Nutrition 30 (2014) 685–689

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Dietary polyunsaturated fatty acid intake during late pregnancy affects fatty acid composition of mature breast milk

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ARTICLE INFO

Article history:
Received 24 September 2013
Accepted 13 November 2013

Keywords:
Human milk
Dietary intake
Pregnancy
Polyunsaturated fatty acids
n-3 fatty acids
n-6 fatty acids
Trans fatty acids

ABSTRACT

Objective: The aim of this study was to investigate how maternal polyunsaturated fatty acid intake at different periods during pregnancy affects the composition of polyunsaturated fatty acids in mature human milk.

Methods: A prospective study was conducted involving 45 pregnant women, aged between 18 and 35 y, who had full-term pregnancies and practiced exclusive or predominant breast-feeding. Mature breast milk samples were collected after the 5th postpartum week by manual expression; fatty acid composition was determined by gas chromatography. Fatty acid intake during pregnancy and puerperium was estimated through multiple 24-h dietary recalls. Linear regression models, adjusted by postpartum body mass index and deattenuated, were used to determine associations between estimated fatty acids in maternal diet during each trimester of pregnancy and fatty acid content in mature human milk.

Results: A positive association was identified between maternal intake of eicosapentaenoic acid (β, 1.873; 95% confidence interval [CI], 0.545, 3.203) and docosahexaenoic acid (β, 0.464; 95% CI, 0.212–0.714) during the third trimester of pregnancy, as well as the maternal dietary ω-3 to ω-6 ratio (β, 0.093; 95% CI, 0.016–0.170) during the second and third trimesters and postpartum period, with these fatty acids content in mature breast milk.

Conclusions: The maternal dietary docosahexaenoic acid and eicosapentaenoic acid content during late pregnancy may affect the fatty acid composition of mature breast milk. Additionally, the maternal dietary intake of ω-3 to ω-6 fatty acid ratio, during late pregnancy and the postpartum period, can affect the polyunsaturated fatty acid composition of breast milk.

Introduction

The long-chain polyunsaturated fatty acids (LC-PUFA), docosahexaenoic acid (DHA, 22:6 ω-3), eicosapentaenoic acid (EPA, 20:5 ω-3), and arachidonic acid (ARA, 20:4 ω-6), are of great importance during child growth and development [1]. The content of these fatty acids in human milk, especially DHA, varies widely [2,3], possibly as a result of diverse food consumption among women and because fatty acid content in maternal diets varies among regions.

Clinical trials suggest that consumption of fatty fish and fish oil supplements during puerperium are associated with an increased content of EPA and DHA in breast milk. However, in studies that employed labeled isotopes, only 30% of the variability in polyunsaturated fatty acid content (LC-PUFA) in breast milk is explained by the estimated dietary intake during the puerperium [4].

Randomized controlled trials (RCTs) suggest a direct relation between the use of ω-3 supplements during the second and third trimesters of pregnancy and its content in mature breast milk [5,6]. However, results reporting the effect of this supplement

RYN and DSS were responsible for the study design, data collection, and analysis. RYN developed the first version of the manuscript. GSFC and AJJ were responsible for data analysis and manuscript review. DSS coordinated the study and reviewed the final manuscript version. All authors have registered curriculum in the Lattes platform from CNPq. There is no conflict of interest to be disclosed.

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http://dx.doi.org/10.1016/j.nut.2013.11.002

during pregnancy on EPA and α-linolenic acid (ALA) concentrations are controversial [5,6].

Several studies using ω-3 (DHA, EPA, and ALA) and ω-6 (ARA and linoleic acid [LA]) fatty acid supplementation during gestational or postpartum periods have found that it has distinct effects on fatty acid content in breast milk [5–11]. Studies investigating the influence of dietary fatty acid during pregnancy on breast milk fatty acid composition are scarce and those that have actually investigated the issue have been limited to food consumption during the third trimester only [12,13].

The association between maternal intake of fatty acids and their content in human milk may differ according to the period of intake, given that according to the gestational stage, the rate by which they are transported by the placenta may vary and maternal metabolism may change as well [14]. We hypothesized that maternal PUFA intake might have distinct effects on the PUFA composition of breast milk depending on the period of maternal intake. This hypothesis has not been tested in previous studies. This study aimed to investigate how fatty acid intake during different pregnancy periods affects the composition of PUFA in mature breast milk.

Materials and methods

Study population

A prospective study was conducted to test the accuracy of a quantitative food frequency questionnaire involving 103 pregnant women, attendees at the Basic Health Units in Ribeirão Preto, São Paulo, Brazil, aged between 18 and 35 y, with prepregnancy body mass index (BMI) between 18.5 and 24.9 kg/m^2, had no diseases that may alter usual food consumption (diabetes mellitus, hypertension). For the validation study, 24-h dietary recalls (24 hRs) were obtained in each trimester of pregnancy. Seventy-five of these women completed the validation study and information on food intake (evaluated by the 24 hRs) throughout their pregnancies were available. Those women were invited to participate in the present study.

The inclusion criteria were: Women who practiced exclusive breast-feeding (exclusive breast milk intake, with no other liquid or solid food intake) or predominant breast-feeding (breast milk intake and other water-based liquid food intake) after the fifth postpartum week, and mothers with full-term pregnancies (birth after the 37th gestational week).

The study was approved by the Municipal Health Secretary of Ribeirão Preto and approved by the Research Ethics Committee from the Centro de Saúde-Escola at FMRP, USP, São Paulo, Brazil (Protocol number 378/CEP-CSE/FMRP-USP).

