Fetal and maternal lipoprotein metabolism in human pregnancy

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1. Lipid, apolipoprotein concentration and composition were determined in maternal venous and umbilical arterial and venous blood at delivery by elective Caesarean section in 13 full-term pregnancies and in 25 healthy non-pregnant females. The indications of Caesarean section were a previous Caesarean section or breech presentation. None of the women was in labour and there were no other complications of pregnancy or fetal distress.

2. The objectives of the study were to establish whether the placenta has a role in feto-maternal cholesterol metabolism through either synthesis or transplacental cholesterol flux. The potential for free cholesterol diffusion between mother and fetus and rates of cholesterol esterification and transfer between lipoproteins were determined and related to the differences in composition between fetal and maternal lipoproteins.

3. Pregnant women had raised levels of all lipid and lipoprotein fractions compared with control subjects. The greatest increases were in free cholesterol and triacylglycerol ($P < 0.0001$). Lipoprotein (a) levels were significantly greater in the pregnant women [112 (12.2) mg/l] than in the control women [50 (10.0) mg/l].

4. The only significant correlation between maternal and fetal lipoprotein concentrations was in lipoprotein (a) levels ($r = 0.791$, $P = 0.002$). In both umbilical venous and arterial blood, concentrations of very-low- and low-density lipoproteins, particularly apolipoprotein B, cholesteryl ester and triacylglycerol, were lower than in maternal blood ($P < 0.0001$), but high-density lipoprotein levels were similar.

5. There was no umbilical arteriovenous differences in lipoprotein concentration or composition. This suggests that cholesterol synthesis or free cholesterol diffusion does not occur in the placenta. The relative concentrations of free cholesterol to phospholipid in maternal and fetal lipoproteins do not indicate the existence of a concentration gradient favouring free cholesterol diffusion across the placenta.

6. The esterification of free cholesterol was significantly reduced in maternal [17.7 (2.4) mmol h$^{-1}$ l$^{-1}$, $P < 0.0001$] and fetal [6.7 (3.5) mmol h$^{-1}$ l$^{-1}$, $P < 0.0001$] compared with control [40.9 (13.2) mmol h$^{-1}$ l$^{-1}$] blood.

7. In fetal compared with maternal high-density lipoproteins the ratios cholesteryl ester/apolipoprotein A-I [0.84 (0.35) versus 0.40 (0.05), $P < 0.01$] and phospholipid/apolipoprotein A-I [1.66 (0.14) versus 0.58 (0.10), $P < 0.0001$] indicated lipid enrichment of these particles in the fetus.

8. Lipid enrichment of high-density lipoprotein is due in part to a marked reduction in transfer of cholesteryl ester in the fetus [1.0 (0.6) mmol h$^{-1}$ l$^{-1}$] compared with maternal [6.15 (1.3) mmol h$^{-1}$ l$^{-1}$, $P = 0.004$] and control [17.3 (7.2) mmol h$^{-1}$ l$^{-1}$, $P < 0.0001$] blood.

9. In conclusion, there was no evidence for involvement of the placenta in cholesterol metabolism during pregnancy. In fetal life high-density lipoproteins are lipid rich, partly because of a reduction in transfer of esterified cholesterol to other particles. Maternal and fetal lipoprotein levels are not correlated, although the results suggested that lipoprotein (a) levels may be related.

**INTRODUCTION**

The association between abnormal levels or structure of blood lipoproteins and cardiovascular disease in adults has been clearly established in epidemiological and prospective studies. While high levels of low-density lipoprotein (LDL) increase cardiovascular risk, there is a paucity of data concerning lipoproteins in fetal life. This is partly due to the technical problems of obtaining fetal blood samples. In addition, the role of the placenta in feto-maternal cholesterol metabolism is not clear. Therefore, we have determined the concentrations of lipoproteins and their components in maternal and fetal blood samples obtained by elective Caesarean section.

