Placenta 87 (2019) 23–29

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Maternal-fetal cholesterol transfer in human term pregnancies

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ARTICLE INFO

Keywords:
Cholesterol metabolism
Placenta
Human
Fetal uptake
Uteroplacental uptake
Maternal-fetal transfer

ABSTRACT

Objectives: The extent to which the human term fetus utilizes cholesterol released from the placenta has remained elusive.

Our aims were to estimate the net mass of cholesterol taken up by the uteroplacental unit, released by the placenta and taken up by the fetus. Thereby we aimed to explore the maternal-fetal cholesterol transfer and hypothesized that maternal levels and uteroplacental uptake were correlated to the fetal uptake of cholesterol.
Methods: A cross-sectional in vivo study of 179 fasting, healthy women with uncomplicated singleton pregnancies. Blood flow in the uterine artery (n = 70) and umbilical vein (n = 125) was measured by Doppler ultrasound. Blood samples from the maternal radial artery, antecubital vein and uterine vein, and the umbilical artery and vein were obtained during cesarean section. Cholesterol was determined enzymatically.
Results: We found a significant uteroplacental uptake (median [Q1,Q3]) of total (3.50 [-36.8,61.1]) and HDL cholesterol (6.69 [-3.78,17.9]) μmol/min, and a fetal uptake of HDL (8.07 [4.48,12.59]), LDL (5.97 [2.77,8.92]) and total cholesterol (13.2 [8.06,21.58]) μmol/min. Maternal cholesterol levels were not correlated to fetal uptake of cholesterol. There was a correlation between uteroplacental uptake of total (rho 0.35, p 0.003) and LDL cholesterol (rho 0.25, p 0.03) and the fetal uptake of LDL cholesterol from the umbilical circulation. The fetal uptake of cholesterol from HDL was higher than from LDL ($p < 0.001$).
Conclusion: Fetal cholesterol uptake is independent of maternal cholesterol levels, but related to the uteroplacental uptake of cholesterol from LDL. This suggests that the placenta influences maternal-fetal cholesterol transfer at term.

1. Introduction

Cholesterol is an essential component of all cell membranes and lipoprotein particles and serves as a precursor for steroid hormones and bile acids. Cholesterol and other sterols also regulate cellular signaling cascades important for the development of various organs and tissues, including the brain and neural tube [1,2]. Together, these diverse roles make cholesterol necessary at all stages of fetal development.

It is currently believed that the fetus establishes its own production of sterols around 20 weeks of gestation [3]. Thereafter, the fetus makes use of cholesterol derived from own synthesis and maternal cholesterol transferred across the placenta [4]. The relative importance of these two sources of cholesterol change during gestation and it has been questioned to which extent the fetus makes use of exogenous cholesterol in late pregnancy [4,5]. Napoli et al. showed that in fetuses younger than 6 months, fetal plasma cholesterol levels correlated with maternal levels [6], whereas this correlation seems to be less consistent near term [6–9]. However, studies of children born with a defective biosynthesis of cholesterol have shown that maternal cholesterol may be transferred across the placenta also at term. The most common defect leads to the Smith-Lemli-Opitz Syndrome. Although affected fetuses have reduced or no ability to synthesize cholesterol, they are born with a certain level of serum cholesterol, and the severity of the syndrome is hypothesized to depend on the amount of trans-placental cholesterol

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https://doi.org/10.1016/j.placenta.2019.09.001
Received 6 March 2019; Received in revised form 16 August 2019; Accepted 2 September 2019

transfer [4,10]. The finding of plant sterols in the fetal circulation also supports that sterols are transferred from the mother to the fetus [11].

The dominating cholesterol carrying lipoproteins in both the maternal and fetal circulations are low density lipoprotein (LDL) and high density lipoprotein (HDL). Several studies have, however, demonstrated that fetal cholesterol levels are markedly lower than maternal levels. Furthermore, the proportion of HDL relative to LDL cholesterol is substantially higher in the fetal than maternal circulation [7,9,12,13]. HDL seems to play an important role in transfer of cholesterol from the villous endothelial cells to the fetal circulation [14]. In addition to this, a net fetal uptake of HDL and LDL cholesterol from the umbilical circulation has been shown in humans [8,15].

It remains unsettled to which extent the human fetus at term takes up cholesterol from the umbilical circulation in normal pregnancy and similarly, how maternal cholesterol levels are related to the actual mass of cholesterol taken up by the fetus.

