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Relationship between folate transporters expression in human placentas at term and birth weights

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ABSTRACT

Background: Adequate folate levels are essential for successful pregnancy outcomes. We aimed to study the relationship between placental mRNA and protein levels of folate transporters to birth weight.
Methods: Placental folate transporters (FOLR1, RFC1 and HCP1/PCFT) mRNA and protein levels in basal (BP) and chorionic plate (CP) of small (SGA), appropriate (AGA) and large (LGA) for gestational age term infants (≥37 weeks gestation, n = 111) were determined by real-time PCR and Western blot respectively.
Results: FOLR1 and HCP1/PCFT mRNA were lower in both plates of SGA and LGA placentas compared to AGA ($p < 0.01$) and RFC1 mRNA was lower only in CP ($p < 0.02$). RFC1 protein levels were lower in BP of SGA ($p < 0.05$) and LGA ($p < 0.01$), and FOLR1 protein levels were lower in CP of SGA ($p < 0.02$) and LGA ($p < 0.01$) groups compared to AGA. HCP1/PCFT protein levels remained unchanged in all groups.
Conclusion: Placentas of SGA and LGA groups showed a reduced mRNA expression and protein levels of folate transporters, with some differences depending on the location within the placenta (BP or CP). This suggests the presence of specific placental regulation mechanisms in gene expression that may be associated to birth weight.

1. Introduction

Folates are naturally present in food. Its synthetic form, folic acid (FA), has been used as a dietary supplement and for the fortification of some foods [1,2]. Folates are essential during pregnancy due to their role in prevention of neural tube defects (NTD) and in many physiological functions, including cell proliferation and differentiation processes, DNA replication, angiogenesis, methylation reactions and antioxidant protection [3,4]. These processes are essential for fetoplacental circulation and normal development of the placenta and fetus, thus enabling successful pregnancy outcomes [1,5].

The current recommendation for daily intake of folates during pregnancy is 600 μg/d. Such intake is expected to cover additional requirements for fetal growth, placental and maternal tissues [6–8]. A maternal deficiency of FA may occur when daily intake falls below 600 μg/d during pregnancy and has been associated to miscarriage, placental abruption and higher risk of preeclampsia, premature birth and fetal growth restriction [9,10]. In addition, folates deficiency has been shown to affect epigenetic mechanisms in the fetus and the placenta, disrupting fetal growth and even modifying the long-term phenotype, increasing the risk for chronic non-communicable diseases later in life [8,11,12].

Because fetal FA concentrations depend on maternal supply, the diet of the mother, the gastrointestinal absorption and the transport of folates through the placenta are fundamental to normal fetal development [13]. So far, three specific transport mechanisms of folate transport are known to operate in the human placenta: a) folate receptor-α (FOLR1), b) reduced folate carrier (RFC1), and c) conveyor coupled to protons (HCP1/PCFT) [13]. These transporters are mainly distributed in the syncytiotrophoblast microvillus plasma membrane (MVM) of the placenta and their expression varies between the first trimester and term [13]. Accordingly, levels of mRNA and protein of these transporters are high in early pregnancy and decrease as gestation progresses [14]. In contrast, folates required for growth reach the highest level in the last trimester [6].

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In addition, there are additional efflux transporters of the ATP-binding cassette (ABC) superfamily that are also distributed in the placenta [14].

The transfer of folate from maternal circulation to the fetus is the result of the coordinated action of FOLR1, RFC1 and HCP1/PCFT. This transfer can be affected by changes in mRNA expression and protein levels, as well as deregulation in the activity of folate transporters as shown in cases of pre-eclampsia [14]. Since folate levels may affect birth weight, we hypothesized that in term pregnancies the SGA condition is associated to lower mRNA expression and protein levels of folate transporters compared to normal birth weight (AGA), thus reducing the folate transport to the fetus. In contrast, the LGA condition may be related with an increased transport of folate levels to the fetus.

Here we studied the mRNA expression and protein levels of folate transporters in term placentas of different birth weight newborns, to assess possible relation between these parameters. This work will help to clarify whether different phenotypes of fetal growth are associated with the mRNA expression and protein levels of specific placental folate transporters.

2. Materials and methods

2.1. Sample collection

Placentas from full term pregnancies (37–40 weeks of gestation) and birth weight between the 10th and the 90th percentiles (AGA, n = 44), below the 10th percentile (SGA, n = 33) and over the 90th percentile (LGA, n = 34) were collected immediately after delivery [15]. Exclusion criteria included maternal hypertension, diabetes, preeclampsia, smoking (>5 cigarettes/day), maternal alcohol or drug consumption, twin or multiple pregnancies and genetic disorders in the newborn. This protocol was approved by the Scientific–Ethical Committee of the Central Metropolitan Health Service and by the Ethics Committee of the Faculty of Medicine and the Institute of Nutrition and Food Technology (INTA) of the University of Chile, Santiago, Chile.