**Key Words:** cholesterol esterification, fetus, lipoproteins, pregnancy.

**Abbreviations:** apo B, apolipoprotein B; apo A-I, apolipoprotein A-I; BMI, body mass index; CE, cholesteryl ester; CET, cholesteryl ester transfer; FC, free cholesterol; HDL, high-density lipoprotein; ICAT, lecithin:cholesterol acyl transferase; LDL, low-density lipoprotein; NEFA, non-esterified fatty acid; NFV, non-pregnant female volunteer; NP, normal pregnant; PL, phospholipid; SQR, semi-interquartile range; TAG, triacylglycerol; TC, total cholesterol; UMA, umbilical arterial blood; UMV, umbilical venous blood; VLDL, very-low-density lipoprotein.

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cular risk, a raised level of high-density lipoprotein (HDL) confers protection [1]. A raised cholesterol level in young adults has recently been linked with cardiovascular disease in later life [2], and as shown in the Bogusia Heart Study, levels of HDL and LDL tend to track from childhood in adult life [3]. Recently, this relationship has been extended into intrauterine life because raised levels of LDL cholesterol in adults have been shown to be inversely related to abdominal girth at birth [4]. Thus, those with 'at-risk' lipoprotein profiles early in life may well be predisposed to later coronary heart disease. The mechanism by which fetal undernutrition [4] may give rise to higher levels of LDL in the adult is unclear, but one suggestion is that lipoprotein metabolism is modified by reduced hepatic sensitivity to growth hormone induced before birth [5, 6]. Whatever the explanation, the epidemiological association described by Barker et al. [7] between small fetal and large placental size and adult cardiovascular disease has engendered much recent interest. These observations emphasize the importance of understanding lipoprotein metabolism during pregnancy and fetal life because this would appear to have implications for later cardiovascular disease.

In the adult, hepatic production of triacylglycerol (TAG)-rich very-low-density lipoproteins (VLDLs) and subsequent TAG hydrolysis by lipoprotein lipase located on the vascular endothelium result in conversion of VLDL to low-density lipoprotein (LDL), which enters the peripheral tissues or the liver by both receptor- and non-receptor-mediated pathways [8]. Excess cholesterol is removed from peripheral sites by a process known as reverse cholesterol transport. There is controversy surrounding the initial acceptor molecule for free cholesterol (FC) from peripheral cells, but undoubtedly it must at some stage enter the HDL free cholesterol pool if it is to undergo esterification. Thence it may either be returned to the liver directly during the circulation of HDL through the liver or be transferred to VLDL and then undergo hepatic uptake after conversion to intermediate-density lipoprotein or LDL [9, 10]. These pathways provide a bidirectional flux of cholesterol between hepatocytes and peripheral cells. During fetal life the system is modified by differences in the contributions from the fetal liver, by the absence of lipid absorption from the fetal gut and by the placenta and maternal factors [11].

The metabolism of non-esterified fatty acids (NEFAs) during pregnancy has been studied previously. Avid placental binding of VLDL [12] and VLDL-TAG hydrolysis [13], in addition to placental fatty acid synthesis [14] followed by transplacental passage [15], gives an umbilical venous–arterial concentration difference in NEFAs [16, 17]. However, similar data do not exist for cholesterol or phospholipids, which have important structural roles in the cell and are required in increased quantities during growth.

### Table 1. Characteristics of the subject groups.

<table>
<thead>
<tr>
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<th>Median SIQR Range</th>
</tr>
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<tbody>
<tr>
<td>Fetal weight (kg)</td>
<td>3.13 0.43 2.6-4.9</td>
</tr>
<tr>
<td>Placental weight (kg)</td>
<td>0.663 0.085 0.42-0.97</td>
</tr>
<tr>
<td>Maternal weight (kg)</td>
<td>66.3 7.4 50-108.5</td>
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<td>Maternal height (m)</td>
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<tr>
<td>Cord pH</td>
<td>7.30 0.03 7.21-7.36</td>
</tr>
<tr>
<td>Cord glucose (mmol/l)</td>
<td>2.45 0.55 1.3-3.5</td>
</tr>
</tbody>
</table>

