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Fatty acid and lipid profiles in primary human trophoblast over 90 h in culture☆

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ABSTRACT

Little is known about the mechanisms underlying the preferential transport of long chain polyunsaturated fatty acids (LCPUFA) to the fetus by the syncytiotrophoblast and the role of cytotrophoblasts in placental lipid metabolism and transport. We studied primary human trophoblast (PHT) cells cultured for 90 h to determine the fatty acid and lipid composition of cytotrophoblast (18 h culture) and syncytiotrophoblast (90 h culture) cells. In cultured PHT total lipid fatty acids were significantly ($P < 0.05$) reduced at 90 h compared to 18 h in culture including lower levels of palmitic acid (PA, 16:0, −37%), palmitoleic acid (POA, 16:1n-7, −30%), oleic acid (OA, 18:1n-9, −31%), LCPUFA arachidonic acid (AA, 20:4n-6, −28%) and α-linolenic acid (ALA, 18:3n-3, −55%). In major lipid classes, OA and most of the n-3 and n-6 LCPUFA were markedly lower at 90 h in TG (−57 to −76%; $p < 0.05$). In the cellular NEFA, n-6 LCPUFA, dihomo-γ-linolenic acid (DGLA, 20:3n-6) and AA were both reduced by −51% and DHA was −55% lower ($p < 0.05$) at 90 h. In contrast, phospholipid FA content did not change between cytotrophoblasts and syncytiotrophoblast except for OA, which decreased by −62% ($p < 0.05$). Decreasing PHT TG and NEFA lipid content at 90 h in culture is likely due to processes related to differentiation such as alterations in lipase activity that occur as cytotrophoblast cells differentiate. We speculate that syncytiotrophoblast prioritizes PL containing AA and DHA for transfer to the fetus by mobilizing FA from storage lipids.

1. Introduction

The syncytiotrophoblast is the primary interface between the maternal and fetal circulations and is responsible for the transfer of all nutrients required for growth by the fetus. Fatty acids (FA) transferred by the syncytiotrophoblast play a crucial role in fetal development by providing energy dense fuels, essential structural components of cell membranes, and acting as ligands for cellular and nuclear hormone receptors. Long chain polyunsaturated fatty acid (LCPUFA) are essential for neural tissue growth and development, and as precursors for other bioactive signaling lipids, including prostaglandins, thromboxanes, leukotrienes, resolvins and protectins, which are involved in an array of biological processes such as inflammation, and vascular reactivity. The fetal capacity to synthesize LCPUFA is so low that uptake from the maternal circulation and transport across the placenta is the primary fetal supply of these vital lipids.

Lipolytic activity in maternal adipose tissue is increased in the third trimester [1] promoting the release of FA, which are incorporated into TG by the maternal liver. These processes are believed to be responsible for the markedly increased TG levels in the maternal circulation towards the end of pregnancy. Transfer to the fetus occurs across the placenta which takes up NEFA released from circulating TG through hydrolysis by maternal lipoprotein lipase (LPL) and endothelial lipase (EL). In addition, a high phospholipase activity has been reported in human placenta at term, leading to a release of FA from maternal circulating phospholipids [2] allowing for uptake by the placenta. NEFA bound to maternal albumin constitutes an additional source of FA for placental transfer. Specific receptors for VLDL, LDL, and HDL are expressed in human placental tissue [2,3] and could be a mechanism to provide fatty acids for placental transfer through the action of phospholipase A_2 and other cellular lipases [4]. Together, NEFA originating from maternal sources, enter the syncytiotrophoblast cells through either passive diffusion or membrane carrier proteins such as fatty acid transport proteins (FATP) isoforms. NEFA then bind to cytosolic fatty-

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acid-binding proteins (FABP) and are shuttled to subcellular organelles, such as the endoplasmic reticulum, mitochondria, lipid droplets and peroxisomes [5]. Each of these organelles assimilates exogenous FA into unique metabolic processes, such as the synthesis of PL, formation of acyl-CoA and eicosanoids at the ER, beta-oxidation in the mitochondria, storage as TG in lipid droplets, and membrane breakdown in the peroxisome [6].

Essential fatty acids (LA and ALA) in the maternal circulation can be converted into LCPUFA by specific elongases and delta-5 and delta-6 desaturases. These enzymes have also been reported to be expressed in fetal liver and placenta [7], however, their enzymatic activities are too low to meet fetal requirements. In particular, DHA and AA which are required for normal brain and retina development [8] must be supplied by the mother and adequate placental transfer of EFA and LCPUFA is critical for optimal growth and development. Higher percentages of DHA and AA have been reported in fetal plasma PL compared to maternal plasma PL [9] a process called biomagnification. The selective fetal enrichment of LCPUFA, in particular DHA, AA, ALA and LA, is believed to be established through a preferential transport of LCPUFA by the placenta via FA transporters (FATP) and binding proteins (FABP) in the placenta [10–13].

