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Associations of birth weight with serum long chain polyunsaturated fatty acids in adolescents; the HELENA study

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ABSTRACT

Objectives: Nutritional factors in early life may have long-term physiologic effects in humans. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play important roles in protecting against cardiovascular disease (CVD) risk. Our aim was to examine the association of birth weight (BW) with serum long chain polyunsaturated fatty acids (LCPUFA) profile in adolescents.

Subjects and methods: A total of 772 European adolescents (56.3% females) aged 14.7 ± 1.2 years were included in this study. Information on BW and gestational age was obtained from parental records. DHA, EPA and arachidonic acid (AA) concentrations were measured in serum phospholipids. Alfa-linolenic (ALA), linoleic (LA), AA, EPA and DHA intakes assessed by a computer based 24 h dietary recall. Gender, gestational age, pubertal status, body mass index, center and total energy and LCPUFA intakes were used as confounders in all the analyses.

Results: BW was significantly associated with serum DHA and EPA (both adjusted $P < 0.05$) independently of potential confounders including their main dietetic source. We did not observe any significant relationship between BW and serum AA levels.

Conclusions: Our findings suggest that early metabolic changes, as a result from prenatal environmental influences, could affect long chain polyunsaturated fatty acid metabolism later in life. These results may contribute to explain the relationship between early nutrition and growth and later metabolic disorders as CVD.

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1. Introduction

There is now compelling evidence that low birth weight (BW) is associated with atherosclerosis and cardiovascular disease (CVD) risk factors such as obesity, hypertension and type 2 diabetes mellitus later in life [1–6]. Environmental and genetic factors, as well as their interactions, seem to partially explain these associations [7,8].

The role of n-3 long chain polyunsaturated fatty acids (LCPUFA) in human health and disease has received special attention during recent decades. Eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) play important roles in human health through various mechanisms, including effects against inflammation, platelet aggregation, hypertension and hyperlipidemia [9]. Thus, an inverse association between EPA and DHA

content of blood phospholipids and CVD risk and subclinical atherosclerosis is a consistent finding in observational studies [10–12]. Furthermore, serum DHA levels have been inversely associated with common carotid artery intima media thickness in subjects with primary dyslipemia [13]. Arachidonic acid (C20:4n-6, AA) is the precursor of important molecules involved in inflammation (e.g. eicosanoids), and is thought to play a role in the atherosclerotic process [14,15]. High concentrations of AA in adipose tissue have been associated with a greater risk of myocardial infarction, which suggests a pro-atherosclerotic role of excess of AA [16,17].

Tissue LCPUFA levels are mainly determined by dietary intake and endogenous synthesis, via the successive elongation and desaturation of dietary polyunsaturated fatty acids precursors, linoleic-acid (C18:2n-6, LA) and α-linolenic acid (C18:3n-3, ALA). ALA is converted to EPA and then to DHA, whereas LA is converted to AA through enzymatic chain elongation and desaturation. Although LCPUFA intakes and metabolic turnover are likely to be the main predictors of DHA and EPA status [18], there are also other factors that influence DHA and EPA levels including age, nutritional status or genetic background [19–22]. Serum phospholipid DHA and EPA are effective biomarkers of DHA and EPA status, respectively [23].

Longitudinal studies suggested that maternal nutritional status during ovum maturation, or even before conception, has a greater effect on the neonatal fatty acid profile than maternal nutrition during the last 2 trimesters of pregnancy [24,25]. It has also been shown that neonatal LCPUFA concentrations at birth seem to significantly affect the postnatal LCPUFA content in blood phospholipids [26]. Several studies suggested that undernutrition and suboptimal growth in pregnancy could change the metabolism of cholesterol and triglycerides [8,27,28]. Information is lacking on the effect of BW on serum LCPUFA concentrations later in life.

The present study aimed to examine the association of BW with serum AA, EPA and DHA levels in European adolescents participating in the Healthy Lifestyle in Europe by Nutrition in Adolescence Study (HELENA).

2. Subjects and methods

2.1. Subjects

The recruitment and phenotyping of the adolescents participating in the HELENA cross-sectional study ("Healthy Lifestyle in Europe by Nutrition in Adolescence", www.helenastudy.com) have been described previously (18). Briefly, a total of 3865 adolescents were recruited between 2006 and 2007. Data were collected in a total of 10 centers from 9 European countries. Subjects were randomly selected from schools by using a proportional cluster sampling methodology and taking age into account. One third of the classes were randomly selected for blood collection; this resulted in a total of 1155 blood samples.