Plasma lipoproteins in cord blood have been studied in at least 20 studies reviewed recently [18]. Few have reported apolipoprotein levels, and none is sufficiently detailed to allow speculation on how fetal lipid metabolism differs from the adult. In this study we have performed comprehensive lipid and apolipoprotein analyses in mother and fetus with simultaneous measurements in maternal venous and umbilical cord arterial and venous blood, the latter providing an indication of the role of the placenta in lipoprotein metabolism in vivo. In addition, we determined the rate at which FC is converted to its ester (cholesterol ester, CE) and transferred between lipoproteins. These data have allowed us to examine the relationship between fetal and maternal lipoprotein levels and composition and the evidence for transplacental passage of cholesterol by reference to the arteriovenous difference and the potential for FC flux.

### MATERIALS AND METHODS

#### Subjects

Twenty-five normal menstruating, non-pregnant female volunteers (NFVs) were studied as a control group. The median age of this group was 29 (range 19–33) years, with a body mass index (BMI) of 22.7 (range 17.2–24.5) kg/m². Smokers and subjects with a medical condition or taking drugs known to affect lipid metabolism, including exogenous oestrogens, were not included.

Thirteen term pregnancies were investigated at a median gestation of 40.2 (range 39–41) weeks. These normal pregnant (NP) women had uncomplicated pregnancies, but were delivered by elective Caesarean section (under epidural anaesthesia) for non-pathological indications (i.e. previous Caesarean delivery, breech presentation). The basic demographic data of this sample are given in Table 1. All fetuses were appropriately grown for gestational age.

#### Ethical committee approval

All subjects gave informed, written consent and ethical approval was granted by the North Staffordshire District Ethical Committee, North Staffordshire Hospitals.
Collection of blood samples

In the NPVs and NP subjects, 30ml of blood was collected by venesection from the antecubital fossa, using a 21-gauge needle, into a polypropylene syringe. All samples were free flowing and obtained without venous stasis. Venesection was performed during the first 7 days of the menstrual cycle in the NPVs, and in pregnant subjects during delivery of the fetus, all subjects having fasted for at least 12h before sampling. Simultaneous samples (20ml) were obtained from the umbilical vein and artery after delivery of the fetus but before placental separation.

Preparation of blood, lipid and lipoprotein assays

The samples were taken into plain and EDTA-containing tubes. After centrifugation, serum and plasma were separated and stored frozen, although an aliquot of plasma was removed before freezing for precipitation of the apolipoprotein B-containing lipoproteins. The resulting supernatant containing HDL was subsequently frozen and stored at −20°C before lipid analyses.

Laboratory methods

Fasting serum levels of triacylglycerol (TAG), phospholipids (PLS), total (TC) and free (FC) cholesterol were measured by commercial enzymatic methods (Boehringer Mannheim, Lewes, Sussex, U.K.) on an automated discrete random access analyser (RA 1000, Technicon, U.S.A.). Supernatants containing total HDL or the HDL subfraction were obtained by precipitation of the other lipoproteins with buffered polyethylene glycol (Quantolip, Immuno Ag, Vienna, Austria). The lipid determinations were made on the supernatants, and increased assay sensitivity was achieved by a three-fold increase in sample volume and the addition of tribromohydroxybenzoic acid in a concentration of 0.5g/l to the assay reagent [19]. Apolipoproteins A-I (apo A-I) and B (apo B) were measured by rate immunonephelometry (Beckman Instruments, CA, U.S.A.) and lipoprotein (a) [Lp(a)] was measured by enzyme-linked immunoassay (Biopool, Umea, Sweden). The rates of cholesterol esterification and its subsequent transfer to apo B-containing lipoproteins were determined by measurement of the rate at which CE appears in whole serum and (VLDL+LDL) following incubation of serum with radiolabelled FC as previously described [20]. LDL-cholesterol was calculated using the Friedewald formula [21], although the validity of this equation has not been investigated for maternal or fetal blood:

\[
\text{LDL-cholesterol} = \text{TC} - \left[ (\text{TAG}/2.19) + \text{HDL-cholesterol} \right]
\]