We have recently established a cohort where we use the 4-vessel sampling method to combine fetal umbilical venous-arterial (v-a) and maternal uteroplacental arteriovenous (AV) concentration differences of several circulating substances with blood flow measurements in the uterine artery and umbilical vein [16]. This enabled us to quantify the net mass of cholesterol taken up by the uteroplacental unit from the maternal circulation as well as the mass released by the placenta to the umbilical circulation and taken up by the human term fetus. Thus, we aimed to explore the correlations between maternal cholesterol levels, uteroplacental and fetal uptake of cholesterol. We hypothesized that maternal cholesterol levels and uteroplacental cholesterol uptake were related to the fetal cholesterol uptake at term.

2. Materials and methods

2.1. Design and study population

We performed a cross-sectional in vivo study of 179 healthy women with uncomplicated singleton pregnancies. The participants have been described in detail in previous publications [16,17]. Briefly, women with planned cesarean section were invited to participate. Exclusion criteria were smoking, significant pre-existing comorbidity, medication (other than levothyroxine), pregnancy complications and onset of labor prior to scheduled cesarean section. Gestational diabetes treated by dietary intervention alone was not an exclusion criterion, since there is no clear metabolic distinction between this group and those defined not to have gestational diabetes.

2.2. Data collection

Clinical data was collected at inclusion and the day of delivery [16,17]. The method including ultrasound examinations and blood sampling has previously been described in detail [16,18].

The blood flow (Q) in the uterine artery and the umbilical vein was measured by Doppler ultrasound 2–3 h before cesarean section [16]. All sonographic examinations were performed by one examiner (GH) using the same machine (Acuson Sequoia 512, Mountain View, CA, USA) [16,18]. Internal vessel diameter (D) was measured in regular B-mode and repeated 5 to 10 times. Time-averaged maximum velocity (TAMX) was measured as close to the site of diameter measurements as possible. The insonation angle was kept low and always $< 30^{\circ}$. The blood velocity was measured over a period of 3–5 s in the vein and over 3 heart cycles in the artery. Blood flow was calculated as: $Q = h \cdot (D/2)^2 \cdot \pi \cdot TAMX$ where h is the coefficient for the spatial blood velocity profile (0.5 in the umbilical vein and 0.6 in the uterine artery).

Blood samples were obtained during cesarean section, which was performed in spinal anesthesia. Just before the uterine incision, blood was drawn from the maternal radial artery (as a proxy for the uterine artery), the uterine vein and the antebrachial vein. Immediately after delivery of the infant, but before cord clamping, blood samples were obtained from the umbilical artery. Blood samples from the umbilical vein were drawn directly after cord clamping and intentionally before delivery of the placenta. The samples were centrifuged at 6 °C, 2500G for 20 min, before the supernatants were carefully removed, leaving 0.5 mL to assure platelet free plasma. We stored the plasma samples at −80 °C.

Samples were analyzed for total cholesterol, low density cholesterol (LDL) and high density cholesterol (HDL) using a colorimetric enzymatic in vitro assay for direct quantification (Roche Diagnostics GmbH, Mannheim, Germany). These assays use both specific blocking and solubility of lipoproteins to separate HDL and LDL cholesterol. Assays were performed by an accredited laboratory according to standard laboratory methods (Department of Medical Biochemistry, Oslo University Hospital, Rikshospitalet). The coefficient of variation for total cholesterol was 2.6%, for LDL 3.5% and for HDL 3.5% if the measured level was < 1 mmol/L and 3.0% if the measured level was > 1 mmol/L.

2.3. Calculations

Assuming similar blood composition in the radial and uterine arteries, we calculated the following AV and v-a concentration differences (mmol/L):

$$\textbf{\textit{Uteroplacental AV difference}}\ [\textbf{cholesterol}] = [cholesterol]_{radial\ artery} - [cholesterol]_{uterine\ vein}$$

$$\textbf{\textit{Antebrachial AV difference}}\ [\textbf{cholesterol}] = [cholesterol]_{radial\ artery} - [cholesterol]_{antebrachial\ vein}$$

$$\textbf{\textit{Fetal umbilical v − a difference}}\ [\textbf{cholesterol}] = [cholesterol]_{umbilical\ vein} - [cholesterol]_{umbilical\ artery}$$

