoproteins, and apolipoproteins with advancing gestational age. Four women were excluded from ANOVA due to the gestational age being unavailable. The data were also analysed by multiple linear regression to take both subject age and gestational age into account.

### Results

Table 1 gives the clinical characteristics and laboratory results for the pregnant and control groups. With the exception of Lp(a) the lipid, lipoprotein, and apolipoprotein concentrations were significantly higher in the pregnant women than in the controls. The pregnant women had mean concentrations of total cholesterol, HDL cholesterol, LDL cholesterol and apolipoprotein AI which were about 30% greater than in the controls. The concentration of apolipoprotein B was 60% greater than in the controls. The most striking increase was that of triglyceride which was almost threefold greater in the pregnant women.

The percentage of pregnant women with concentrations of total cholesterol above 5.2, 6.5, and 7.8 mmol/l was 87%, 63%, and 20%, respectively, compared with 24%, 0%, and 0% for the controls. Forty four per cent of the pregnant women, but only three per cent of the controls, had concentrations of LDL cholesterol above 4.0 mmol/l. These concentra-

tions are associated with an increased risk of CHD and are the treatment levels recommended by the British Hyperlipidaemia Association.<sup>14</sup>

Table 2 shows the mean concentrations of lipids, lipoproteins, and apolipoproteins at different stages of gestation. There was little change in the concentration of HDL cholesterol and its principal apolipoprotein, AI, after weeks 21–26. The concentrations of total cholesterol, LDL cholesterol, and apolipoprotein B peaked between weeks 34 and 36. Thereafter, there was a slight decrease to term. The concentration of triglyceride continued to increase throughout gestation. One-way analysis of variance showed that the concentrations of total cholesterol, triglyceride, apolipoprotein AI and apolipoprotein B differed significantly between the different gestational age bands. Interpretation of data after 36 weeks is difficult because of the small number of subjects. Multiple linear regression analysis showed that the women's age was not related to the mean concentrations in the pregnant women after taking gestational age into account. After taking age into account, only apolipoprotein B and triglyceride showed a significant trend throughout gestation ( $p = 0.03$  and  $p < 0.001$ , respectively).

### Discussion

This study shows that increases in serum lipids are common during the second half of pregnancy. Many pregnant women had serum concentrations of total cholesterol which in non-pregnant women would be associated

Table 1 Clinical characteristics and mean (SD) fasting lipid and lipoprotein concentrations in pregnant women and controls with 95% confidence intervals for the difference between mean values

	Pregnant (n = 178)	Non-pregnant (n = 58)	Unpaired <i>t</i> test ( <i>p</i> value)	95% CI for difference
Age (years)	28.7(5.2)	31.2(5.9)	0.009	(0.64, 4.31)
Weight (kg)	77.9 (16.6)	71.1 (19.7)	0.02	(-12.6, -0.9)
Gestation (weeks)	29.9 (4.1)			
Total cholesterol (mmol/l)	6.83(1.4)	4.72(0.75)	<0.001	(-2.4, -1.82)
Triglyceride (mmol/l)*	2.08(1.33, 2.24)	0.85(0.77, 0.95)	<0.001	(0.36, 0.47)
HDL cholesterol (mmol/l)	1.91(0.46)	1.36(0.32)	<0.001	(-0.65, -0.43)
LDL cholesterol (mmol/l)	3.90(1.30)	2.90(0.62)	<0.001	(-1.26, -0.74)
Apo B (g/l)	1.28(0.32)	0.84(0.16)	<0.001	(-0.51, -0.38)
Apo AI (g/l)	1.95(0.29)	1.44(0.22)	<0.001	(-0.59, -0.45)
Apo AII (g/l)	0.59(0.09)	0.55(0.09)	0.003	(-0.07, -0.016)
Lp (a) (g/l)*	0.25(0.22, 0.29)	0.18(0.13, 0.25)	0.08	(0.50, 1.04)
Total cholesterol/ HDL cholesterol	3.80(1.27)	3.60(0.85)	0.19	(-0.49, 0.10)
Apo B/apo AI	0.67(0.20)	0.59(0.20)	<0.001	(0.03, 0.12)

Mean (SD), \*geometric mean with 95% confidence interval calculated from log transformed data. The final column shows the confidence interval for the ratio of the geometric means.