Data analysis

Statistical analysis was performed using the NCSS package (J. Hintze, Kaysville, UT, U.S.A.). As the data did not fit a normal distribution, results are expressed as median and semi-interquartile range (SIQR) and non-parametric tests were used. Numerical variables were compared between groups using the Mann-Whitney U-test and differences between umbilical arterial and venous blood were sought using Wilcoxon's signed-rank test. Correlations were performed using Spearman's rank correlation coefficient, except for Lp(a), for which Pearson's coefficient was calculated after logarithmic transformation. Values are given as medians (interquartile ranges).

RESULTS

Lipoprotein profile in NP subjects versus NFV subjects

The plasma lipids in the NP subjects were typical of those expected in the third trimester of pregnancy and characterized by increased concentrations of all lipid fractions as compared with NFV subjects (Table 2). The most marked differences were a significant increase in pregnant subjects of FC [NFVs 1.04 (0.14) versus NP subjects 1.97 (0.69)nmol/l, P<0.0001] and triacylglycerol [NFVs 0.70 (0.27) versus NP subjects 2.88 (0.61)nmol/l, P<0.0001]. Phospholipids and CE in plasma were significantly increased in NP subjects but to a lesser extent, largely because of the increase in LDL-cholesterol (NFVs 3.07 (0.31) versus NP subjects 6.19 (0.76)nmol/l, P<0.0001) (Table 2). Apo A-I was slightly increased in NP subjects [NFVs 1.84 (0.33) versus NP subjects 2.19 (0.16)mg/l, P=0.036], while apo-B demonstrated a two-fold increase [NFVs 0.85 (0.11) versus NP subjects 1.92 (0.24)mg/l, P<0.0001]. Lp(a) was significantly increased in the plasma of NP subjects [NFVs 50 (10.0) versus NP subjects 112.5 (12.2)mg/l, P=0.0037].

The HDL concentrations are somewhat lower than previously reported for women of this age, probably because a different analytical method for HDL determination was used in this compared with previous studies [22]. Compared with the control subjects the total cholesterol content was higher in the NP subjects owing to increased CE in the larger, less dense HDL subfraction (Table 2). The HDL-PL level was not different from that in the NPVs, despite a rise in total plasma total PL. HDL-FC was significantly reduced in NP subjects (Table 2).

As the number of apolipoprotein molecules within each lipoprotein particle is relatively stable, the concentration gives information on particle numbers. The lipid contents of HDL and VLDL+LDL were expressed as a ratio to apo A-I and apo B respectively to indicate the lipid content per particle (Table 3). In NP subjects, HDL FC/apo
cholesterol/apo B was significantly lower in NP women \( C_{3.06 (0.057)} \) than in NFVs \( C_{3.84 (0.432)} \), \( p < 0.001 \), whereas TAG/apo B was significantly lower in NP women \( C_{0.74 (0.27)} \) than in NFVs \( C_{2.88 (0.61)} \), \( p < 0.001 \].

Table 3. Lipid composition of HDL and VLDL+LDL fractions with reference to the protein content. Significance of differences from maternal blood are given: \( *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 \).