It is likely that a net transfer of water between the maternal, placental and fetal compartments may affect the calculated concentration differences. We have previously published results from representative sub cohorts (maternal circulation n = 40, umbilical circulation n = 30) where we observed significant differences in hemoglobin (Hb) concentrations between the outgoing and incoming vessels on the maternal and fetal sides of the placenta [17]. These findings are consistent with a net uteroplacental uptake of water from the maternal circulation and a net placental release to/fetal uptake of water from the umbilical circulation. In order to take the net passage of water across the placenta into account, we adjusted the uteroplacental AV and umbilical v-a concentration differences in each mother-fetus pair by using the median maternal and fetal Hb ratios from these sub cohorts:

$$\textbf{\textit{Adjusted uteroplacental AV difference}}\ [\textbf{cholesterol}] = [cholesterol]_{radial\ artery} - \left(\frac{[cholesterol]_{uterine\ vein}}{1.017}\right)$$

$$\textbf{\textit{Adjusted umbilical v − a difference}}\ [\textbf{cholesterol}] = [cholesterol]_{umbilical\ vein} - \left(\frac{[cholesterol]_{umbilical\ artery}}{1.048}\right)$$

According to Fick's principle we calculated the mass of cholesterol (μmol/min) taken up by the fetus and by the uteroplacental unit by multiplying the adjusted AV concentration differences (mmol/L) by the volume blood flow in the umbilical vein and uterine artery, respectively [19]. We assumed steady state and considered placental release to be equal to fetal uptake of cholesterol.

$$\textbf{\textit{Uteroplacental uptake}} = Adjusted\ uteroplacental\ AV\ difference\ [cholesterol] \times Q_{uterine\ artery}$$

$$\textbf{Fetal uptake} = \text{placental release} = \text{Adjusted umbilical } v - a \text{ difference [cholesterol]} \times Q_{\text{umbilical vein}}$$

2.4. Statistics

Descriptive data are reported as numbers with percentages, and mean values with standard deviation (SD) if normally distributed, or as median with quartiles (Q1, Q3) if skewed. Comparisons of the paired cholesterol concentrations and mass uptake of cholesterol were performed by paired t-tests or Wilcoxon signed rank test as appropriate. Correlations are reported with Pearson's, or Spearman's correlation coefficients according to the data distribution. A two-sided p-value $<$ 0.05 was considered significant. We performed all analyses using Statistical Package for the Social Sciences, Version 25.0 (SPSS Inc., IBM).

2.5. Study approval

All participants signed a written informed consent prior to inclusion. The study was approved by the Institutional Review Board at Oslo University Hospital and the Regional Committee for Medical and Health Research Ethics, South East Norway 2419/2011. Application number S-07174a.

3. Results

3.1. Clinical characteristics

The clinical characteristics of the women and their infants are shown in Table 1. The women had a mean age of 35.4 years and the majority was multiparous and well-educated. Their infants were born at term with a mean birthweight of 3546 g and a mean placental weight of 617 g.

3.2. Volume blood flow

In a subset of 77 mother-fetus pairs blood flow in the uterine artery was determined by Doppler ultrasound. Likewise, we determined the blood flow in the umbilical vein in 128 mother-fetus pairs. These measurements have been published elsewhere [20]. The median blood flow in the uterine artery was 488.4 mL/min [358.5, 604.4] and in the umbilical vein 196.2 mL/min [158.3, 232.3]. Intra observer variation measured as intra class correlation coefficient for umbilical vein diameter was 0.97 [21]. The equivalent coefficient for uterine artery diameter was 0.97 (n = 16).

3.3. Cholesterol concentrations, AV differences (mmol/L) and calculated uptake (μmol/min)

The mean concentrations of maternal and fetal total, LDL and HDL cholesterol in the four vessels as well as the maternal antebrachial vein at the time of cesarean section are listed in Table 2. The mean maternal AV and fetal umbilical v-a differences are presented both unadjusted and adjusted for changes in hemoglobin concentrations (Table 2). Unadjusted we found a negative uteroplacental AV concentration difference of total, LDL and HDL cholesterol, indicating a uteroplacental release to maternal circulation. However, when we adjusted for changes in hemoglobin concentration we found a significant uteroplacental uptake of total and HDL cholesterol, but no significant uptake or release of LDL cholesterol. We found positive and significant umbilical v-a differences for LDL and HDL cholesterol both with and without adjustment (Table 2).