Socio-demographic characteristics and lifestyle

A structured questionnaire applied at the first interview during the prospective study (first trimester of pregnancy) was used to collect data on age, education, assets, and skin color. The economic classification of each participant was assessed according to the Brazilian Economic Classification Criterion (CCEB), which defines classes from A (highest socioeconomic level) to E (lowest socioeconomic level) [15].

Assessment of maternal BMI, weight gain, and gestational age

The nutritional status was assessed using the body weight of each participant in each trimester during prenatal and postpartum care appointments using an electronic digital scale (Plenna, model MEA 07700, São Paulo-SP, Brazil) with a capacity of 150 kg and 100 g gradation. Heights were measured using an anthropometric scale ruler (Cauduro, model 101 PL, Cachoeira do Sul-RS, Brazil).

Gestational weight gain was calculated as the difference between the weight at the last prospective data collection (third trimester) and prepregnancy weight (reported by the participant). Maternal pregestational and postpartum BMI were obtained using the height measured at the first prenatal visit and self-reported pregestational weight and measured postpartum weight, respectively.

Gestational age was calculated preferably based on the date of the last menstrual cycle on record, which was correlated to data from the ultrasound exam performed up to the twentieth gestational week.

Estimation of polyunsaturated fatty acid intake during pregnancy

PUFA (ω-3 fatty acids [DHA, EPA, and ALA] and ω-6 fatty acids [ARA and LA]) intake during pregnancy were evaluated through three 24 hR applied throughout the pregnancy, one 24 hR in each trimester of pregnancy, and two 24 hRs during the postpartum period. A second 24 hR was applied in each trimester of pregnancy in a subsample of the participants to correct for intraindividual variability. The 24 hR were applied by trained nutritionists using the multiple pass technique [16].

The NutWin software (NutWin software, Nutrition Support Program, version 1.5, Escola Paulista de Medicina, São Paulo, Brazil, 2002) was used for the analysis of PUFA content based on the 24 hR information, employing the Brazilian Food Composition Table-TACO [17], and the U.S. Department of Agriculture's Food Composition Table [18].

Determination of fatty acids in breast milk

Samples (5–10 mL) of mature breast milk (after postpartum week 5) were obtained by hand expression by the participant in the morning, immediately after the baby's first feeding and before mother's breakfast. Milk samples were stored at −80°C until analysis.

Fatty acids were extracted from 800 μL of milk, to which methanol and chloroform (v/v, 1:1) were added according to a previously described method [19]. After lipid extraction, the chloroform phase was evaporated under nitrogen current and fatty acids were methylated with potassium hydroxide in methanol (0.5 M) and removed with hexane. One μL of fatty acid methyl esters was injected in a SHIMADZU GC-2014 gas chromatographer (Shimadzu Europe, Duisburg, Germany) equipped with AOC-20 i auto-injector (Shimadzu Europe, Duisburg, Germany) and separated with a polyethylene glycol-SUPLELCOWAX 30 m capillary column (30 m, 25 mm × 0.25 μm; Supelco Inc., Bellefonte, PA, USA).

Helium was used as the carrier gas at a flow rate of 1 mL/min. Synthetic air was used for flame ionization detection at 280°C. The injections were performed in split mode. The injector and detector temperatures were 250°C. The initial column temperature was maintained for 1 min at 100°C, followed by an increase of 13°C/min until reaching 195°C, when it was maintained for 5 min, and subsequently elevated to 240°C, at the rate of 15°C/min, and maintained at this temperature for 30 min.

The identification standard used was composed of a mixture of fatty acid methyl esters from Supelco (Supelco 37 Component FAME Mix; Supelco Inc., Bellefonte, PA, USA) with the addition of 9 c, 11 t-CLA and 10 t, 12 c-CLA. The quantification was performed by area normalization and results presented in weight percentages.

Data analysis

Linear regression models were used employing the breast milk fatty acid content as the dependent variable and the maternal fatty acid intake, during each trimester of pregnancy and puerperium, as the independent variables.

Univariate and multiple linear models were adjusted by postpartum BMI and deattenuation. Deattenuation consists of correction of the linear regression coefficient by the intra- and interpersonal variability error. It was calculated using the formula: $Bt = bo\,[1 + (\sigma\,intra/\sigma\,inter)/n]$ [20], where bt is the true regression coefficient, bo is the observed regression coefficient, σ intra and σ inter are the intra- and interindividual variances, respectively, and n is the number of 24 hR replicates (in this case, n = 2 per trimester).

The confounding variables tested in the models, using the milk fatty acid content as the dependent variable, were age, family income, education, economic class, skin color, alcohol consumption, smoking, physical activity, prepregnancy BMI, postpartum BMI, gestational length, gestational weight gain, and baby's weight at birth. However, only the postpartum BMI was directly related to the milk fatty acid content and therefore was employed as the adjusting variable in the final model.

The time between the 24 hR applications and milk collection was tested as a covariate in the linear regression model; however, no association was observed.

The statistical analyses were carried out using the SPSS software (SPSS Software, Version 17.0 SPSS Inc., Woking, Surrey, UK).

Results

Seventy-five women completed the prospective study, of whom 20 were excluded. Eighteen were not practicing exclusive or predominant breast-feeding and two developed gestational diabetes. Among the 55 eligible women, 2 refused to participate in the study, 2 were not located, 5 relocated to another town, and 1 did not complete the 24 hR during puerperium; therefore, 45