Is normal pregnancy atherogenic?

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ABSTRACT

Serum cholesterol, triacylglycerols and low-density lipoprotein (LDL) subfractions were determined in 120 primagravid women during normal gestation (40 in each trimester) and in 20 non-pregnant age-matched controls. LDL subfractions were determined by PAGE, and an LDL score was calculated. The higher the score, the smaller the subfractions. The objective of the study was to determine the effects of the hyperlipidaemia, high oestrogen concentrations and insulin resistance known to exist in normal pregnancy on LDL subfraction formation. Pregnant women had an increased mean serum cholesterol concentration [5.78 (S.D. 1.09) mmol/l] in the first trimester compared with the non-pregnant controls [5.11 (0.77) mmol/l; \(P < 0.01\)]. The serum cholesterol concentration increased progressively throughout gestation to a mean of 8.14 (1.39) mmol/l in the third trimester (\(P < 0.001\) compared with the second trimester). Triacylglycerol concentrations in the first trimester were similar to those of controls, and there was a non-significant increase by the second trimester to 1.32 (0.44) mmol/l. However, by the third trimester the mean triacylglycerol concentration had doubled [2.58 (0.98) mmol/l; \(P < 0.001\) compared with the first and second trimester]. During gestation the LDL score increased dramatically, from 1.17 (0.39) during the first trimester to 2.01 (0.37) in the second trimester (\(P < 0.001\)) to 2.73 (0.48) in the third trimester (\(P < 0.001\) compared with the second trimester). Thus an atherogenic lipid profile develops during normal gestation. The significance of these changes remains unclear, but they may have important implications for mother and foetus.

INTRODUCTION

Low-density lipoprotein (LDL), an important risk factor for coronary heart disease, is made up of particles of various sizes and densities [1]. Clinical studies have established that individuals with a small, dense LDL subfraction profile are more likely to develop coronary heart disease [2–8]. These small, dense LDL subfractions are more susceptible to oxidation than large, lighter ones and, once oxidized, promote foam-cell formation, initiate endothelial dysfunction and thereby promote atherogenesis in a variety of ways [9–12].

Although high triacylglycerol concentrations are largely responsible for promoting the formation of small, dense LDL particles [3], both insulin resistance [13] and oestrogens (in women) may be important. There is a striking difference in the LDL profile between men and women, with pre-menopausal women tending to have larger, lighter subfractions [14], and this may be due to the influence of oestrogen. In contrast, hormone replacement therapy in post-menopausal women seems to promote the formation of small, dense subfractions, at least in the limited number of studies that have been performed [15,16]. In normal pregnancy a unique metabolic state...
exists, comprising insulin resistance and high concentrations of oestrogens, which may alter the LDL profile in the latter part of pregnancy [17]. This effect is exaggerated in pre-eclampsia, and small, dense LDL subfractions have been implicated in the pathogenesis of this condition [18]. To date only two small studies have investigated the changes in LDL profiles during normal pregnancy [19,20]. Each study investigated 10 women during pregnancy, and noted a change in subfractions in six and seven patients respectively. In the present paper we investigate the changes in lipids and in the LDL profile in a much larger cohort of women to determine whether a change to a more atherogenic lipid profile is an inevitable consequence of normal pregnancy.

METHODS

Subjects and sample collection
Serum lipids and LDL subfractions were analysed prospectively in 120 Caucasian women (40 in each trimester) of mean age 31.8 (S.D. 3.7) years in normal gestation, and in 20 age-matched controls. Non-fasting venous blood was sampled and serum was separated by low-speed centrifugation (3000 g for 15 min) and frozen at −80 °C.

Ethics
All subjects gave informed written consent. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and ethical approval was given by the South Birmingham Local Research Ethics Committee.

Laboratory methods
LDL subfraction patterns were determined by a well-established, quick and reliable electrophoretic method using pre-cast 3 % (w/v) acrylamide gels (Lipoprint LDL System; Quantimetrix Co., Redondo Beach, CA, U.S.A.), which has been validated previously in our laboratory [7]. Although LDL subfractions are usually separated by density-gradient ultracentrifugation or gradient PAGE, both methods are time consuming (taking over 24 h), technically demanding (gradient 2–16 % gels must be self-prepared) and unsuitable for simple, routine and quick assessment of patients. By comparison, the continuous disc PAGE used in this study allows separation of seven LDL subfractions within 90 min.