<table>
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<tr>
<th></th>
<th>NPV</th>
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<th>NP</th>
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<th>UMA</th>
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<th>UMV</th>
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<td></td>
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<td>SIQR</td>
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<td>SIQR</td>
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<td>SIQR</td>
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<td>Apo B (g/l)</td>
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<td>0.28</td>
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<td>5.22</td>
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<td>TAG</td>
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<td>LDL</td>
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<td>0.76</td>
<td>0.96***</td>
<td>0.15</td>
<td>0.81***</td>
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<td>0.69</td>
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<tr>
<td>HDL-FC</td>
<td>0.15*</td>
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<td>0.08</td>
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<td>HDL-CE</td>
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<td>HDLz-PL</td>
<td>0.96</td>
<td>0.18</td>
<td>1.11</td>
<td>0.17</td>
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<td>0.11</td>
<td>0.87*</td>
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<td>0.07</td>
<td>&lt;0.01</td>
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<tr>
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<td>0.24*</td>
<td>0.10</td>
<td>0.42</td>
<td>0.06</td>
<td>0.24*</td>
<td>0.09</td>
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<tr>
<td>HDLz-FC</td>
<td>0.03</td>
<td>0.08</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>0.01</td>
<td>0.06</td>
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<tr>
<td>HDLz-PL</td>
<td>0</td>
<td>0</td>
<td>0.16</td>
<td>0.09</td>
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<td>HDLz-TAG</td>
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<td>0.03</td>
<td>0.03</td>
<td>0</td>
<td>0.02</td>
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<td>LP (a) (mg/l)</td>
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<td>1.00</td>
<td>112.5</td>
<td>122</td>
<td>7.4***</td>
<td>4.85</td>
<td>7***</td>
<td>4.45</td>
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</table>

Lipoproteins in umbilical cord blood

The median values for the lipoproteins and apolipoproteins in umbilical venous (UMV) compared with umbilical arterial (UMA) blood did not show a significant difference in either concentration (Table 2) or composition (Table 3). Compared with their mothers (NP women), many lipid and apolipoprotein concentrations in the fetal blood were significantly lower. The concentrations of apo-B, CE and TAG, that is those components associated with VLDL and LDL, showed the most profound decrease [apo-B, NP subjects 2.19 (0.16) versus UMA 0.70 (0.04) versus UMV 0.68 (0.02) mg/l, \( P < 0.0001 \]; CE, NP subjects 5.22 (0.64) versus UMA 1.02 (0.28) versus UMV 1.01 (0.21) nmol/l, \( P < 0.0001 \); TAG, NP subjects 2.88 (0.61) versus UMA 0.31 (0.07) versus UMV 0.30 (0.07) nmol/l, \( P < 0.0001 \). In contrast, the HDL-lipid fractions were not significantly different from the NP subjects, with the exception of PL and apo A-I, which was significantly lower [0.015 (0.32)] than in NFVs [0.078 (0.045), \( P < 0.05 \). In the apo B-containing lipoproteins (VLDL+LDL), total cholesterol/apo B was significantly lower in NP women [3.06 (0.057)] than in NFVs [3.84 (0.432), \( P < 0.001 \), whereas TAG/apo B was significantly increased [1.38 (0.321) versus NFVs 0.813 (0.207), \( P < 0.01 \).
showed a slight but significant reduction in the fetus (Table 2). Compared with maternal blood, umbilical cord blood HDL appears to transport a greater proportion of its cholesterol in the HDL₃ subfraction, the levels being the same as in the mothers. Levels of HDL₂ were reduced compared with the mother, but equivalent to the NPV values (Table 2). The most marked differences between the blood samples from NP subjects and umbilical cord blood were in lipid/protein composition. The umbilical cord plasma HDL (both arterial and venous) was at least two-fold enriched in lipid as compared with maternal blood [NP subjects, 0.68 (0.17); UMA, 1.42 (0.65) mmol/l, P < 0.05] and was reduced a further sixfold in the cord blood compared with the NPVs [NFVs, 17.3 (7.2) versus UMA, 6.70 (1.75) and UMV, 6.15 (0.35) mmol/l, P < 0.05]. The rate of esterification of endogenous cholesterol by lecithin-cholesterol acyl transferase (LCAT) and subsequent cholesteryl ester transfer (CET) from HDL to apo B-containing lipoproteins was determined by direct assay in six of our pregnant subjects. Compared with healthy control subjects, there was a marked reduction in the cholesterol esterification rate during pregnancy [NFVs, 40.9 (13.2) versus NP subjects, 17.65 (1.2) µmol h⁻¹ l⁻¹, P < 0.001]. Esterification was reduced to an even greater extent in cord than in maternal blood [NP subjects, 17.65 (1.2) µmol h⁻¹ l⁻¹; UMV, 6.70 (1.75) and UMV, 6.15 (0.35) µmol h⁻¹ l⁻¹; both P < 0.004]. Similarly, transfer of CE from HDL to VLDL + LDL was reduced to about one-third of normal in the pregnant subjects [NFVs, 17.3 (7.2) versus NP subjects, 17.65 (1.2) µmol h⁻¹ l⁻¹; UMA, 6.70 (1.75) and UMV, 6.15 (0.35) µmol h⁻¹ l⁻¹; both P < 0.004].

