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ORIGINAL ARTICLE

The effect of long-chain polyunsaturated fatty acids intake during pregnancy on adiposity of healthy full-term offspring at birth

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OBJECTIVE: The adjusted effect of long-chain polyunsaturated fatty acid (LCPUFA) intake during pregnancy on adiposity at birth of healthy full-term appropriate-for-gestational age neonates was evaluated.
STUDY DESIGN: In a cross-sectional convenience sample of 100 mother and infant dyads, LCPUFA intake during pregnancy was assessed by food frequency questionnaire with nutrient intake calculated using Food Processor Plus. Linear regression models for neonatal body composition measurements, assessed by air displacement plethysmography and anthropometry, were adjusted for maternal LCPUFA intakes, energy and macronutrient intakes, prepregnancy body mass index and gestational weight gain.
RESULT: Positive associations between maternal docosahexaenoic acid intake and ponderal index in male offspring ($\beta = 0.165$; 95% confidence interval (CI): 0.031–0.299; $P = 0.017$), and between n-6:n-3 LCPUFA ratio intake and fat mass ($\beta = 0.021$; 95% CI: 0.002–0.041; $P = 0.034$) and percentage of fat mass ($\beta = 0.636$; 95% CI: 0.125–1.147; $P = 0.016$) in female offspring were found.
CONCLUSION: Using a reliable validated method to assess body composition, adjusted positive associations between maternal docosahexaenoic acid intake and birth size in male offspring and between n-6:n-3 LCPUFA ratio intake and adiposity in female offspring were found, suggesting that maternal LCPUFA intake strongly influences fetal body composition.

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INTRODUCTION

Insufficient intrauterine supply of energy, macronutrients and/or micronutrients, during a period of rapid growth, may result in metabolic and body composition alterations.[1] Both n-3 and n-6 long-chain polyunsaturated fatty acids (LCPUFA) are claimed as indispensable for fetal growth and development, despite the insufficient scientific evidence on their individual effect on fetal body composition.[2] In particular, the fetus has limited ability to synthesize docosahexaenoic acid (DHA), and supply is almost entirely dependent upon maternal transfer.[3]

Randomized controlled trials evaluating the effect of maternal LCPUFA intake on offspring health have mainly been focused on maternal n-3 LCPUFA intake, and the offspring growth has been assessed as a secondary outcome.[1,2,4–6] Systematic reviews on these trials have found associations between n-3 LCPUFA intake and offspring birth size.[2,4,5,7] These associations have been interpreted as a consequence of prolonged gestation by 2 to 2.5 days rather than a direct effect on fetal growth, reflected by increase in birth weight around 50 g;[2,4,7] however, this effect disappears when adjusted for gestational age.[8] The effect of LCPUFA on fetal growth has been usually analyzed without adjustment for main cofactors with potential influence on fetal growth,[6] including prepregnancy body mass index (BMI), energy and macronutrient intake, and gestational weight gain (GWG).[9,10]

Eicosanoids derived from arachidonic acid (n-6 family) appear to have an adipogenic effect, by providing a molecular link between fatty acid uptake and preadipocytes differentiation during early hyperplasic growth stages of adipose tissue, whereas eicosanoids derived from eicosapentaenoic acid and DHA (n-3 family) seem to counteract this process.[1,4,6,11] Data from many observational and interventional studies in humans, even those measuring fatty acids profile in maternal blood, are insufficient to support the hypothesis that maternal n-3 LCPUFA diets exert an antiadipogenic action on the fetus, and are inconsistent with some findings in animal studies.[12] Most studies have relied on birth weight and indirect growth variables such as BMI or BMI Z-scores, which do not distinguish between the major components of body mass.[6] Other studies[13,14] have used skinfolds to estimate body fat mass, but limitations of this method in predicting body fat in neonatal age have been reported.[15] Assessment of body composition, rather than birth size alone, should give better insight into quality of growth and contribute to clarify the lack of consistency between studies on the effect of maternal LCPUFA intake on fetal growth.[16]