Before analysis, all samples required ultracentrifugation (300000 g for 45 min) to separate chylomicrons and large very-low-density lipoprotein (VLDL) particles present in samples from non-fasting subjects, which may block the gel pores [21,22]. Serum (25 μl) was applied in duplicate to gel tubes, followed by loading gel containing the lipid stain Sudan Black. Polymerization was achieved by exposure of the gel tubes to fluorescent light for 30 min. Electrophoresis was performed at a constant current of 3 mA per gel tube for 70 min, and gels were then scanned directly at a wavelength of 610 nm. Using this system, seven LDL subfractions can be identified (LDL-0 to LDL-6), based on their specific electrophoretic mobility. An LDL score is calculated from the relative percentage area under the curve (AUC) for each LDL band present in a single gel run, multiplied by the LDL band number:

\[ 1 \times \text{AUC}_{\text{LDL-0}} + 2 \times \text{AUC}_{\text{LDL-2}} + 3 \times \text{AUC}_{\text{LDL-3}} \ldots \]

The higher the score, the greater the proportion of small, dense LDL subfractions present in the sample. The reproducibility and repeatability of the method were determined by calculating the intra- and inter-assay coefficients of variation; these were 0–2.7 % and 0–4.6 % respectively. The scoring system has previously been validated by Rajman et al. [7], who demonstrated that patients with angiographically proven coronary artery disease had a raised score compared with negative controls. Serum total cholesterol and triacylglycerol levels were measured using standard colorimetric and enzymic assays.

Statistics
The statistical significance of differences between groups was determined by the unpaired Student’s t test, and the level of significance was taken as \( P < 0.05 \). Regression analysis was performed to investigate associations between LDL scores and lipoprotein parameters.

RESULTS

The mean serum total cholesterol concentration was significantly higher in the pregnant women in the first trimester [5.78 (S.D. 1.09) mmol/l] than in the control group [5.11 (0.77) mmol/l; \( P < 0.01 \)]. Serum cholesterol increased progressively during pregnancy, to a mean concentration of 8.14 (1.39) mmol/l in the third trimester \( (P < 0.001 \) compared with the second trimester; Table 1). In eight women, serum cholesterol exceeded 9 mmol/l in the third trimester. On average, mean cholesterol increased by 40 % during gestation. Triacylglycerol concentrations in the first trimester were similar to the normal range observed in the general population. There was a non-significant rise in the mean serum triacylglycerol concentration in the second trimester [1.32 (S.D. 0.44) mmol/l, compared with 1.19 (0.53) mmol/l in the first trimester]. By contrast, in the third trimester there was a striking increase, with a doubling of the mean concentration [2.58 (0.98) mmol/l; \( P < 0.001 \) compared with the first and second trimesters].
Table 1 Total serum cholesterol, triacylglycerols and LDL score in pregnant women during gestation compared with non-pregnant controls

Values are means (S.D.) for \( n = 40 \) women in each trimester, and for \( n = 20 \) controls. \( * \) \( P < 0.01 \) compared with controls; \( † \) \( P < 0.001 \) compared with the first trimester; \( ‡ \) \( P < 0.001 \) compared with the second trimester.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total cholesterol (mmol/l)</th>
<th>Triacylglycerols (mmol/l)</th>
<th>LDL score (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td>5.78 (1.09)( ^* )</td>
<td>1.19 (0.53)( ^* )</td>
<td>1.17 (0.39)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>6.88 (1.16)( † )</td>
<td>1.32 (0.44)</td>
<td>2.01 (0.37)( † )</td>
</tr>
<tr>
<td>Third trimester</td>
<td>8.14 (1.39)( ‡ )</td>
<td>2.58 (0.90)( ‡ )</td>
<td>2.73 (0.48)( ‡ )</td>
</tr>
<tr>
<td>Controls</td>
<td>5.11 (0.77)</td>
<td>0.80 (0.28)</td>
<td>1.05 (0.22)</td>
</tr>
</tbody>
</table>

In eight women, triacylglycerol concentrations exceeded 3.5 mmol/l in the third trimester.

During gestation, the LDL score increased dramatically, from 1.17 (S.D. 0.39) during the first trimester to 2.01 (0.37) in the second trimester (\( P < 0.001 \)) to 2.73 (0.48) in the third trimester (\( P < 0.001 \) compared with the second trimester). The change in LDL score during pregnancy was positively correlated with serum triacylglycerols (\( r = 0.5 \); \( P < 0.01 \)), although the increased score observed in the second trimester occurred in the absence of a significant increase in triacylglycerols (Table 1; Figure 1).

**DISCUSSION**

Previous studies are consistent with our findings of marked hyperlipidaemia during the course of normal pregnancy; serum cholesterol and triacylglycerols have been found to increase by approx. 25–50% and 200–300% respectively [23–28]. Total cholesterol and triacylglycerol concentrations have been shown to be positively correlated with 17β-oestradiol, progesterone, human placental lactogen and insulin levels throughout the whole period of gestation [29]. Although the significance of the changes in serum lipids is uncertain, they are likely to relate to the maintenance of nutrient fuel to the mother and foetus.