Data were collected on a detailed case report form, in accordance with standardized procedures. In each center, trained researchers carried out complete physical examinations, including weight, height and blood pressure measurements. The protocol was approved by the appropriate investigational review board for each investigating center. Written, informed consent was obtained from each adolescent and both of his/her parents or legal representatives. Participation in the study was voluntary.

For the purpose of this study, adolescents with at least data on serum AA, EPA and DHA levels, body mass index (BMI) and neonatal data were included in the analysis. To be born at term (95.5%) was an additional inclusion criterion. Based on these criteria, the final study sample comprised 772 adolescents (56.3% females). The final

sample did not differ in key characteristics (i.e., BW and age) from the original sample (all $P > 0.1$).

2.2. Neonatal data

A questionnaire was developed for parents to collect information on the adolescents' BW and gestational age [29]. Parents were specifically asked to recall this information from the health booklets of their son/daughter. This questionnaire was sent to the parents together with the study information letter and consent form, and collected at school on the first day of the examinations. If information from the parental questionnaire was lacking, the local investigators were advised to send the questionnaire to the parents again to obtain the required information.

2.3. Physical examination

Harmonization and standardization of anthropometric measurements used to assess body composition in the HELENA Study were strictly controlled and have been previously described [30]. Weight (Type SECA 861; range, 0.05–130 kg; precision, 0.05 kg), and height (Type SECA 225; range, 60–200 cm; precision, 1 mm) were measured. Thereafter, BMI was calculated. Identification of pubertal status (stages I–V) was assessed by a medical doctor according to Tanner and Whitehouse [31].

2.4. Serum phospholipid LCPUFA analysis

Blood samples were drawn at school according to standardized collection protocol (after 10-h overnight fast)[32]. Samples were centrifuged directly at school (3500 rpm for 15 min at room temperature) and serum stored at $-20\,^{\circ}$C. Serum samples (1 mL) were defrosted at room temperature and 50 μL internal standard (1,2-dipentadecanoyl-sn-glycero-3-phosphocholine, Sigma–Aldrich, Deisenhofen, Germany) were added. A methanol/chloroform solution was used to extract proteins and transfer lipid classes into a non-aqueous solution according to the modified methods of Folch [33]. Briefly, 1 mL of methanol and 2 mL of chloroform were added to the sample. The solution was centrifuged (2000 rpm, 10 min, 4 $^{\circ}$C). The upper layer was removed via aspiration and the interphase by filtration. The filtrate was evaporated to dryness under a N_2 flow and the residue was dissolved in chloroform. After each step, N_2 was blown over the samples to avoid fatty acid (FA) oxidation. The serum phospholipid fraction was separated by thin-layer chromatography according to the method of Christophe and Matthijs [34]. The phospholipid band was scraped off and the FA were converted into their methyl esters by transesterification using 2 mL of a mixture of methanol/hydrochloric acid as methylating solution for 4 h at 95 $^{\circ}$C. After cooling ($-20\,^{\circ}$C, 10 min), distilled water was added. The methyl esters were extracted with petroleum ether (bp 40–60 $^{\circ}$C) and evaporated to dryness under a N_2 flow. The residue was redissolved in heptane and analyzed. The FA methyl esters of the phospholipid fraction were analyzed using a temperature-programmed capillary gas chromatograph (Model 3900, Varian GmbH, Darmstadt, Germany) with a 30 m $\times$ 0.25 mm $\times$ 0.25 μm polyethylene glycol column (Zebron ZB-WAXplus, Phenomenex Ltd., Aschaffenburg, Germany). Helium was used as carrier gas with a constant flow of 4 mL/min, the split ratio was set to 1:20. Injector and flame ionization detector temperatures were 200 $^{\circ}$C and 250 $^{\circ}$C, respectively. The oven temperature was programmed as follows: the initial temperature (100 $^{\circ}$C) was held for 2 min and increased to 210 $^{\circ}$C at rates of 25 $^{\circ}$C/min, followed by 0.5 $^{\circ}$C/min, 1.0 $^{\circ}$C/min, and 5.0 $^{\circ}$C/min, respectively. After reaching 210 $^{\circ}$C, the temperature was increased to 240 $^{\circ}$C at a rate of 10 $^{\circ}$C/min. The final temperature of 240 $^{\circ}$C was held for 3 min. Peaks of interest were

Table 1
Characteristics of the study sample.