To our knowledge, no reliable validated method has been used to date to assess the effect of maternal LCPUFA intake on adiposity of offspring at birth.[1] Air displacement plethysmography (ADP) is a reliable method validated in neonates[17] to assess body composition in a two-compartment model; ADP thus allows for an accurate assessment of adiposity, defined by the percentage of body fat.[18] This method is convenient for use in neonates since it

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is noninvasive, relatively rapid to perform and not affected by movements.[16]

This study explores the effect of LCPUFA intake during pregnancy on the adiposity at birth of healthy full-term appropriate-for-gestational age neonates, assessed by ADP and anthropometry, while taking major maternal nutrition-related cofactors potentially affecting fetal growth and body composition into account. We hypothesize that imbalanced intake of LCPUFA during pregnancy, resultant of excessive n-6 intake and/or insufficient n-3 intake, increases adiposity of the fetus.

METHODS

This study is part of a broader study on the influence of maternal nutrition on body composition of offspring, conducted in the maternity and the nutrition laboratory of a level III pediatric hospital. Ethical committee approval and parental-informed consent were obtained.

The cross-sectional study design and baseline subject characteristics have been previously reported.[10] To summarize, a convenience sample of mother and infant dyads was systematically recruited, with the following criteria for inclusion: proper medical surveillance of pregnancy ending with a singleton neonate and full-term ($\geqslant$37 and $\leqslant$41 weeks of gestation), appropriate-for-gestational age (birth weight $>$ 3rd and $<$ 97th percentiles for gestational age and sex)[19] neonates considered healthy. Some major factors influencing maternal and fetal LCPUFA profile in term pregnancies, such as being small-for-gestational-age, grand multiparity ($\geqslant$5 pregnancies) and multiple pregnancies were controlled in the study design by exclusion.[20] In addition, the recruitment excluded neonates whose mothers had adverse conditions potentially affecting fetal nutrition and growth, such as type 2 diabetes mellitus, abnormal oral glucose tolerance test, inborn errors of metabolism, severe renal and cardiovascular disease, diastolic blood pressure during pregnancy $>$ 90 mm Hg or any reported consumption of alcohol, tobacco or illicit drugs.[21]

Maternal diet was assessed by a semiquantitative 86-item food-frequency questionnaire (FFQ) validated for Portuguese pregnant women.[22] There were nine response options that ranged from 'never/less than one per month' to 'six or more portions per day'. Women answered this questionnaire once, in the immediate postpartum period, to recall their usual dietary intake during the whole pregnancy. To estimate food consumption, the reported frequency for each food item was multiplied by their predetermined average portion pattern in grams. Food Processor Plus 6.0 (ESHA Research, Salem, OR, USA) was used to convert the consumption of food items into nutrients, based on thes United States Department of Agriculture food composition data, and was complemented with nutrient composition from the Portuguese Table of Food Composition.[23] Mothers were specifically questioned on the intake of LCPUFA supplements, including fish oil.

Daily intakes of eicosapentaenoic acid, DHA and arachidonic acid (expressed in mg) and intakes of n-6:n-3 LCPUFA ratio were included for analysis.

Neonatal anthropometric measurements were performed within the first 72 h after birth. Direct measurements were taken for weight, length and midarm circumference using the recommended procedures.[24] Indices derived from weight and length were calculated to estimate body adiposity: weight/length index as weight (g)/length (cm), BMI as weight (kg)/squared length (m) and ponderal index as weight (g)/cubed length (cm).[24] WHO Anthro (version 3.2.2, 2011; www.who.int/childgrowth/software/en/) was used for calculation and analyses of age- and sex-specific Z-scores of anthropometric data.[25]