**Concentration gradient for free cholesterol diffusion**

The ratio of FC to PL concentration dictates the direction of FC diffusion [23]. We found no significant difference in this ratio between mother and fetus for whole plasma. However, this ratio in HDL was significantly lower in NP subjects than in NPVs and also lower than in the fetuses, although this difference did not reach statistical significance (Table 4). In VLDL + LDL, the median level was two-fold greater in fetal than in maternal lipoproteins (NP subjects, 0.68 (0.17) versus UMA, 1.42 (1.06) versus UMV, 1.06 (0.77)). This was significantly only in the placental venous blood (P < 0.05) owing to the greater variability of the ratio in umbilical arterial blood.

**Cholesterol esterification and transfer**

The ratio of free to esterified cholesterol provides an index of the rate of cholesterol esterification. We calculated this ratio for whole plasma and the fractions separately but found no difference between the groups (Table 4). The rate of esterification of endogenous cholesterol by lecithin-cholesterol acyl transferase (LCAT) and subsequent cholesteryl ester transfer (CET) from HDL to apo B-containing lipoproteins was determined by direct assay in six of our pregnant subjects. Compared with healthy control subjects, there was a marked reduction in the cholesterol esterification rate during pregnancy [NFVs, 40.9 (13.2) versus NP subjects, 17.65 (1.2) µmol h⁻¹ l⁻¹, P < 0.001]. Esterification was reduced to an even greater extent in cord than in maternal blood [NP subjects, 17.65 (1.2) µmol h⁻¹ l⁻¹; UMV, 6.70 (1.75) and UMV, 6.15 (0.35) µmol h⁻¹ l⁻¹; both P < 0.004]. Similarly, transfer of CE from HDL to VLDL + LDL was reduced to about one-third of normal in the pregnant subjects [NFVs, 17.3 (7.2) versus NP subjects, 17.65 (1.2) µmol h⁻¹ l⁻¹; UMA, 6.70 (1.75) and UMV, 6.15 (0.35) µmol h⁻¹ l⁻¹; both P < 0.004].

**Relationships between maternal and fetal lipid levels and fetal characteristics**

A high degree of correlation was found between all parameters measured in umbilical cord arterial and venous blood (data not shown). All lipid parameters were tested for concordance between maternal and fetal levels. No significant correlations were found, with the exception that the maternal level of Lp(a) was significantly correlated with the fetal level in umbilical cord blood (NP subjects versus UMA, r = 0.791, P = 0.002; NP subjects versus UMV, r = 0.556, P = 0.048) (Fig. 1). The Lp(a) concentrations in umbilical arterial and venous blood were highly correlated (r = 0.900; P = 0.0001). Correlations were sought between fetal and maternal lipid parameters and anthropometric measurements, but no significant relationships were found between the lipids and fetal and placental weight.