	Males ($n = 337$)	Females ($n = 435$)	All ($n = 772$)
Age (year)	14.7 ± 1.2	14.7 ± 1.1	14.7 ± 1.2
Birth weight (kg)	3.4 ± 0.6	3.3 ± 0.6	3.3 ± 0.6
Body mass index (kg/m^2)	21.2 ± 3.9	21.3 ± 3.5	21.2 ± 3.7
AA (μmol/L)	343.4 ± 89.4	363.4 ± 98.7	354.6 ± 95.1
EPA (μmol/L)	18.6 ± 13.6	19.1 ± 14.1	18.9 ± 13.9
DHA (μmol/L)	94.3 ± 40.0	112.0 ± 40.7	104.2 ± 41.5
Total energy intake (kcal/day)	2570 ± 895	1892 ± 520	2189 ± 784
LA intake (mg/day)	10800 ± 5641	8260 ± 3221	9373 ± 4618
ALA intake (mg/day)	1697 ± 1162	1255 ± 590	1449 ± 913
AA intake (mg/day)[*]	321 ± 182	251 ± 152	282 ± 170
EPA intake (mg/day)[*]	70 ± 61	63 ± 54	66 ± 58
DHA intake (mg/day)[*]	166 ± 142	159 ± 140	162 ± 141

Data are expressed as means ± standard deviation unless otherwise stated. AA: arachidonic acid; ALA: alfa-linolenic fatty acid; DHA: docosahexaenoic fatty acid; EPA: eicosapentaenoic fatty acid; LA: linoleic fatty acid.

[*] $N = 511$ adolescents (224 males and 287 females).

identified by comparison with authentic FA methyl ester standards (Sigma–Aldrich, Deisenhofen, Germany). The absolute FA composition was expressed in μmol/L. The coefficients of variations for the analyses were below 4.4% for all FA.

For the present study, the following fatty acids were relevant: AA; EPA; and DHA. Other measured fatty acids were not considered because they were beyond the scope of our study.

2.5. Dietary intake assessment

To obtain dietary intake data, the HELENA-DIAT 24-h dietary recall software was used. This 24-h recall assessment tool is based on six meal occasions referring to the day before the interview. The adolescents completed the questionnaire during school time, after dieticians/researchers instructed them on how to fill in this 24-h recall as accurately as possible. The participants were allowed to ask questions and assistance and after completion, the recall was checked for completeness. Every participant was asked to fill in the HELENA-DIAT on arbitrary days, twice in a time-span of 2 weeks. Since the questionnaire was filled in during school time, no data could be collected about the dietary intake on Fridays and Saturdays. A validation study by Vereecken et al. [35] indicated that the YANA-C, a former version of the HELENA-DIAT, showed good agreement with an interviewer-administered YANA-C interview. The HELENA-DIAT tool has been indicated as a good method to collect detailed dietary information from adolescents and was received well by the study participants [35]. Furthermore, a repeated 24-h recall was selected as the most suitable method to get population means and distributions by the European Consumption Survey Method (EFCOSUM) project [36]. To calculate energy and nutrient intake, data of the HELENA-DIAT was linked to the German Food Code and Nutrient Data Base (BLS (Bundeslebensmittelschlüssel), version II.3.1, 2005).

In addition, the adolescents completed a brief Food Frequency Questionnaire (FFQ) asking for the frequency in consumption of different food items (e.g. fish consumption). The frequency categories included the options 'never', 'less than once per week', 'once per week', '2 to 4 times per week', '5 to 6 times per week', 'once per day' and 'more times per day'. Usual food intakes were estimated for each adolescent based on the 24-h recalls and the FFQ-information by using the Multiple Source Method (MSM) (https://nugo.dife.de/msm/). The MSM calculates dietary intake for individuals first and then constructs the population distribution based on the individual data. With this method dietary data was corrected for between and within person variability.

For the purpose of this study we used as confounders ALA, DHA and EPA as the main dietetic sources of plasma DHA and EPA levels, and LA and AA as the main dietetic sources of plasma AA levels, respectively. We also recorded total energy intake (kcal/day).