The body composition of the neonates was measured using ADP (Pea Pod; Life Measurement Instruments, Concord, CA, USA), a two-compartment model measuring body mass (kg) with precision of 0.1 g of fat mass and fat-free mass. The percentage of body fat was calculated from body density assuming the density of fat to be 0.9007, and age- and sex-specific densities of fat-free mass were computed based on the data of Fomon.[17]

Results are expressed as mean (standard deviation) or median (minimum, maximum), as appropriate. An analysis was conducted to identify interactions between sex and LCPUFA intake regarding the percentage of fat mass (%FM) as main outcome. Linear multiple regression analyses were performed including variables identified by univariate analysis ($P < 0.15$). Models for either neonatal anthropometry or ADP measurements considered gestational age, maternal intakes of eicosapentaenoic acid, DHA, arachidonic acid and n-6:n-3 LCPUFA ratio as independent variables. In these models, energy and macronutrient intake, prepregnancy BMI (as a continuous variable and categorized for the threshold 25 kg m^{-2}) and GWG (as a continuous variable and categorized for the threshold 10 kg) were also considered as independent variables according to previously described criteria.[10] Daily maternal DHA intake was used as a continuous variable and categorized for the threshold of recommended $\geqslant$ 200 mg daily intake.[3] The level of significance was $\alpha = 0.05$. Data were analyzed using the Statistical Package for the Social Sciences 19.0 for Windows (SPSS, Chicago, IL, USA).

RESULTS

Neonates had a mean birth weight of 3.360 (0.359) kg and 55 (55%) of them were female. Median postnatal age of assessment was 50 (min. 23; max. 75) hours. The mean age of the mothers was 29.7 (6.1) years. Male offspring were found to have significantly greater weight, length and fat-free mass compared with females, and a significant positive correlation was found between anthropometric measurements and ADP measurements. These and additional results are published elsewhere.[10]

The LCPUFA intakes during pregnancy, considered as continuous variables, are described in Table 1. It was found that 39% of the mothers consumed less than the recommended daily intake of 200 mg per day.[3] No mother received supplementation of LCPUFA, including fish oil.

Upon analysis, several interactions were identified between sex and LCPUFA intake in regard to %FM, particularly more pronounced ($P = 0.015$) between sex and n-6:n-3 LCPUFA ratio intake. This distinct effect of the estimated n-6:n-3 LCPUFA ratio intake on the %FM of male and female offspring highlighted the need to stratify the analysis by sex:

- In male offspring, the ponderal index was positively associated with maternal DHA intake (β-estimate = 0.165; 95% confidence interval (CI): 0.031, 0.299; $P = 0.017$), prepregnancy BMI (β-estimate = 0.188; 95% CI: 0.050, 0.326; $P = 0.009$) and GWG (β-estimate = 0.150; 95% CI: 0.016, 0.285; $P = 0.029$). This indicates that when maternal DHA intake was sufficient ($\geqslant$200 mg), the male offspring ponderal index was an average 0.165 g cm^{-3} higher after adjusting for BMI and GWG (Table 2).
- In female offspring, the n-6:n-3 LCPUFA ratio intake was positively associated with fat mass (β-estimate = 0.021; 95% CI: 0.002, 0.041; $P = 0.034$) and %FM (β-estimate = 0.636; 95% CI: 0.125, 1.147; $P = 0.016$). This indicates that for each unit increase on the estimated maternal n-6:n-3 LCPUFA ratio intake, the female offspring fat mass was an average of 21 g heavier and the %FM was an average of 0.64% higher (Table 3).

DISCUSSION

This cross-sectional study conducted in an Atlantic European country, was designed to analyze the influence of nutrition during pregnancy on the body composition at birth of full-term appropriate-for-gestational age neonates born to mothers without known potential factors affecting the intrauterine growth.[10] The presented subanalysis focuses on the adjusted effect of maternal LCPUFA intake on the body composition of those neonates.