**Table 4. Rates of cholesterol esterification and transfer and the ratio of free cholesterol to its ester and phospholipids in plasma, HDL and VLDL + LDL.**

<table>
<thead>
<tr>
<th></th>
<th>NPV Median</th>
<th>SIQR</th>
<th>NP Median</th>
<th>SIQR</th>
<th>UMA Median</th>
<th>SIQR</th>
<th>UMV Median</th>
<th>SIQR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCAT (µmol h⁻¹ l⁻¹)</td>
<td>4.09**</td>
<td>13.2</td>
<td>17.7</td>
<td>1.2</td>
<td>6.7**</td>
<td>1.8</td>
<td>6.1**</td>
<td>0.3</td>
</tr>
<tr>
<td>CET (µmol h⁻¹ l⁻¹)</td>
<td>17.3***</td>
<td>7.2</td>
<td>6.2</td>
<td>0.7</td>
<td>1.0**</td>
<td>0.3</td>
<td>1.1**</td>
<td>0.4</td>
</tr>
<tr>
<td>Plasma FC/CE</td>
<td>0.37</td>
<td>0.057</td>
<td>0.45</td>
<td>0.16</td>
<td>0.32</td>
<td>0.29</td>
<td>0.33</td>
<td>0.37</td>
</tr>
<tr>
<td>Plasma FC/PL</td>
<td>0.45</td>
<td>0.037</td>
<td>0.49</td>
<td>0.14</td>
<td>0.42</td>
<td>0.21</td>
<td>0.42</td>
<td>0.20</td>
</tr>
<tr>
<td>HDL FC/CE</td>
<td>0.17</td>
<td>0.09</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td>0.08</td>
<td>0.22</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>HDL FC/PL</td>
<td>0.13**</td>
<td>0.07</td>
<td>&lt;0.01</td>
<td>0.05</td>
<td>0.05</td>
<td>0.10</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>(VLDL + LDL) FC/CE</td>
<td>0.38</td>
<td>0.10</td>
<td>0.35</td>
<td>0.20</td>
<td>0.40</td>
<td>0.50</td>
<td>0.44</td>
<td>0.73</td>
</tr>
<tr>
<td>(VLDL + LDL) FC/PL</td>
<td>0.74</td>
<td>0.15</td>
<td>0.68</td>
<td>0.17</td>
<td>1.42</td>
<td>1.06</td>
<td>1.1*</td>
<td>0.77</td>
</tr>
</tbody>
</table>
may be FC acceptors. These results suggest that the gradient for FC diffusion is from fetus to mother and not vice versa.

The results of this study show a reduction in the rate of cholesterol esterification in maternal and fetal blood. Unlike CE, FC is relatively polar, diffusible and rapidly equilibrates between lipoproteins and tissues [23], therefore reduced esterification facilitates cholesterol delivery to fetal and maternal tissues. Previous calculations predict rapid exchange of FC between HDL and tissues, despite its low concentration [31], although reduced esterification might be expected to increase the plasma ratio of FC to CE, as reported previously in cord blood [32]. In this present study of term pregnancies, no significant difference in this ratio was noted between non-pregnant, pregnant or fetal samples in either whole plasma, HDL or the VLDL+LDL fractions. This is probably due to the reduction in the FC/CE ratio towards term [32], presumably as a result of maturation of cholesterol esterification together with an increased demand for FC for fetal growth. The reason for the lower esterification rate is not known. It may be due to immaturity of hepatic LCAT production in the fetus or inadequate activation of LCAT owing to a low surface density of apo A-I in lipid-rich fetal HDL [33], but, as there is also low activity in the mother, oestrogen-dependent suppression may be a factor.