At 18 h in culture, isolated villous cytotrophoblasts cells from term placentas are predominantly mononucleated and secrete low amounts of human chorionic gonadotropin (hCG) [14]. Over time cytotrophoblast cells fuse to generate multinucleated syncytiotrophoblasts by 66 h to 90 h in culture, and this differentiation is accompanied with increased secretion of hCG [14,15]. The metabolism of FA in human trophoblast cells was studied by Coleman et al. using ^{14}C-acetate and ^{14}C-oleate. These investigators compared syncytiotrophoblast and cytotrophoblast and reported that syncytialization was associated with decreasing specific activities of enzymes critical for TG and phosphatidylcholine synthesis [16]. Interestingly, Kolahi and co-workers provided evidence that rapid esterification of LCPUFA and incorporation into lipid droplets occur in the cytotrophoblast rather than in the syncytiotrophoblast [17], but little is known about the FA composition in specific lipid classes pre- and post-syncytialization in PHT cells. Identification of differences in the FA composition of total lipids and in the major lipid classes in cytotrophoblasts and syncytiotrophoblasts will help us better understand the unique contributions of these two cell types to placental FA metabolism.

We hypothesized that the FA concentration and composition in both total lipids and in major lipid classes is distinct in syncytiotrophoblast as compared to cytotrophoblasts and that these changes can be attributed to the process of differentiation.

2. Methods

2.1. Study subjects

Informed written consent was obtained from all participants for collection of placental tissue and for the use of their protected health information, under a protocol approved by the Institutional Review Board at University of Colorado, Denver (COMIRB 14-1073). Women were recruited to donate their placenta to a data/bio repository for use in multiple research studies. Table 1 summarizes the maternal and infant characteristics of the study subjects. De-identified samples and data were transferred to the research team based on inclusion criteria including pre-pregnancy/early pregnancy BMI range 18–27; ultrasound confirmation of gestational age at 14–18 weeks; singleton pregnancy; maternal age 18–45 years. Exclusion criteria included concurrent inflammatory, vascular, or metabolic diseases (such as diabetes and polycystic ovary disease), conception by assisted reproductive technology, current use of tobacco, street drugs or medications (including corticosteroids), fetal malformations, history of pregnancy loss, and pre-term delivery.

Table 1
Maternal and infant characteristics.

Variable	n = 5
Maternal BMI (kg/m^2)	25.6 ± 0.6
Ethnicity	3 White Caucasian, 2 African American
Age at delivery (years)	31.8 ± 2.9
Gestational age (weeks)	39.1 ± 0.1
Delivery mode	4C-section with no labor, 1 NSVD
Placental weight (g)	660 ± 31
Birth weight (g)	3374 ± 31
Infant sex	3 females, 2 males

Mean ± SEM, C-section for Caesarean-section, NSVD = normal spontaneous vaginal delivery.

2.2. Placenta collection and isolation and culture of primary human trophoblast cells

Placentas were collected immediately after delivery from five pregnancies and villous cytotrophoblast cells were isolated and purified as previously described [14]. Briefly, cells were isolated using DNAse/trypsin digestion and purification on a Percoll gradient. Primary human trophoblasts (PHTs) were plated at a density of 5 × 10^6 per 60 mm plate. Cells were cultured in media containing Ham's F-12 and high glucose Dulbecco's modified Eagles Medium (DMEM, Sigma-Aldrich, MO, USA) (1:1, v/v), 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA), and antibiotics (penicillin, gentamicin and streptomycin) for 90 h according to a well-established protocol [18–20]. Media were changed daily. At 18 h and 90 h after plating, PHTs were washed four times in ice-cold PBS containing $MgCl_2$ and $CaCl_2$ (DPBS, Sigma-Aldrich). One mL of cold 0.9% saline solution (NaCl) was added and the cells were scraped on ice using a rubber tipped spatula. Cells were stored at −80 °C until lipid analysis. Release of chorionic gonadotropin (hCG) from trophoblast cells was used as a biochemical marker of differentiation. The concentration (mIU/mg protein) of β-hCG in the cell culture media was measured by ELISA (DRG Instruments, Marburg, Germany) at 18, 42, 66, and 90 h after plating the cells and is shown in Fig. 1S in Supplementary data.

2.3. Total lipid extraction

All organic solvents used, were HPLC grade. Four hundred μL of dispersed cells in NaCl was added to 1 mL of methanol (MeOH, Sigma-Aldrich). Total lipids were extracted by adding 1 mL of dichloromethane (DCM, Sigma-Aldrich), vortexing, followed by addition of 400 μL of water, vortexing, and centrifugation at 24 °C for 10 min at 500Xg. The DCM layer was collected, transferred to new glass tubes, and dried under nitrogen (N_2) at 40 °C. Lipids were resuspended in 300 μL of isooctane/ethyl acetate (3:1, v/v) (Sigma-Aldrich), and 100 μL was used for total lipid composition and 200 μL for separation and FA composition in four lipid classes.

2.4. Lipid class separation

Blended internal standards containing uniformly labelled [U^{13}C16:1]-palmitoleic acid (from Cambridge Isotope laboratories, MA, USA), TG (15:0/15:0/15:0), cholesteryl ester (17:0) (from Sigma-Aldrich) and [D_{62}]-phosphatidylcholine (16:0/16:0) (Avanti Polar, AL, USA) were added at equimolar amounts of 500 ng/μL to each sample. Samples were dried under N_2 and resuspended immediately in 200 μL hexane/chloroform ($CHCl_3$)/MeOH (95:3:2, v/v/v) (Sigma-Aldrich). Solid Phase Extraction NH_2 cartridges (Phenomenex, CA, USA) were conditioned by 1 mL acetonitrile (Fisher Scientific, NH, USA) and 2 × 1 mL hexane and 200 μL of sample was added to SPE column. The elution of the **cholesteryl ester (CE)** fraction was performed with 3 mL of hexane in new glass tubes. TG fraction was eluted with 3 mL of 1%