



# Cholesterol acceptor capacity is preserved by different mechanisms in preterm and term fetuses



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## ABSTRACT

Fetal serum cholesterol and lipoprotein concentrations differ between preterm and term born neonates. An imbalance of the flow of cholesterol from the sites of synthesis or efflux from cells of peripheral organs to the liver, the reverse cholesterol transport (RCT), is linked to atherosclerosis and cardiovascular disease (CVD). Pre-term delivery is a risk factor for the development of CVD. Thus, we hypothesized that RCT is affected by a diminished cholesterol acceptor capacity in preterm as compared to term fetuses. Cholesterol efflux assays were performed in RAW264.7, HepG2, and HUVEC cell lines. In the presence and absence of ABC transporter overexpression by TO-901317, umbilical cord sera of preterm and term born neonates ( $n = 28$  in both groups) were added. Lipid components including high density lipoprotein (HDL), low density lipoprotein (LDL), apolipoprotein A1, and apolipoprotein E were measured and related to fractional cholesterol efflux values. We found overall, fractional cholesterol efflux to remain constant between the study groups, and over gestational ages at delivery, respectively. However, correlation analysis revealed cholesterol efflux values to be predominantly related to HDL concentration at term, while in preterm neonates, cholesterol efflux was mainly associated with LDL. In conclusion cholesterol acceptor capacity during fetal development is kept in a steady state with different mechanisms and lipid fractions involved at distinct stages during the second half of fetal development. However, RCT mechanisms in preterm neonates seem not to be involved in the development of CVD later in life suggesting rather changes in the lipoprotein pattern causative.

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## 1. Introduction

Over the past decades preterm birth rates have considerably risen in Europe and the United States of America and at present, about 9.6% of all live births occur before 37 weeks of gestation worldwide [1]. Preterm delivery is associated with an increased cardiovascular risk later in life, including the development of high blood pressure in early adulthood [2–5]; the underlying mechanisms are not fully understood. Beyond hypertension, cardiovascular events in general are linked to an altered cholesterol metabolism. Elevated serum concentrations of the low density lipoprotein (LDL), and low concentrations of the high density lipoprotein (HDL) are strongly associated with the development of atherosclerotic diseases [6,7], and are commonly used as surrogate endpoints for atherosclerosis in clinical trials. Moreover, serum lipid profiles in childhood are predictive of those in adulthood [8,9], and it has been assumed that this association may originate at birth [10,11]. The cord blood lipid profile at birth is to a large extent dependent on

the gestational age at delivery. Both total cholesterol (TC) and LDL concentrations are higher in preterm as compared to neonates born at term [12–14]. On the contrary, fetal HDL levels remain constant throughout gestation. As a result, the atherogenic index given by the ratio of LDL/HDL in cord blood is higher in preterm as compared to term neonates. Of note, the concentrations of the main apolipoprotein (apo) A-I associated with HDL, progressively rise between 21 and 34 weeks of gestation suggesting changes in HDL composition during fetal development [14,15].

Lipoproteins are involved in the development of atherosclerosis. An increased cholesterol influx and the oxidation of LDL to oxLDL particles are prerequisites for rapid accumulation of LDL in macrophages of the vascular wall, leading to foam cell formation and fatty plaque development [16]. The major mechanism by which the vascular wall is protected from cholesterol overload is reverse cholesterol transport (RCT) [17]. RCT is defined as the transport of cholesterol originating from peripheral organs or macrophages to an acceptor in plasma, followed by its transfer to the liver where it is either eliminated via bile acids or recycled; RCT is tightly regulated and kept in a steady state. Several pathways that regulate cholesterol efflux (CE), which is the first and most probable rate limiting step of RCT, have been described so far. First, the ATP binding cassette transporter ABCA1-

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dependent pathways lead to the transfer of cholesterol to a lipid-free apoA-I. Next, the ABCG1 and the scavenger receptor B1 (SR-B1) pathways involve cholesterol loading to mature HDL [18,19]. Finally, simple diffusion of cholesterol, mainly to non-specific proteins like albumin, occurs through the plasma membrane [20].