In adults, following esterification of FC on HDL, CE either remains with HDL or is transferred to VLDL in exchange for TAG. The results in this work show for the first time a significant reduction in this process during pregnancy, with an even greater reduction in the fetus. This results in differences in lipoprotein composition which are particularly marked in the fetus and in some respects resemble species that are deficient in CET activity [34]. As the apolipoprotein content of a given lipoprotein class varies little, for example LDL and VLDL contain only one molecule of apo B and HDL an average of three apo A-I molecules per particle, the protein component may be used as a measure of particle number and the lipid/apolipoprotein ratio as a measure of lipoprotein composition. In this study, apo B-containing lipoproteins were grouped together (LDL+VLDL). During pregnancy there was an increase in the number of VLDL+LDL particles with a slightly reduced CE content and increased TAG compared with NPVs. This reflects increased production of TAG-rich VLDL with a relative reduction in CE production on HDL and transfer to VLDL in exchange for TAG. In fetal blood, the number of apo B-containing particles is markedly reduced, but they are of similar composition to the adult. In contrast, fetal HDL particles demonstrate marked enrichment in CE and PL, with TAG depletion. The HDL composition changes can be explained in part by the marked reduction in CET activity, although there is no evidence that suppression of CET
CET may also explain the finding of increased apo E [36] as observed in CET deficiency [37] and the overall impression of a lipoprotein profile of low atherogenicity. Lipid enrichment of HDL, particularly of phospholipid, could be due to reduced hepatic lipase activity. This enzyme hydrolyses HDL-PL and HDL-TAG [38] and correlates inversely with the lipid/protein ratio in HDL [39]. Although no data on the activity of this enzyme in vivo are available, its activity could be suppressed by oestrogens in utero [40].

Surprisingly, lipid-rich fetal HDL particles do not precipitate as HDL₂ in our assay, and others, using gradient gel electrophoresis, have not shown an increase in large HDL [41]. Together these observations suggest that, in the fetus, HDL particles are predominately small but relatively lipid rich and therefore markedly different from adult HDL particles. Longitudinal studies in newborn infants show a fairly rapid progression to an adult lipid profile by the end of the neonatal period [42, 43]; this is partly due to feeding [44, 45], but in addition maturation of cholesterol esterification and transfer may also be factors. The causal link between fetal lipid levels and adult cardiovascular disease suggested by Barker et al. [4] can only be speculative, particularly as we have shown that intrauterine and adult lipid metabolism differ markedly. It is possible that persistence in part of the fetal lipoprotein pattern, with low CET, could lead to higher HDL levels in adults. Alternatively, intrauterine nutritional status may relate to social standing and reflect the prevalence of cardiovascular risk factors later in life.

There was no relationship between maternal and fetal lipoproteins except perhaps for Lp(a). Lipoprotein (a) is a lipoprotein resembling LDL but possessing a unique additional apoprotein designated apo (a) [46]. Its levels are genetically determined and its athero- and thrombogenic effects make it a strong, independent predictor of atherosclerotic cardiovascular disease [47]. Low levels of Lp(a) in umbilical cord blood have been reported previously [48, 49], but in this study, despite the low concentration, there appeared to be a good correlation with maternal levels. However, this may be a chance finding resulting from the small number of subjects, as it suggests that the paternal gene has little influence on cord levels, which is unlikely. The increased Lp(a) level observed in the pregnant subjects may be pregnancy related, but as Lp(a) plasma levels are substantially genetically determined subject selection could account for this. Larger studies are needed to investigate these issues.

In summary, this prospective study has shown lower plasma lipid and apolipoprotein concentrations in the fetoplacental circulation (both arterial and venous) than in paired maternal samples from uncomplicated term pregnancies, except for HDL, which approached maternal levels. Cholesterol esterification was reduced, but additionally this study has shown minimal transfer of CE from HDL to VLDL, which explains in part the lipid enrichment of fetal HDL. An absent arteriovenous gradient across the placenta suggests that transplacental passage or placental synthesis of cholesterol is not significant, furthermore the FC/PL ratios do not favour transfer of FC from mother to fetus, which supports suggestions of abundant fetal synthesis of cholesterol. Conclusions based on single measurements of lipoprotein composition at the time of delivery are limited, but it is possible that recent advances in the application of stable isotopes [50] may be used to shed further light on lipid metabolism during pregnancy and in utero.

REFERENCES