The calculated mass of cholesterol taken up by the uteroplacental unit and the fetus is presented in Table 3. The fetal uptake (placental release) of HDL cholesterol from the umbilical circulation was significantly higher than LDL cholesterol (Table 3). Further, the placental release of HDL cholesterol to the umbilical vein as a fraction of total cholesterol release (HDL cholesterol release/total cholesterol release) was higher than the corresponding placental release of LDL cholesterol (LDL cholesterol release/total cholesterol release) (0.42 vs 0.33, $p < 0.001$). Fetal, but not uteroplacental cholesterol uptake was correlated to birthweight (rho 0.21, $p < 0.05$ and rho ranging from -0.05 to 0.05, p-values 0.7 to 0.9, respectively), and to placental weight (rho ranging from 0.18 to 0.26, $p < 0.05$; rho -0.02 to 0.05, p-values 0.7 to 0.9, respectively).

Table 1
Clinical characteristics of the participating women and infants.

	N (%)	Mean (SD)	Median [Q1,Q3]	Range
Women	179			
Age, years		35.4 (3.8)		23–44
Para 0	44 (24.6)			
Para 1	79 (44.1)			
Para > 1	56 (31.3)			
BMI pregestational, kg/m^2			22.3 [20.8, 25.4]	17.0–47.6
Gestational weight gain, kg			15.1 [12.0, 17.8]	−1.2–31.3
Smoking during pregnancy[a]	0			
Higher education, > 15years	155 (86.6)			
Married or partnership	174 (97.2)			
IVF	15 (8.4)			
Gestational diabetes[b]	4 (2.2)			
Infants	179			
Gestational age, weeks		39.2 (0.6)		37.1–42.0
Birthweight, g		3546 (443)		2297–4955
Apgar score < 7 after 5 min	0			
Placental weight, g		617 (133)		310–1115
Male sex	99 (55.3)			

Abbreviations: SD, Standard Deviation; Q1, First Quartile; Q3, Third Quartile; BMI, Body Mass Index; kg, kilograms; g, grams; IVF, In Vitro Fertilization.

[a] 7 women stopped smoking when pregnancy was confirmed in first trimester.

[b] Defined after WHO-criteria (1999) of fasting plasma glucose ≥ 7.0 mmol/L and plasma glucose ≥ 7.8 mmol/L 2 h after oral glucose tolerance test of 75 g glucose. None of the 4 needed any other treatment but diet.

Table 2
Cholesterol concentrations and AV concentration differences (mmol/L) (n = 179).

	Total cholesterol		LDL cholesterol		HDL cholesterol	
	Mean (SD) or (95% CI)	p-value	Mean (SD) or (95% CI)	p-value	Mean (SD) or (95% CI)	p-value
Radial artery	5.94 (1.05)		3.59 (0.97)		1.70 (0.41)	
Uterine vein	6.02 (1.06)		3.66 (0.99)		1.72 (0.42)	
Antebrachial vein	5.94 (1.03)		3.59 (0.97)		1.70 (0.41)	
Umbilical vein	1.56 (0.36)		0.55 (0.19)		0.750 (0.24)	
Umbilical artery	1.55 (0.35)		0.54 (0.19)		0.738 (0.23)	
Paired uteroplacental AV difference	-0.075 (-0.100 – -0.450)	< 0.001[a]	-0.067 (-0.081 – 0.053)	< 0.001[a]	-0.021 (-0.027 – -0.015)	< 0.001[a]
Paired adjusted uteroplacental AV difference [b]	0.027 (0.003 – 0.052)	0.03[a]	-0.005 (-0.019 – 0.009)	0.49[a]	0.008 (0.002 – 0.015)	0.01[a]
Paired antebrachial AV difference	-0.003 (-0.026 – 0.044)	0.90[c]	-0.009 (-0.031 – 0.0125)	0.40[c]	0.003 (-0.009 – 0.015)	0.62[c]
Paired umbilical v-a difference	0.079 (-0.003 – 0.019)	0.16[d]	0.009 (0.005 – 0.013)	< 0.001[d]	0.012 (0.006 – 0.018)	< 0.001[d]
Adjusted umbilical v-a difference[b]	0.080 (0.068 – 0.091)	< 0.001[d]	0.034 (0.029 – 0.039)	< 0.001[d]	0.046 (0.039 – 0.052)	< 0.001[d]

Abbreviations: SD, standard deviation; CI, confidence interval; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; AV, arteriovenous, v-a, veno-arterial.
[a] Comparison between concentrations in the radial artery and uterine vein.
[b] Adjusted for change in hemoglobin concentration.
[c] Comparison between concentrations in radial artery and antebrachial vein.
[d] Comparison between concentrations in the umbilical vein and artery.