This study has two strong points that should be emphasized: the assessment of adiposity at birth by measuring the %FM using

Table 1. Estimated daily LCPUFA intakes during pregnancy

Daily intakes	*Median*	*Min; max*
AA (mg)	168.5	61; 536
EPA (mg)	114.5	2; 502
DHA (mg)	266.0	10; 1210
n-6:n-3 LCPUFA ratio	7.0	3.9, 19.0

Abbreviations: AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LCPUFA, long-chain polyunsaturated fatty acids.

Table 2. Association between PI at birth in male offspring and estimated DHA intake during pregnancy, adjusted to prepregnancy BMI and GWG

Maternal variables	*β Estimate (95% CI)*	P-*value*
DHA intake	0.165 (0.031, 0.299)	0.017
Prepregnancy BMI (threshold 25 kg m^{-2})	0.188 (0.050, 0.326)	0.009
GWG (threshold 10 kg)	0.150 (0.016, 0.285)	0.029

Abbreviations: CI, confidence interval; PI, ponderal index; GWG, gestational weight gain. Variables considered for the multivariable model: DHA intake, prepregnancy BMI and GWG (as categorical variables).

Table 3. Association between %FM and FM at birth in female offspring and estimated n-6:n-3 LCPUFA ratio intake during pregnancy

Neonatal measurements	*n-6:n-3 LCPUFA ratio*	P-*value*
	β estimate (95% CI)	
%FM[a]	0.636 (0.125, 1.147)	0.016
FM[b]	0.021 (0.002, 0.041)	0.034

Abbreviations: CI, confidence interval; %FM, percentage of fat mass, FM, fat mass, LCPUFA, long-chain polyunsaturated fatty acids. Variables considered for the multivariable model. [a]Prepregnancy BMI (as categorical variable) and n-6:n-3LCPUFA ratio intake. [b]n-6:n-3LCPUFA ratio intake as the only significant variable identified by univariate analysis.

ADP, as a reliable and precise method with recent increasing use;[16,17,26,27] and the assessment of the effect of maternal LCPUFA intake on the body composition at birth adjusted for three major maternal nutrition cofactors (prepregnancy BMI, energy and macronutrient intakes during pregnancy, and GWG).

Some limitations have to be acknowledged. A systematically recruited convenience sample was studied. Despite its convenience size, most of the associations obtained in this sample are strong, as evidenced by the statistical significance and narrow confidence intervals. To avert the potential effect of non-assessed placental pathology on outcome measurements, neonates born to mothers with adverse conditions known to affect fetal nutrition and growth were excluded, and only assumed healthy full-term appropriate-for-gestational age neonates were included. Owing to safety concerns, it was not feasible to perform the assessments in the first hours after birth; thus, the physiological water loss occurring in the first days after birth is an uncontrollable bias. A semiquantitative validated FFQ was administered in the immediate postpartum period to assess maternal diet during pregnancy. In spite of the potential recollection bias inherent to the FFQ, the quality of the n-3 fatty acids estimates derived from the FFQ has been judged as acceptable according to the European micronutrient Recommendations Aligned scoring system.[28] In fact, in several observational studies assessing the effect of maternal LCPUFA intake on fetal health, the FFQ has been administered either during pregnancy or following delivery. [5,29–31]

The neonatal body composition assessed within the first 72 h after birth allowed the best feasible estimate of the intrauterine nutritional status,[32] while minimizing the effect of postnatal factors such as the feeding regimen. Most of studies analyzing the effect of maternal LCPUFA intake have not restricted the intervention to the prenatal period but continued the exposure during lactation, thus making it difficult to interpret the potential independent effects of variations in LCPUFA supply on intrauterine developing fat depots.[6]

Only few studies have adjusted the analysis for major cofactors influencing fetal body composition at term birth, such as maternal BMI,[33–37] total energy intake during pregnancy[35] and GWG.[31] Data previously reported on the same sample[10] demonstrated positive adjusted associations between both prepregnancy BMI and energy intake from carbohydrate and offspring body size, positive association between prepregnancy overweight and adiposity in male offspring, and positive association between GWG and body size in female offspring. In this study, using the same reliable method for measuring %FM, a significant interaction was identified between sex and estimated n-6:n-3 LCPUFA ratio intake. Moreover, it has been reported that females have significantly greater fat mass and %FM at birth than males.[18] Therefore, further analysis was done to stratify by sex.