These different pathways involved in CE are cell-type specific. In macrophages, CE occurs mainly to delipidated apoA-I with the ABCA1 pathway predominantly contributing to cholesterol export. SR-B1 and other molecules play a supplementary role [18,21]. Accordingly, overexpression of ABCA1 in macrophages increases CE [19,22]. In other cell types like the human HepG2 hepatoma cells CE to HDL particles is primarily mediated by SR-B1 [21,23]. The mechanisms in endothelial cells (such as human umbilical vein endothelial cells (HUVECs)) are conflicting. Some authors have suggested that neither ABC transporters, nor SR-B1 seems to mainly contribute to apoA1 mediated CE [19,24], and therefore it has been speculated that aqueous diffusion and yet unidentified, additional factors might be involved [20]. However, a recent publication provided evidence for a role of ABCG1 in HUVECs [25].

Apart from cell specific efflux pathways, the amount and composition of acceptors in the plasma strongly influences CE. HDL and apoA1 are generally regarded as the main cholesterol acceptors. However, levels of HDL and apoA1 accounted for less than 40% of the variation observed in response to whole patient's serum, supporting the hypothesis that other molecules are involved in mediating CE apart from total HDL and apoA1 concentrations [23,26,27].

Since cord blood lipid composition differs between preterm and term born neonates, we hypothesized that CE to cord blood is less effective in preterm as compared to term neonates. If maintained, ineffective CE could well contribute to later life cardiovascular risk. To test the hypothesis, we first aimed to analyze the fetal serum composition obtained from cord blood of term and preterm neonates, and second, to test the CE from various cell types to fetal serum in different conditions to measure acceptor-specific CE.

## 2. Material and methods

### 2.1. Patients

The study was approved by the Ethics' Committee of the Medical Faculty of the Technical University of Aachen (EK119/08, EK154/11). Written informed consent was obtained from each patient (parents). Biomaterial of Caucasian patients and their neonates delivered at our hospital was sampled between October 2008 and March 2012. Gestational age was calculated by last menstrual period and verified

by first trimester scan documentation. Fetal and neonatal birth weight percentiles were determined according to the population-based, newborn weight charts [28]. Only neonates of singleton pregnancies with birth weight adequate for gestational age (more than the 10th and lower than the 90th percentile) were included. The mothers were confirmed to be free of gestational hypertension/preeclampsia, suspected diabetes, intrauterine growth restriction, or severe chronic diseases. Neonates with fetal anomalies, abnormal fetal karyotype, and positive TORCH screening results were excluded. Clinical characteristics and cord blood lipid values were in part already described elsewhere [29]. 56 neonates born at term ( $n = 28$ ) and preterm ( $n = 14$  in each preterm group) were chosen for availability of sufficient sample volume, and to allow matching for fetal gender, mode of delivery, and maternal BMI as much as possible. Neonates were clustered into three groups according to their gestational age at delivery: group (i) early preterm neonates,  $24 + 0$  to  $31 + 6$  weeks of gestation (WOG), group (ii) late preterm neonates,  $32 + 0$  to  $36 + 6$  WOG, and group (iii) term neonates,  $37 + 0$  to  $40 + 6$  WOG. Additional experiments have been performed with half of the cohort in an explorative manner. Study groups for these complementary analyses have been renamed in accordance to the primary study groups (i'), (ii'), and (iii').

### 2.2. Sample collection and preparation

Umbilical vein serum was sampled and stored as previously described [29]. Immediately after delivery blood was drawn from the double clamped cord using monovette syringes (S-Gel-Monovette, Sarstedt, Germany). After incubation at room temperature for 15–30 min, samples were subjected to sedimentation of blood cells by centrifugation at 2000 g at room temperature for 15 min. Serum was aspirated and divided into aliquots, which were stored at  $-80^{\circ}\text{C}$ . Altogether, time between blood sample collection and storage of serum aliquots averaged below 1 h. Sample storage duration ranged from 1.5 to 5.3 years as indicated in Table 1.