Table 3
Calculated uteroplacental and fetal cholesterol uptake (μmol/min).

	Uteroplacental uptake from maternal circulation (n = 70)[a]		Fetal uptake from umbilical circulation (n = 125)[b]	
	Median	[Q1, Q3]	Median	[Q1, Q3]
Total cholesterol	3.50	[-36.8, 61.1]	13.2	[8.06, 21.58]
LDL cholesterol	3.87[c]	[-16.11, 27.49]	5.97	[2.77, 8.92]
HDL cholesterol	6.69	[-3.78, 17.9]	8.07[d]	[4.48, 12.59]

Abbreviations: Q1, First Quartile; Q3, Third Quartile; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein.
[a] In 7 of the cases which included maternal flow measurements maternal arterial blood sampling was unsuccessful.
[b] In 3 of the cases with available fetal flow measurements fetal arterial blood sampling was unsuccessful.
[c] No significant median LDL cholesterol uptake was demonstrated.
[d] Fetal uptake of HDL cholesterol was significantly higher than the uptake of LDL cholesterol compared by Wilcoxon signed rank test ($p < 0.001$).

3.4. Correlations between maternal and fetal cholesterol

Apart from a negative correlation between maternal HDL cholesterol concentrations and LDL cholesterol levels in the umbilical vein (r −0.16, $p < 0.05$, all three maternal vessels), we found no correlations between concentrations of cholesterol (total, LDL or HDL) in the maternal radial artery and the umbilical vein (r ranging from 0.00 to 0.12, *p*-values 0.99 to 0.12). However there was a correlation between maternal and fetal HDL/LDL ratios (rho 0.21, *p* 0.007).

The maternal arterial cholesterol levels were not correlated to the umbilical v-a concentration differences (mmol/L) of cholesterol on the fetal side of the placenta (r ranging from 0.13 to 0.10, *p*-values 0.27 to 0.11). Further, the maternal cholesterol levels were not correlated to the fetal uptake of cholesterol (μmol/min) (rho ranging from 0.00 to 0.05, *p*-values 0.60 to 0.99). The uteroplacental uptake of HDL cholesterol was not correlated to fetal uptake of cholesterol of any fractions (Table 4). Even if we could not demonstrate a significant uteroplacental uptake of LDL cholesterol, there was a correlation between the uteroplacental uptake of total and LDL cholesterol from the maternal circulation and the fetal uptake of LDL cholesterol from the umbilical

Table 4
Correlations between uteroplacental and fetal cholesterol uptake (n = 70).

		Uteroplacental uptake of total cholesterol rho	p-value	Uteroplacental uptake of LDL cholesterol rho	p-value	Uteroplacental uptake of HDL cholesterol rho	p-value
Fetal uptake total cholesterol	Uptake μmol/min	0.19	0.12	0.16	0.18	−0.01	0.94
	v-a difference mmol/L	0.27	0.03	0.19	0.11	0.06	0.65
Fetal uptake LDL cholesterol	Uptake μmol/min	0.35	0.003	0.25	0.03	0.18	0.13
	v-a difference mmol/L	0.37	0.002	0.28	0.02	0.20	0.09
Fetal uptake HDL cholesterol	Uptake μmol/min	0.21	0.08	0.21	0.08	0.07	0.54
	v-a difference mmol/L	0.25	0.04	0.28	0.02	0.13	0.30

Abbreviations: rho, Spearman's rank correlation coefficient; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; AV, arteriovenous, v-a, veno-arterial.

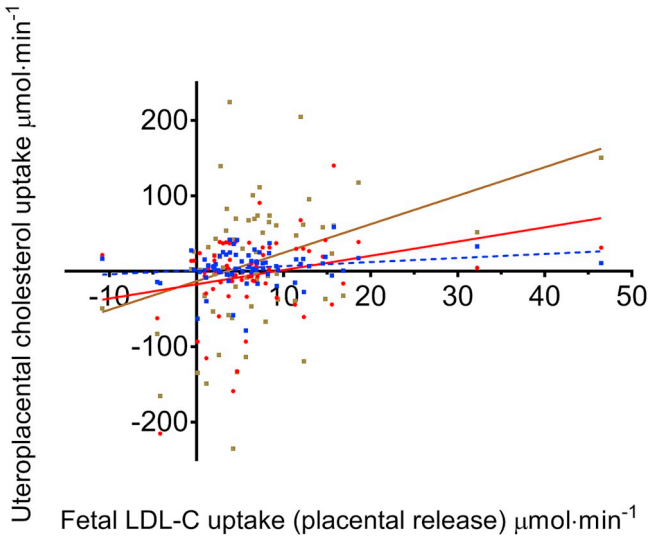

Fig. 1. Uteroplacental uptake of total (golden) and LDL (red), but not HDL (blue) cholesterol correlates with fetal cholesterol uptake (n = 70).

