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Altered maternal proportions of long chain polyunsaturated fatty acids and their transport leads to disturbed fetal stores in preeclampsia ☆

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ABSTRACT

Our previous cross-sectional studies have shown altered proportions of long chain polyunsaturated fatty acids (LCPUFA) in preeclampsia (PE) at the end of pregnancy when the pathology has already progressed. The present longitudinal study for the first time reports fatty acid proportions from 16th week of gestation till delivery and placental transport in PE. This is a hospital based study where women were recruited in early pregnancy. Maternal blood was collected at 3 time points i.e. T1 = 16–20th week, T2 = 26–30th week and T3 = at delivery. Cord blood and placenta were collected at delivery. This study reports data on 140 normotensive control (NC) and 54 PE women. In PE we report lower proportions of DHA in maternal plasma at T1, cord plasma and placenta ($p < 0.05$ for all). The mRNA levels of placental Δ5 desaturase, fatty acid transport proteins -1, -4, were lower ($p < 0.05$ for all) in PE. There was also a positive association between cord and maternal plasma DHA and total omega-3 fatty acids at T1. This study demonstrates that women with PE have lower fatty acids stores at 16–20th week of gestation and lower placental synthesis and transport. It is likely that supplementation of omega-3 fatty acids during the 16–20th week of gestation may help in improving fatty acid status in infants born to mothers with PE.

1. Introduction

Long chain polyunsaturated fatty acids (LCPUFA) of the omega-3 and omega-6 series, such as docosahexaenoic acid (DHA) and arachidonic acid (AA) are required in adequate amounts for fetal growth and brain development [1,2]. These LCPUFA are critical during the third trimester of pregnancy, when the fetus intensifies its nutrient demand for exponential growth [3]. To satisfy its need for fatty acids, the fetus depends on the maternal diet as well as on placental metabolism and transport [1] since the fetal synthesis of LCPUFA is very low [4]. Studies indicate that a better understanding of fatty acid metabolism in the feto-placental unit will help prevent adverse fetal development [5].

Our previous cross-sectional studies in pregnancy complication like preeclampsia (PE) have shown reduced maternal plasma, erythrocyte and placental DHA proportions [6–9]. However, these fatty acid proportions were analyzed at the end of pregnancy and may be confounded by the disease. Thus, it is not possible to determine whether the differences in the LCPUFA proportions are a cause or consequence of the PE [10]. Therefore, there is a need to undertake longitudinal studies which will help resolve the above issues.

It is well established that the Δ5 desaturase and Δ6 desaturase enzymatic chain promotes the formation of the omega-3 and omega-6 LCPUFA from their essential shorter chain precursors [11]. There are some studies which have examined the levels of desaturases in the human placenta [12,13], perfused placenta [14], human placental microsomes [15], rat placental microsomes [16,17], placental cell lines [18], or in animal placentas [19,20] and reports are contradictory. We have recently reported that mRNA levels of desaturases are altered by maternal micronutrients in the rat placenta [21].

A number of fatty acid binding proteins (FABP) facilitate the transfer of fatty acids across membranes [22]. The presence of several membrane fatty acid transport proteins [plasma membrane fatty acid binding protein (FABP) pm, fatty acid translocase (FAT), fatty acid transport protein (FATP)] and cytoplasmic fatty acid-binding proteins [liver-type (L-FABP, FABP1) and heart-type

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(H-FABP, FABP3)] has been reported in trophoblasts isolated from human placenta in uncomplicated pregnancy [23,24]. The complex interaction between these transport proteins has been suggested to lead to enrichment of LCPUFA in the fetal circulation compared with the maternal side [25]. The placental fatty acid transport is recently suggested to play a vital role in determining birth weight by either promoting or limiting fatty acid transfer to the fetus [3]. These fatty acids are transported across the placenta by a series of membrane bound and cytosolic fatty acids binding and transport proteins [26]. In PE, there is insufficient development of the uteroplacental unit called "shallow placentation" [27]. It is unclear whether this shallow placentation affects the various fatty acid transport proteins which in turn could be responsible for the altered proportions of placental LCPUFA observed in the preeclamptic pregnancies.

We hypothesize that the synthesis and transfer of fatty acids will be altered in conditions like PE. The present longitudinal study analyzes cord and maternal plasma proportions of fatty acids at three different time points in preeclamptic pregnancy and compares them with normotensive pregnancy. This study also reports for the first time the placental mRNA levels of Δ5 desaturase, Δ6 desaturase enzymes and FATP1, FATP4 and FABP3 in PE.