The change in triacylglycerols in normal pregnancy is important in relation to lipoprotein subclasses, such as LDL. These lipoproteins contain discrete subfractions of various sizes, densities and compositions, which differ in their ability to initiate atherogenesis [12]. For example, small, dense LDL particles do not bind readily to the LDL receptor and therefore remain in the circulation for longer [11]. They also penetrate the arterial intima better than do larger ones [30] and they are more readily oxidized, possibly because they contain less vitamin E and other antioxidants [31]. Finally, their uptake into macrophages to create foam cells, and thus initiate atherogenesis, is facilitated [32]. This may explain their identification as an independent risk factor for coronary heart disease [2]. Plasma triacylglycerol is the major determinant of small, dense LDLs, accounting for 40–60% of the variability of this fraction in the plasma [3].

Pregnancy provides a unique opportunity to observe changes in LDL subfractions in relation to a progressive increase in triacylglycerols. We have shown that, as pregnancy progresses and plasma triacylglycerols increase, the LDL score also increases dramatically, suggesting a change to a more atherogenic lipid profile. Several factors during gestation may promote this change. For example, pregnancy is associated with a progressive increase in oestrogen levels; in addition, fasting insulin levels, although normal during the first half of pregnancy, rise after week 23 to reach a plateau at week 31 [29,33]. The mechanisms for the formation of small, dense LDL particles remain unclear, but may involve the process of neutral lipid exchange and lipolysis [3]. VLDL represents the major precursor of LDL and reflects plasma triacylglycerol levels. Two subclasses of VLDL have been defined: a large and buoyant fraction enriched with triacylglycerols (VLDL\(_{1\alpha} \)) and a smaller, denser fraction (VLDL\(_{2\alpha} \)). It follows from the association between LDL subclasses and raised triacylglycerols that VLDL\(_{1\alpha} \) may be important as a vehicle in the process of neutral lipid exchange and generation of small, dense LDLs. Cholesterol esters are transferred from LDL and
high-density lipoprotein to VLDL, by cholesterol ester transfer protein, in exchange for triacylglycerols. Triacylglycerol-enriched LDL particles subsequently undergo a size reduction through the action of hepatic lipase and lipoprotein lipase, resulting in the formation of small, dense LDL subfractions.

The activity of cholesterol ester transfer protein has been shown to increase significantly by the second trimester of pregnancy, before declining towards the end of gestation [34]. In keeping with this, the esterification of free cholesterol was significantly reduced in the maternal blood at delivery by elective Caesarian section of 13 full-term pregnancies compared with controls [35]. However, the activity of this enzyme is not thought to be rate-limiting [34]. In contrast, it is likely that increased concentrations of VLDL, due to hypersecretion by the liver promote triacylglycerol transfer into LDL during pregnancy [19]. This hypersecretion of VLDL, particles by the maternal liver is probably a secondary effect of the steroid hormones oestradiol, oestrone and progesterone that are secreted by the foetus and placenta [23]. Subsequent remodelling of LDL particles towards smaller, denser species appears to happen in pregnancy despite decreased hepatic lipase activity associated with high oestrogen levels [36]. Relative insulin resistance is a feature of late gestation, and this may also contribute to increased VLDL levels, since insulin normally impairs VLDL secretion by the liver [37]. Reduced catabolism of VLDL may be a further factor in late pregnancy, due to a marked reduction in lipoprotein lipase activity, probably also caused by insulin resistance [17,23,37].

The changes in lipid profile observed during pregnancy may be of potential importance for a woman’s long-term health, because elevated serum triacylglycerols are an independent risk factor for coronary heart disease in women [38]. During pregnancy all women develop a transient hyperlipidaemia, and a significant proportion go on to develop hypertension associated with pre eclampsia, gestational diabetes and maternal obesity. The long-term consequences of these conditions for coronary heart disease are not clear. There is, however, an increased prevalence of angina, cholesterol gallstones, diabetes and obesity in post-menopausal women who have had several pregnancies [39]. In keeping with this, the change in serum cholesterol during pregnancy is associated with parity so that, for each additional previous pregnancy, a further increase in cholesterol of 0.11 mmol/l is observed [40]. Furthermore, it is not clear whether, post partum, serum lipids and subfractions normalize as rapidly and completely in multiparous women who are at increased risk of cardiovascular disease [41].

In conclusion, we have demonstrated that normal pregnancy is associated with a shift towards small, dense LDL subfractions, along with raised triacylglycerols and cholesterol. By the third trimester most women have a lipid profile which would be considered highly athero-

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