circulation (Fig. 1 and Table 4). The same association was also present when fetal uptake was adjusted for birthweight (µmol/min/kg) (rho 0.33, $p < 0.05$ and rho 0.23, p 0.055) respectively.

3.5. Correlation between umbilical cholesterol concentration and fetal uptake of cholesterol

We found a significant positive correlation between the cholesterol level of a given cholesterol fraction in the umbilical vein and the corresponding fetal uptake of the same cholesterol fraction (Table 5). Furthermore, the levels of different cholesterol fractions in the umbilical vein were also significantly correlated with the fetal uptake of the other cholesterol fractions (Table 5).

4. Discussion

In contrast to our hypothesis we found no strong or direct relation between maternal levels of total, LDL and HDL cholesterol and the fetal uptake (placental release) of cholesterol. Still we demonstrated that the uteroplacental uptake (µmol/min) of total and LDL cholesterol from the maternal circulation was related to the fetal uptake (placental release) (µmol/min) of LDL cholesterol. This finding was supported by significant correlations between umbilical v-a concentration differences (mmol/l) (both the LDL and HDL cholesterol fractions) and the uteroplacental uptake of total and LDL cholesterol. Thus, even though fetal cholesterol uptake seems to operate independently of maternal cholesterol levels, our results indicate that the placenta influences fetal cholesterol uptake at term.

The ability of the placenta and the syncytiotrophoblast to take up cholesterol from the maternal circulation has been demonstrated in previous in vitro and animal studies [22–26]. Tracer studies have demonstrated transfer of cholesterol from the maternal to the fetal circulation in early pregnancy [27], and transfer has been suggested by assessment of cholesterol precursors in second trimester of pregnancy [28]. The significance of maternal-fetal cholesterol transfer in late gestation, as fetal cholesterol synthesis grows more important, has been questioned [5]. In line with several earlier studies [6–8], we found no positive correlations between maternal and fetal cholesterol levels. Whether cholesterol of placental origin (from stores or de novo synthesis) influences these relations is unknown. Nevertheless, our data indicated a uteroplacental uptake of cholesterol at term, significant for total cholesterol and the HDL cholesterol fraction, although the result should be interpreted with caution as the calculation was dependent on adjustment for placental water transfer. The fact that we could not demonstrate a significant uteroplacental uptake of cholesterol from LDL in the whole group was unexpected given that the syncytiotrophoblast is capable of uptake of LDL particles by endocytosis [29]. Our finding does, however not exclude placental uptake of maternal LDL. At the time of sampling, LDL cholesterol was taken up by the placenta in some individuals, whereas it was released by the placenta in others. In each individual, the placental handling of maternal LDL seemed to be reflected in what was released to the fetal circulation, as demonstrated by the significant correlation between uteroplacental uptake of LDL cholesterol and fetal cholesterol uptake. Importantly, as discussed below, the uteroplacental uptake should be interpreted with caution, because the variability of the uteroplacental uptake of cholesterol between individuals is high and may reflect both biological and methodological variability.

In an in vitro model using a polarized trophoblast cell line, Schmid et al. studied the cholesterol transport across a placental monolayer. The authors found increasing cholesterol efflux to the basolateral surface with increasing levels of cholesterol on the apical side [30]. Napoli et al. showed that maternal hypercholesterolemia during pregnancy was associated with fatty streak like changes in the fetal aorta, changes that may lead to atherosclerotic lesions later in life [31]. Both these studies indicate a connection between maternal cholesterol levels and trans-placental cholesterol transfer. This is in contrast to our findings, which demonstrate that fetal levels and uptake of cholesterol are independent of maternal levels at term. We did however find a significant inverse correlation between maternal HDL and fetal LDL cholesterol levels, and a correlation between the maternal and fetal HDL-LDL ratios, even in our healthy pregnancies, indicating a link between maternal and fetal cholesterol profiles which might be in line with an inherited atherosclerotic risk profile as demonstrated by Napoli [31].