To estimate arterio-venous differences of CE response we sampled arterial cord blood in a subset of neonates ( $n = 4$ ). Moreover, since CE experiments using fetal sera have not been performed in such a detail before, we aimed to estimate and characterize CE kinetics by comparing fetal serum cholesterol acceptor capacity to sera of their mothers. Maternal serum from women delivered at term (38 and 39 WOG,  $n = 2$ ) and preterm (32 and 33 WOG,  $n = 2$ ) was taken antenatally, and 11 to 20 weeks post-partum ( $n = 3$ ), respectively from the antecubital vein. Sera of the mothers were processed equally

**Table 1**  
Maternal and neonatal clinical baseline characteristics. Clinical data are presented as means and 95% confidence interval (95% CI) or as percentages. WOG = weeks of gestation at delivery.

|                                                          | (i) 24–32 WOG<br>( $n = 14$ ) (ii) |             | 33–36 WOG<br>( $n = 14$ ) |             | (iii) 37–41 WOG ( $n = 28$ ) |             |
|----------------------------------------------------------|------------------------------------|-------------|---------------------------|-------------|------------------------------|-------------|
|                                                          | Mean or %                          | 95% CI      | Mean or %                 | 95% CI      | Mean or %                    | 95% CI      |
| Maternal age (y)                                         | 30.4                               | 27.6–33.3   | 30.7                      | 27.6–33.8   | 32.3                         | 30.4–34.3   |
| Maternal BMI before pregnancy ( $\text{kg}/\text{m}^2$ ) | 24.1                               | 20.7–27.4   | 22.4                      | 20.7–24.1   | 23.9                         | 22.1–25.6   |
| Primiparity (%)                                          | 42.9                               |             | 64.3                      |             | 35.7                         |             |
| Systolic blood pressure prior to birth (mm Hg)           | 113                                | 107.5–118.4 | 117                       | 111.5–121.9 | 115                          | 110.5–119.8 |
| Diastolic blood pressure prior to birth (mm Hg)          | 60                                 | 54.1–66.5   | 61                        | 56.2–66.6   | 70                           | 66.2–73.7   |
| Smoking status during pregnancy (%)                      | 21.4                               |             | 14.3                      |             | 10.7                         |             |
| Smoking status before pregnancy (%)                      | 35.7                               |             | 21.4                      |             | 21.4                         |             |
| Gestational weeks at delivery (wk)                       | 27.7                               | 26.3–29.2   | 34.3                      | 33.6–35.0   | 39.1                         | 38.7–39.5   |
| Mode of delivery (in % vaginal birth)                    | 14.3                               |             | 57.1                      |             | 35.7                         |             |
| Fetal gender (in % of female)                            | 50                                 |             | 42.9                      |             | 50                           |             |
| Fetal birth weight (g)                                   | 1146                               | 928–1364    | 2387                      | 2161–2613   | 3421                         | 3289–3553   |
| Fetal birth weight percentile                            | 48.9                               | 38.2–59.6   | 48.9                      | 35.4–62.5   | 51.6                         | 42.9–60.4   |
| Umbilical artery pH                                      | 7.36                               | 7.33–7.39   | 7.32                      | 7.26–7.37   | 7.29                         | 7.27–7.32   |
| Neonatal 5 min Apgar score                               | 8.5                                | 7.7–9.3     | 9.7                       | 9.4–10.0    | 9.7                          | 9.4–10.0    |
| Betamethasone treatment within the last 5 days (%)       | 50.0                               |             | 21.0                      |             | 0.0                          |             |
| Years of sample storage at $-80^{\circ}\text{C}$         | 3.27                               | 2.76–3.78   | 3.30                      | 2.84–3.76   | 3.14                         | 2.94–3.34   |

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