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Randomized control trials

Efficacy of docosahexaenoic acid-enriched formula to enhance maternal and fetal blood docosahexaenoic acid levels: Randomized double-blinded placebo-controlled trial of pregnant women with gestational diabetes mellitus

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SUMMARY

Background & aims: Gestational diabetes mellitus (GDM) compromises the level of docosahexaenoic acid (DHA) in phospholipids of maternal and fetal red blood cells and fetal plasma. This is of some concern because of the importance of DHA for fetal neuro-visual development. We have investigated whether this abnormality could be rectified by supplementation with DHA-enriched formula.

Methods: Women with GDM (n = 138) recruited from Newham University Hospital, London received two capsules of DHA-enriched formula (active-group) or high oleic acid sunflower seed oil (placebo-group) from diagnosis until delivery. Maternal (baseline and delivery) and fetal (cord blood) red blood cell and plasma phospholipid fatty acid composition, and neonatal anthropometry were assessed.

Results: One hundred and fourteen women (58 active, 56 placebo) completed the trial. The active-group compared with the placebo-group had significantly enhanced level of DHA in plasma phosphatidylcholine (4.5% vs 3.8%, $P = 0.011$), red blood cell phosphatidylcholine (2.7% vs 2.2%, $P = 0.022$) and phosphatidylethoanolamine (9.5% vs 7.6%, $P = 0.002$). There was no difference in cord plasma and red blood cell phospholipid DHA between the two groups. The neonates of the two groups of women had comparable anthropometric measurements at birth.

Conclusion: Daily supplementation of 600 mg DHA enhances maternal but not fetal DHA status in pregnancy complicated by GDM. The inefficacy of the supplement to improve fetal status suggests that the transfer of DHA across the placenta maybe impaired in women with the condition. Regardless of the mechanisms responsible for the impairment of the transfer, the finding has implications for the management of neonates of women with GDM because they are born with a reduced level of DHA and the condition is thought to be associated with a risk of neuro-developmental deficits. We suggest that babies of women with GDM, particularly those not suckling, similar to the babies born prematurely require formula milk fortified with a higher level of DHA.

1. Introduction

The impacts of gestational diabetes mellitus (GDM) on short- and long-term health of women and their offspring are increasingly recognized [1]. In Europe, the estimated prevalence of GDM ranges from 2 to 6% and it is expected to rise with increasing obesity, a strong risk factor for developing the condition [2]. Other determinant factors are a family history of type 2 diabetes, advanced maternal age and ethnicity. Certain ethnic groups are more predisposed to GDM [3].

We have previously reported that GDM reduces the levels of docosahexaenoic acid (DHA) and arachidonic acid (AA) in maternal red blood cell [4,5] and fetal red blood cell and plasma [5–7]

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phospholipids. DHA and AA, which are highly unsaturated fatty acids of the omega-3 and omega-6 family, respectively, are vital structural and functional components of cellular and sub-cellular membranes as well as precursors of diverse bioactive compounds. In addition, based on animal models of obesity and cell culture studies, it is postulated that DHA might be a potent anti-adiposity agent [8] and enhance glucose utilization by modulating insulin secretion and action [9,10].

The importance of DHA and AA in fetal life has been extensively documented [11,12]. Of these two fatty acids, DHA is considered to be the most limiting nutrient in pregnancy and lactation because it is scarce or absent in land animal and plant food sources. Moreover, the synthesis of DHA from the parent compound α-linolenic acid is inefficient. In normal pregnancy, DHA is preferentially transferred by placental selection from maternal to placental circulation. However, recent studies have reported that the placental uptake and transfer of DHA is impaired in pregnancy complicated by GDM [13,14]. There are no published communications whether or not the impact of this impairment could be ameliorated by DHA supplementation. In this report, we present the findings of a randomized, double-blind, placebo-controlled trial in which women with GDM were supplemented with DHA-enriched formula or high oleic acid sunflower seed oil. The primary outcome measure was the red blood cell membrane phospholipid DHA levels in the women and their neonates at delivery.

2. Materials and methods

The study was approved by East London & The City HA Local Research Ethics Committee 3 (REC reference no. 06/Q0605/89) and registered with ISRCTN Register (registration no. ISRCTN68997518). Written informed consent was obtained from all participants and the investigation was carried out in accordance with the principles of the Declaration of Helsinki as revised in 2007. Participants, midwives, and all investigators were blinded to allocation until all the data collated and analyzed.