In 1935, Boyd and Wilson reported that the human fetus at birth appeared to absorb very large quantities of phospholipids from umbilical blood together with free and esterified cholesterol [15]. Net fetal uptake of cholesterol from the umbilical circulation defined as v-a concentration differences has been reported previously in non-fasting women with vaginal deliveries [8,32]. Parker et al. found umbilical v-a differences of cholesterol that were 5–6 times higher than in the present study [8]. Yoshimitsu et al. showed that umbilical venous serum lipid levels increased during vaginal delivery [33]. Thus, the divergent findings may be explained by labor and maternal dietary intake. Despite these discrepancies, our study confirms a significant fetal uptake of cholesterol from the umbilical circulation at term. Another common feature of the studies is individual variation in fetal cholesterol uptake. In a detailed study of umbilical lipoprotein fractions, Neary and coworkers did not find umbilical v-a differences for any of the lipoprotein fractions in 13 full-term pregnant women [7]. Given the variation in

Table 5
Correlations between umbilical cholesterol levels (mmol/L) and fetal cholesterol uptake (µmol/min) (n = 125).

	Fetal uptake of total cholesterol r	p-value	Fetal uptake of LDL cholesterol r	p-value	Fetal uptake of HDL cholesterol r	p-value
Total cholesterol umbilical vein	0.36	< 0.001	0.44	< 0.001	0.44	< 0.001
LDL cholesterol umbilical vein	0.21	0.02	0.39	< 0.001	0.16	0.07
HDL cholesterol umbilical vein	0.29	0.001	0.34	< 0.001	0.53	< 0.001

Abbreviations: r, Pearson's correlation coefficient; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein.

cholesterol uptake, their contrasting results could be due to lower statistical power.

The apolipoprotein composition in the fetal circulation may be of importance for the trans-placental cholesterol transfer. It has previously been reported that HDL seems to play a relatively dominant role in the transfer of cholesterol between the placenta and the fetus [9,14], with HDL_3 as the main cholesterol acceptor in fetal blood. The present in vivo work is in accordance with this notion, because the fetal uptake of cholesterol from HDL was significantly higher than that from LDL. The proportion of HDL relative to LDL cholesterol is substantially higher in the fetal than adult circulation, which may facilitate trans-placental transfer of cholesterol. Together these findings indicate that HDL serves different functions in the fetoplacental unit than in born individuals. Stefulj et al. demonstrated that human placental endothelial cells released cholesterol to lipid free Apo-1 and HDL particles [14]. They further demonstrated two possible synergistic mechanisms for regulation of maternal-fetal cholesterol transfer to HDL. The release of cholesterol to ApoA-1 and HDL was increased both as a response to ligands to liver-x-receptors (oxysterols) and if the placental endothelial cells were preloaded with cholesterol. Thus, placental cholesterol transfer to the umbilical circulation might be regulated both by cholesterol availability and possibly by fetal demands. The correlation between uteroplacental cholesterol uptake and fetal uptake in our data provides some support for this notion, but placental release was independent of cholesterol availability in the maternal circulation. As suggested by Stefulj et al., HDL seems to be involved in transfer of cholesterol from endothelial cells in the placental vasculature, and may also play a role in maintenance of endothelial functions [34]. As a consequence, HDL might have a local cytoprotective function [35]. Averna et al. found that HDL was strongly positively correlated with apoE, apoCII and apoCIII, none of which were correlated with HDL in adult sera [12]. van Biervliet speculated that the high fraction of HDL enriched in apoE, apoAII and cholesterol might compensate for the relatively low LDL levels and function as an additional source for cholesterol delivery to the fetal peripheral tissue via the apo (B,E)-receptor [36].