Adjustment for maternal nutritional factors and gestational age, with the use of both ADP and anthropometry, revealed clinically significant positive associations in regard to the effect of maternal LCPUFA intake on offspring body composition. Among male offspring, with mean ponderal index of 2.8 g cm^{-3},[10] the ponderal index was on average 0.165 g cm^{-3} higher (circa 5.9%) when maternal DHA intake reached sufficient requirements.[3] Among female offspring, with mean fat mass of 370 g and %FM of 11.6%,[10] the fat mass increased 21 g (circa 5.7%) and adiposity (%FM) increased 0.64% (circa 5.5%) for each one unit increase in maternal n-6:n-3 LCPUFA ratio intake (ranging from 3.9 to 19.0). Although weight:length ratios at birth, such as BMI and ponderal index, may give a more complete insight into size at birth, they do not distinguish between the major components of body mass and therefore provide poor assessment of adiposity.[6] Of note, increase in fat-free mass such as bone cannot be accounted for using BMI and/or ponderal index. DHA, in sufficient intake, seems to modulate the differentiation of mesenchymal bone marrow cells toward osteoblastogenesis rather than adipocytes.[38] It is possible that positive associations seen between adequate DHA intake and BMI and/or ponderal index could be related to changes in bone mass rather than increased adiposity. It has been suggested that ponderal index may correlate better with lean mass rather than adiposity.[16] Correspondingly, Much *et al.*[14] found neonatal n-3 LCPUFA status to be negatively associated with fat mass at birth.

The positive association found between estimated maternal n-6:n-3 LCPUFA ratio intake and adiposity in female offspring may be explained by adipogenic effect resulting from proportionally increased n-6 LCPUFA intake and/or reduced n-3 LCPUFA intake.[1,4,6,11] This positive association is not consistent with the findings of either Hauner *et al.*[13] or Much *et al.*,[14] who did not find significant effect of the variation in n-6:n-3 LCPUFA ratio intake during pregnancy and lactation on offspring fat mass during the first year of life. Hauner *et al.*[13] estimated total body fat using skinfold measurements and Much *et al.*[14] evaluated the adiposity using both skinfolds and regional fat estimated by ultrasound measurements of abdominal subcutaneous fat and preperitoneal fat thickness. Notwithstanding, skinfolds have limitations in predicting body fat at least in the neonatal period.[15] Hauner *et al.*[13] did not find evidence of an interaction between maternal n-6:n-3 ratio intake and sex, considering fat mass as the outcome. Nevertheless, sexual dimorphism in offspring response to maternal n-3 LCPUFA intake has been reported at least in relation to brain development.[39] It has been suggested that human females need greater amounts of adipose tissue, stored in early life, in order to provide sufficient amounts of DHA to support growth of long brains in their developing fetus.[39]

To summarize, in coherence with the reported adipogenic effect of n-6 family and anti-adipogenic effect of n-3 family,[1,4,6,11] the adiposity of female offspring (elicited by %FM and fat mass) was found to be positively associated with a maternal LCPUFA ratio intake favoring n-6 over n-3 LCPUFA. Consistent with reports of increased birth size associated with maternal n-3 LCPUFA intake,[2,4,5,7] the ponderal index of male offspring was positively associated with maternal DHA intake.

In conclusion, the assessment of body composition of healthy full-term offspring at birth using both anthropometry and ADP revealed significant positive associations between estimated maternal DHA intake and body size (reflected by ponderal index) in male offspring, and between estimated maternal n-6:n-3 LCPUFA intake and adiposity (elicited by %FM and fat mass) in female offspring, suggesting that maternal LCPUFA intake strongly influences fetal body composition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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