2.1. Subjects and intervention

The study was carried out in Newham Borough, an inner city area of Greater London with a high proportion of immigrant community, predominantly South Asian and African/Caribbean ethnic group. Subjects were recruited during their visit to the antenatal or diabetic clinics at Newham University Hospital between October 2007 and February 2012. The inclusion criteria were 17–45 years old women with singleton pregnancies diagnosed with GDM. Those who were planning to receive tocolytic or corticosteroid therapy or had been taking omega-3 supplement during the period of time leading to their current pregnancy were excluded. Because of the high incidence of GDM and type 2 diabetes in the area, the hospital tests for GDM for women with high risk factors for the condition from early weeks of pregnancy. The test involves an overnight fast followed by drinking a solution of 75 g of glucose and drawing blood at 60 and 120 min for glucose determination. GDM is diagnosed if the glucose level at 120 min is greater than 7.8 mmol/l.

The eligible women who consented to participate in the study were randomized to receive daily 2 capsules of either “DHA-enriched formula” or “placebo (high oleic acid sunflower seed oil)”. Each active supplement capsule contained 300 mg of DHA, 42 mg of eicosapentaenoic acid (EPA) and 8.4 mg of AA, and placebo 721 mg of oleic acid. Randomization was carried out using a random code generated by the supplement provider (Equazen/Vifor Pharma Ltd., Glattbrugg, Switzerland). Both supplements contained vitamin E (d-alpha tocopherol) as an antioxidant and were encapsulated in an identical oblong soft gelatin capsule (750 mg in size).

2.2. Sample size

Sample size was calculated based on our previous case–control study [7] which found a 35% difference in red blood cell phosphatidylcholine DHA between neonates of pregnant women with GDM (4.0%) and those without the condition (5.4%). We assumed that supplementation of women with GDM with DHA-rich fish oil would increase the level of the nutrient in their neonates to the level of those born to women who had uncomplicated pregnancy. The power calculation indicated that a minimum of 40 subjects (neonates) per group would be required to detect the changes in red blood cell phosphatidylcholine DHA level with 80% power. The sample size and power calculation was performed using G*Power 3 [15] and based on a two independent groups, two-tailed t-test with an alpha of 0.05.

2.3. Blood collection and fatty acid analysis

Non-fasting venous blood samples (5–10 ml) were obtained from the subjects at recruitment (17^{th}–33rd gestational weeks) and delivery (maternal and cord) in EDTA vacutainer tubes. Samples collected during the day were transported promptly to the Lipidomics and Nutrition Research Centre (LNRC) laboratory for processing. Blood samples collected at night or on weekends were processed at the Pathology Laboratory, Newham University Hospital and subsequently transported to the LNRC laboratory. All samples were stored at −70 °C until analysis.

Fatty acid composition of red blood cell and plasma phospholipids was determined using the standardized method developed by our laboratory [7]. Briefly, total lipids were extracted by homogenizing the samples in chloroform and methanol, and the phospholipid fractions from the resulting total lipid were separated by thin-layer chromatography. Fatty acid methyl esters prepared from the phospholipids were separated using a gas–liquid chromatograph (HRGC MEGA 2 Series; Fisons Instruments, Milan, Italy) and quantified using a chromatography data system (Agilent EZChrom Elite Chromatography Data System v3.2, Scientific Software, Inc., Pleasanton, CA, USA).

2.4. Neonatal anthropometric measurement

Weight and length of the newborn babies were recorded by a midwife who attended the delivery as per routine practice and head-, shoulder-, mid-arm-, and abdominal circumferences by research midwives (JH and IN). Seca 210 portable measuring mat and Seca 201 ergonomic circumference measuring tape (Seca UK, Birmingham, UK) were used.

2.5. Statistical analysis

Data are presented as mean ± standard deviation (SD), median (range), and number of occurrence as appropriate and statistical significance was set at $P < 0.05$. All analyses were performed based on intention-to-treat principle. Independent t-test was used to compare the difference in fatty acids and anthropometric measurement between the active and placebo groups. The differences in pregnancy outcome were tested with Chi-square test. The change in DHA level within the group (baseline versus delivery) was assessed both by independent (including all subjects) and paired-sample (including only matched samples) t-test. All analyses were carried out with IBM SPSS Statistics version 20 (IBM Corporation, Armonk, NY, USA).