2. Materials and methods

2.1. Subjects

This study was conducted at the Department of Obstetrics and Gynecology, Bharati Hospital and Gupte Hospital and Research Centre, Pune, India. This study was approved by the Bharati Vidyapeeth Medical College Institutional Ethical Committee and a written consent was taken from each subject. The present study is hospital based and is part of a large ongoing prospective study where women willing to participate in the study were recruited in early pregnancy. At delivery, they were classified as normotensive control (NC) delivering at term (total gestation $\geq$37 weeks and baby weight $\geq$2.5 kg) and those that develop PE during pregnancy. The incidence of PE in this population is about 8–10% of total pregnancies. However, in the present study, the samples were randomly selected for analysis.

Thus, this study includes data on 140 NC women delivering at term and 54 women with PE. PE was defined by systolic and diastolic blood pressures greater than 140 and 90 mmHg, respectively, with the presence of proteinuria ($>1+$ or 300 mg per 24 h) on a dipstick test. Blood pressure was measured with a mercury sphygmomanometer and PE was confirmed by repeated recording of the blood pressure with an interval of 6 h at the time of diagnosis. Treatment of preeclamptic women included antihypertensive drugs and arginine supplementation.

Women were excluded from the study if there was evidence of other pregnancy complications such as gestational diabetes, eclampsia, chronic hypertension, type I or type II diabetes mellitus, seizure disorder and renal or liver disease. All study participants neither consumed alcohol nor smoked. This has been confirmed when the women were interviewed for demographic and nutritional history. All women were routinely given iron tablets, folic acid, calcium and multivitamin tablets. Gestational age was calculated from the last menstrual period and confirmed by routine ultrasonography.

Maternal characteristics and clinical information such as maternal age, maternal body mass index (BMI), maternal systolic and diastolic blood pressure, gestation and parity were recorded at various time points i.e. T1 (16–20th week of gestation), and subsequently at T2 (26–30th week of gestation) and at the time of delivery.

2.2. Dietary assessments

Pregnant women were interviewed with a food frequency questionnaire during T1, T2 and at delivery to estimate the frequency of intake of foods rich in alpha linolenic acid (ALA), DHA and omega-3 fatty acids. All pregnant women had to indicate the frequency of each food consumed during the last 1 month for which scores were calculated. For example, an item consumed once a week has a score of 4 while that consumed daily has a score of 30. These foods were identified using "Nutritive Values of Indian Foods" [28–31]. The food frequency questionnaire has been used by us in a number of studies on pregnant women [7,32,33].

2.3. Sample collection and processing

Fasting blood samples (10 mL) were collected into ethylenediaminetetraacetic acid (EDTA) tubes at the time of each prenatal visit, scheduled at 4-week intervals until delivery. The first sample was obtained between 16 and 20 weeks of gestation (T1), the second between 26 and 30 weeks of gestation (T2) and the third sample was taken just before going to the labor room (T3). Umbilical cord was also collected just after delivery. All blood samples were immediately layered on histopaque (Sigma-Aldrich, St Louis, MO, USA) and centrifuged at 626*g* for 30 min to separate the plasma and erythrocytes and were stored at $-80\,^{\circ}C$ until further analysis. Fresh placental tissues were obtained from normal and preeclamptic pregnancies within half an hour of delivery of the neonate. Fetal membranes were trimmed off and small pieces were randomly cut out from the placental cotyledons. Tissues were rinsed in phosphate buffered saline (PBS) to wash off maternal and fetal blood, snap frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until assayed.

2.4. Biochemical estimations

All biochemical analyses were performed at laboratories separately from subject recruitment sites. Investigators were blinded to subject identity, which was indicated by a code number maintained by the clinical staff until analysis was completed.

2.5. Fatty acid analysis

The procedure for fatty acid analysis used in our study was as reported in our several previous studies [6–9,34]. Briefly, placental tissue was homogenized with chilled PBS and spun at 10621*g* at 4 °C for 20 min. Supernatant and cell membrane fractions were separated. Transesterification of cell membrane phospholipid fraction and the total plasma fatty acids was performed using hydrochloric acid–methanol. These were separated using a Perkin Elmer gas chromatograph (SP2330, 30 m capillary Supelco column). Helium was used as carrier gas at 1 ml/min. Oven temperature was held at 150 °C for 10 min, programmed to rise from 150 to 220 °C at 10 °C/min and at 220 °C for 10 min. The detector temperature was 275 °C and the injector temperature was 240 °C. Methyl esters were separated using a Perkin Elmer gas chromatograph (SP 2330, 30 m capillary Supelco column, Perkin Elmer, Shelton, CT, USA). Peaks were identified by comparison with standard fatty acid methyl esters (Sigma-Aldrich). Fatty acids were expressed as g per 100 g fatty acid. The saturated fatty acids (SFA) included myristic acid (Myr), palmitic acid (Pal) and stearic acid (Ste), while the monounsaturated fatty acids (MUFA) included myristoleic acid (Myro), palmitoleic acid (Palo), oleic acid (Ole) and nervonic acid (NA). The total omega-3 fatty acids included ALA,