Each placenta was sectioned transversally (approximately 7 × 5 cm) near the cord insertion site and, the decidual layer, chorionic surface and membranes were removed. The tissues were divided into segments from the chorionic plate (CP, fetal side) and basal plate (BP, maternal side). Each sample was rinsed thoroughly in cold sterile saline solution and frozen in liquid nitrogen and stored at −80 °C for RNA analyses.

2.2. RNA extraction, cDNA synthesis and real-time PCR

Total RNA from the both plates of placental tissues was extracted with Trizol (Invitrogen, Carlsbad, CA) and chloroform. RNA was frozen at −80 °C until cDNA synthesis. For cDNA synthesis M-MLV kit reverse transcriptase (RT, Promega, Wisconsin, USA) was used following manufacturer instructions. For mRNA quantification of *folr1*, *rfc1* and *hcp1/pcft*, the Eco qPCR System (Illumina San Diego, CA, U.S.A) was used. Results were analyzed by Eco real time PCR System Software v4.1 (Illumina) and calculated by the $2^{-\Delta\Delta}$CT method [16] following the MIQE Guidelines [17]. Results were expressed in relation to the geometric mean expression of 3 of the most stable housekeeping genes (*GAPDH*, *YWHAZ* and *β-actin*) [18,19] using the AGA group as control. Gene primers are described in Table 1. Life Technologies supplied Primers for *folr1* (assay code: Hs01124177_m1).

2.3. Tissue protein extraction and analysis of folate transporter proteins by western blot

Frozen placental explants (approx. 100 mg) of each sample were macerated in dry ice. Proteins were extracted with lysis buffer (Bio Source, Invitrogen) supplemented with detergent Triton X-100 and 1% protease inhibitor cocktail (Roche) and frozen at −80 °C until western blotting. Protein concentrations were determined by the Pierce ® BCA Protein Kit Assay (Thermo Scientific, USA) using a standard curve of fetal bovine albumin (BSA 1000 mg/mL). Ten μg of each sample was separated by electrophoresis in polyacrylamide gels (Tris-glycine SDS-polyacrylamide 8%) and then electro-transferred to nitrocellulose membranes (0.2 μm) at 4 °C. Primary antibodies and dilutions were: FOLR1 (1:10,000, R&D Systems; MN, USA), RFC1 (1:25,000, Abcam, Inc. Cambridge, UK), HCP1/PCFT (1:5.000, Abcam Inc. Cambridge, UK), β-actin (1:2,000,000, Sigma), Vinculin (1:100,000, Santa Cruz, CA, USA). Second antibodies for FOLR1 were anti-goat (1:20,000, R&D Systems; MN, USA), RFC1 (1:20,000, anti-rabbit, Abcam Inc., Cambridge, UK), HCP1/PCFT (1:5,000, anti-rabbit, Abcam Inc. Cambridge, UK), β-actin (1:25,000, anti-mouse, Sigma, MO, USA), Vinculin (1:50,000, anti-mouse, Sigma, MO, USA). Chemiluminescence detection and protein quantification were performed with Westar EtAc – enhanced chemiluminescent substrates for Western blot (Cyanagen, Bologna, Italy) using the equipment 10gD (Ultralum Inc, CA, USA) and the software UltraQuant 6.0 (version 6.0.0.344).

2.4. Statistical analysis

To assess the normality of variables we used the Kolmogorov–Smirnov test. The nonparametric Kruskal Wallis followed by Mann Whitney test to comparing differences between groups was used. All results are expressed as median (min–max). Based in the FOLR1 mRNA expression in the CP, with an α error of 0.05, a number of 23 (SGA), 25 (AGA) and 24 (LGA) samples, a mean of 0.66, 1.26 y 0.72 per SGA, AGA and LGA respectively, and an estimated error variance of 0.52, the power of the sample was 0.8. Spearman correlation test and the statistical software SPSS version 21.0 for Windows were used. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Anthropometric characteristics of women and newborn

As conceived, all placentas samples were recruited from term pregnancies (>37 gestation weeks) (Table 2). Placentas were separated in relation to newborn birth weight into SGA, AGA and LGA according to standard clinical criteria between the 10th and the 90th percentiles for gestational age, defined as AGA, below the 10th percentile as SGA and over the 90th percentile as LGA using Chilean birth weight references [15] (Table 2). Placental weights were correlated with birth weights as expected. The body mass index weight (BMI) at the last medical control (BMI 2) was significantly higher in pregnant mothers delivering LGA compared with SGA newborn.

3.2. mRNA expression and protein levels of folate transporters in term placentas of SGA, AGA and LGA newborn

The mRNA expression of all folate transporters was lower in the CP and BP of placentas in SGA and LGA when compared to AGA samples with the exception of *rfc1*, which was lower only in the CP of SGA and LGA groups (Figs. 1A, 2A and 3A). The profile of protein levels of folate transporters was different from the mRNA expression of the genes (Figs. 1–3 B, C, and D). RFC1 levels decreased only in the