Table 2 Mean concentrations of lipid, lipoprotein, and apolipoproteins in controls and in pregnant women at different periods of gestation

Gestational age (weeks)	n	Total cholesterol (mmol/l)	Triglyceride (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	Apo B (g/l)	Apo AI (g/l)	Apo AII (g/l)	Lp(a) (g/l)
Controls	58	4.72	0.85	1.36	2.90	0.84	1.44	0.55	0.18
Pregnant women									
<20	7	5.58	1.41	1.73*	3.21	1.07	1.73*	0.56	0.24
21–26	10	6.27*	2.03*	2.09*	3.26	1.14*	1.95*	0.64	0.26
27–30	84	6.74*	2.08*	1.93*	3.96*	1.24*	1.96*	0.58	0.35
31–33	41	7.15*	2.51*	1.91*	4.05*	1.36*	1.95*	0.58	0.39*
34–36	24	7.29*	2.85*	1.79*	4.12*	1.42*	1.93*	0.63	0.40*
>37	8	6.90*	2.99*	2.06*	3.80	1.30*	2.30*	0.60	0.33
Overall <i>p</i> value by ANOVA		0.04	<0.001	0.45	0.39	0.03	0.02	0.05	0.38

ANOVA results show differences in the concentrations with advancing gestational age. Results which are significantly different from the mean of the control group by the unpaired *t* test ( $p < 0.05$ ) are asterixed.

with an increased risk of CHD. We have also shown that the concentrations of LDL and HDL cholesterol, and of their corresponding apolipoproteins B, and AI and AII, respectively, are increased in pregnancy. A novel finding was that the concentration of Lp(a) was not significantly increased during pregnancy.

The increases in total and LDL cholesterol and triglyceride are similar to those reported by others,<sup>3-10</sup> but the increase in HDL cholesterol has not been widely observed. Our results for HDL cholesterol agree with those of Desoye *et al*<sup>7</sup> and with those of Piechota and Staslewski.<sup>10</sup> Other workers have reported little change in HDL cholesterol during pregnancy.<sup>8,9</sup> The lack of response of Lp(a) to pregnancy suggests that this protein is controlled by different metabolic mechanisms to those of the other lipoproteins and apolipoproteins. This is compatible with studies in non-pregnant subjects showing that Lp(a) is unaffected by hormones and drugs known to influence the metabolism of the major lipoproteins.<sup>15,16</sup> High plasma concentrations of endogenous oestrogens are well known to lower LDL cholesterol, but do not affect plasma Lp(a) concentrations.<sup>17</sup> Our results for Lp(a) differ from those of Desoye *et al*, who in a longitudinal study reported peak increases for Lp(a) of 200%.<sup>18</sup> This discrepancy may have been due to differences in the selection of patients and in study design.

Our study does have certain limitations. Firstly, the subjects were not observed longitudinally. Secondly, the pregnant group was highly selected, the subjects being recruited from women presenting for oral glucose tolerance tests. The results may therefore not be representative of the general population. However, women with impaired glucose tolerance were excluded from the study, and although the pregnant women may have been consuming a high carbohydrate diet which could account for the higher concentration of triglycerides, we are not aware that the nutrient intake varied significantly between the pregnant and control groups. Thirdly, recruitment from these patients has led to a clustering of subjects between the gestational ages of 27 and 33 weeks, thereby restricting the examination of the effects of gestational age. Fourthly, the concentration of LDL cholesterol was obtained by calculation, which assumes that the composition of lipoproteins in pregnancy is the same as in normal metabolic states. The calculated value also includes any IDL cholesterol. Finally, the subjects were not matched for the degree of obesity. These limitations could be overcome by a longitudinal study in randomly selected pregnant women with measurement of LDL and other cholesterol fractions by ultracentrifugation.

The mechanism whereby pregnancy induces hyperlipidaemia has not been fully elucidated. The complementary and opposing actions of the individual pregnancy hormones and their changing concentrations during pregnancy would be expected to lead to pro-

nounced alterations in lipoprotein metabolism as gestation progresses. Desoye *et al* found a positive correlation between changes in the lipid and lipoprotein concentrations and the changes in the concentrations of the pregnancy hormones oestradiol, progesterone, and human placental lactogen (HPL) during gestation.<sup>7</sup> Triglyceride was also positively correlated with increasing concentrations of insulin in the second half of gestation.