LDL is predominantly a cholesterol donor. It has been shown that LDL is an important source of cholesterol in the fetal adrenal steroidogenesis [37], and that fetal liver expresses LDL-receptors. This indicates that LDL supplies cholesterol to fetal organs as it does in born individuals [38,39]. The relative concentrations of LDL and HDL cholesterol appear to change in the last trimester [39]. In particular there is a decrease in LDL cholesterol attributed to increased utilization of cholesterol in the fetal adrenal steroid synthesis [40,41]. We found a significant fetal uptake (placental release) of LDL cholesterol at term. To our knowledge it has never been reported that LDL may be enriched with cholesterol by direct transfer of cholesterol molecules from cells as has been established for HDL. Thus, one implication of our findings is that the placenta may release LDL, i.e. apoB. The syncytiotrophoblast has been reported to release apoB in vitro [42,43], making further studies on trophoblast release of apoB highly warranted. The apparent placental release of LDL cholesterol to the fetal circulation may have other explanations. The presence of cholesterol ester transfer proteins, as well as lecithin-cholesterol acyltransferase (LCAT) has been reported in the fetoplacental circulation [35,44,45]. A transfer of free cholesterol from the villous endothelium to HDL, immediately followed by esterification and exchange of cholesterol esters to LDL for triglycerides could be an alternative explanation of the apparent release of LDL cholesterol from placenta.

As previously reported, we found a significantly higher hemoglobin concentration in the uterine vein compared to the radial artery, and in the umbilical artery compared to the vein [16,17]. We believe that the most likely explanation is a net flux of water from the maternal circulation to the placenta and from the umbilical circulation to the fetus. Consequently, placental water exchange needs to be considered in studies of transfer of compounds across the placenta. This assumption is supported by the fact that the calculation of the uteroplacental uptake at the maternal side of the placenta was highly dependent on adjustment for observed AV differences in hemoglobin concentrations. Thus, the calculation of the uteroplacental cholesterol uptake is uncertain. Nevertheless, on the fetal side a net uptake of cholesterol from both LDL and HDL was demonstrated independently of adjustment, although the quantitative estimates differed.

Strengths of this study include the large sample size of mother-fetus pairs, the arteriovenous blood sampling on both sides of the placenta and flow measurements under standardized conditions with fasting women delivered by pre labor cesarean section. By measuring the blood flow and adjusting for the change in hemoglobin concentrations following passage through the fetus, the present study adds a significant advancement compared to previous attempts to evaluate the cholesterol transfer between the mother, the placenta and the umbilical circulation.

Our study cannot account for fetal or possible placental cholesterol synthesis and the in vivo design has some limitations. The blood flow measurements were obtained prior to surgery and before spinal anesthesia, both of which may affect blood flow in the uteroplacental unit, and thus our estimates of cholesterol uptake. Even though the biological variability in our data is plausible, as discussed above, the calculation of cholesterol uptake depends on several measured parameters causing variance to accumulate. Hence, variability will increase and statistical power may decrease, and there is a risk that we fail to discover certain relations. Nonetheless, we demonstrated that uteroplacental and fetal cholesterol uptake were correlated. The cholesterol concentrations in the radial artery and the antebrachial vein were identical, adding validity to our approach. Furthermore, the quantitative estimates of the uteroplacental and fetal cholesterol uptakes must be considered with caution, not only for methodological reasons, but also for the fact that they were obtained at one single time point during maternal fasting. Our measurements do not differ between lipoprotein sub fractions which might potentially demonstrate more subtle relations. These relations do not; however, seem to affect the overall relation between maternal cholesterol levels, uteroplacental cholesterol uptake and fetal cholesterol uptake. Nevertheless, there is a need to reproduce these findings on a more sensitive lipid analysis platform. Finally, the relations between maternal and fetal cholesterol might be different earlier in pregnancy, as both the lipid and the apolipoprotein composition of the lipoproteins in maternal and fetal circulations differ across gestation [13,46,47].

5. Conclusion

In healthy, human term pregnancies the fetus takes up cholesterol released by the placenta. We found a substantial placental release and a corresponding fetal uptake of both LDL and HDL cholesterol, with HDL being the quantitatively dominant external source of cholesterol for the fetus. The fetal uptake appeared independent of the maternal cholesterol levels, but was related to the uteroplacental uptake of cholesterol from LDL in the maternal circulation indicating that the placenta might be involved in regulating cholesterol transfer.

Funding

The South-Eastern Norway Regional Health Authority (grant number 2013043 A.M.H., grant number 2016066 M.B.H. and grant number 2015025 T.M.M.) and the Norwegian National Advisory Unit on Women's Health (M.C.P.R. and T.M.M.) provided financial support to conduct the research. H.H. was funded by Oslo University Hospital and the University of Oslo. The authors declare no conflict of interest related to the performed work. The funders had no involvement in the study design, data collection, analyses or interpretation. Further, they were not involved in the writing or the decision to publish this paper.

Declaration of interests

None.

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