Oestrogen seems to be responsible for most of the alterations in lipoprotein metabolism during pregnancy, but its actions are complemented and opposed by the other pregnancy hormones, and in late pregnancy by increasing insulin resistance. Oestrogens can increase the concentration of plasma triglyceride by stimulating hepatic production of the triglyceride-rich very low density lipoproteins (VLDL)<sup>19</sup> and by inhibition of hepatic and adipose tissue lipoprotein lipases.<sup>20</sup> Oestrogens increase the concentration of HDL cholesterol by directly stimulating the production of apolipoproteins AI and AII,<sup>20</sup> and indirectly by reducing the catabolism of HDL<sub>2</sub> to HDL<sub>3</sub> by hepatic lipase. Production of LDL and of apolipoprotein B is stimulated by oestrogen,<sup>21</sup> but the net effect is to reduce plasma concentrations as the clearance of LDL is enhanced owing to increased activity of the hepatic LDL receptors.<sup>22</sup>

The role of progesterone in pregnancy associated hyperlipidaemia is questionable. Progestogens have been shown to oppose the actions of oestrogens on lipoprotein metabolism, leading to increased concentrations of LDL cholesterol and decreased concentrations of HDL cholesterol.<sup>23</sup> Some authors have suggested that the oestrogen:progesterone ratio, which is low in early and in very late pregnancy is important in the balance of alterations in lipoprotein metabolism throughout pregnancy.<sup>7,21</sup> The actions of exogenous progestogens on lipoprotein metabolism seem, however, to depend on the androgenicity of the preparations used.<sup>23,24</sup> Natural progesterone is not androgenic and has not been shown to affect lipoprotein concentrations,<sup>24</sup> and so may not be involved in the alterations in lipoprotein metabolism during pregnancy.

In late pregnancy rising concentrations of prolactin inhibit adipose tissue lipoprotein lipase activity<sup>25</sup> resulting in a rise in the concentration of plasma triglyceride. HPL has lipolytic activity<sup>26</sup> which may increase substrate supply of free fatty acids for the increased maternal hepatic VLDL production. The hypertriglyceridaemia in late pregnancy may be further enhanced by hyperinsulinaemia due to insulin resistance which is known to be associated with higher concentrations of triglyceride.<sup>27</sup> Hypoalbuminaemia is a further possible contributory cause of hyperlipidaemia in pregnancy, but our subjects did not have sufficiently low serum albumin concentrations to result in secondary hyperlipidaemia.

It has been suggested that the alterations in lipid metabolism during gestation may be important in the control of delivery to and

uptake of nutrients by the fetus, particularly during rapid fetal weight gain in the second half of gestation. During early pregnancy adipose stores are enlarged and used by the mother in late gestation so as to spare glucose for the fetus. Maternal hyperlipidaemia may therefore have a beneficial influence on fetal development. Whether the maternal hyperlipidaemia has any pathological importance is not known. A positive association between parity and European heart disease in later life has been reported<sup>28,29</sup> and a recent study has shown that lipid concentrations after delivery are higher, and that the total cholesterol to HDL cholesterol ratio is higher in women who have had five or more pregnancies compared with those who have had only one.<sup>30</sup> Our results suggest that in spite of the mitigating effect of increased HDL cholesterol concentrations, the increased apolipoprotein B : AI ratio may have an atherogenic effect. Pregnancy may also precipitate severe chylomicronaemia in women with the rare familial lipoprotein lipase deficiency<sup>31</sup> or with other causes of hypertriglyceridaemia. Such women may be previously undiagnosed and may present with acute pancreatitis during pregnancy. Patients have been successfully managed by the exclusion or restriction of fat, and the measurement of lipids during pregnancy would enable those women at risk to be identified. Of additional interest is the possibility that the extent of the hyperlipidaemic response to pregnancy may be genetically determined. If this is so, measurement of lipids and lipoproteins during the third trimester of pregnancy may provide a sensitive test for identifying women without overt hyperlipidaemia who are at risk of future coronary heart disease and of atheroma. This hypothesis needs to be evaluated in a follow up study of these women before routine screening for hyperlipidaemia in pregnancy can be recommended.

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