3. Statistical analysis

Statistical analyses were performed using "Statistical Package for the Social Sciences (SPSS)" software 17.0 (SPSS, Chicago, IL), and the threshold for statistical significance was set at $P < 0.05$. Physical and biochemical characteristics of the study sample by sex are presented as means and standard deviation, unless otherwise stated. Variables with skewed distribution, i.e., AA, EPA, and DHA levels, were log transformed to obtain a more symmetrical distribution.

Regression analysis was used to examine the association between BW and serum phospholipid LCPUFA levels. These relationships were analyzed in three separate regression models. The unadjusted model included the predictor (BW) and the dependent variables (serum AA, EPA and DHA levels). Model 2 included BW and the dependent variable adjusted for gestational age, pubertal

Table 2
Multiple regression standardized coefficients (β) and standard errors (SE) examining the association of birth weight with serum long chain polyunsaturated fatty acid concentrations.

	Unadjusted ($n = 772$)			Model 2 ($n = 772$)			Model 3 ($n = 511$)		
	β	SE	P	β	SE	P	β	SE	P
AA (μmol/L)[a]	-0.003	0.016	0.874	0.021	0.016	0.199	0.028	0.019	0.140[b]
DHA (μmol/L)[a]	0.051	0.024	0.035	0.057	0.023	0.013	0.054	0.026	0.039[c]
EPA (μmol/L)[a]	0.156	0.041	<0.001	0.076	0.037	0.039	0.088	0.041	0.031[c]

AA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid. Model 2 controls for pubertal status, sex, study location and BMI. Model 3: includes model 1 and additionally controls for total energy intake and the corresponding polyunsaturated fatty acid dietetic source.

[a] Analysis was performed with log-transformed data.
[b] Adjusted for linoleic and arachidonic acids intake.
[c] Adjusted for alfa-linolenic, docosahexaenoic and eicosapentaenoic acids intake.

status, sex, center (entered as dummy variable) and BMI. For model 3, analyses were additionally controlled for dietary intake variables. Likewise, regression analysis examining the association between BW and serum AA levels was controlled for LA, AA and energy intake variables, and the relationships between BW and serum EPA and DHA levels were additionally adjusted for ALA, EPA, DHA, and energy intake variables:

4. Results

The descriptive characteristics of the participants are presented in Table 1.

Table 2 presents linear regression statistics showing the observed change in mean serum phospholipids LCPUFA levels per kilogram increase in BW in adolescents. BW was positively associated with serum EPA ($P < 0.05$) and DHA ($P < 0.001$) levels in the unadjusted model and these relationships remain statistically significant after additional adjustment for sex, gestational age, BMI, pubertal status and center (model 2, Table 2). Further controlling for total energy, ALA, EPA and DHA intakes did not substantially change the outcome (model 3, Table 2).

BW was not significantly associated with serum AA levels in any of the studied regression models (Table 2).

5. Discussion

The findings of the present study show significant associations between BW and serum DHA and EPA levels in adolescents independently of potential confounders, including ALA, DHA and EPA intakes. To our knowledge, there are no previous studies examining the association of BW on later serum LCPUFA profile, which hamper between studies comparisons.

It is biologically plausible that environmental intrauterine factors, such as insufficient fetus nutrition and growth, may partially program the activity and/or expression of the enzymes required for the metabolic endogenous synthesis of these LCPUFA. In utero challenges, such as relative under-provision of nutrients or placental insufficiency, can result in the long-term programming of individual metabolism, tissues and whole organ systems, with adverse consequences for their function in later life. Alternatively, the familial resemblance of dietary habits between the mothers and their children could be responsible for long term LCPUFA tracking. Likewise, to obtain LCPUFA the fetus depends on placental transfer and, thus, on the LCPUFA status of the mother. Indeed, an adverse maternal fatty acid profile early in pregnancy has been associated with reduced fetal growth [35]. Previous reports [35–38] estimated that low maternal serum concentrations of n-3 PUFA increased the risk of being small for gestational age in 40–50%, and it was associated with decreased BW on an average of 50–60 g.

It could also be argued that the main determinants of serum phospholipid fatty acid profile in the short term are dietary habits, yet the results were consistent even after adjustment for DHA and EPA intake, as well as for their precursor ALA intake. It has been suggested that there are other factors influencing serum DHA and EPA levels. Previous studies reported considerable variability among individuals in the conversion rates of ALA and LA on their respective n-3 and n-6 metabolites, even when the subjects followed similar background diets [22,39]. Other reports showed that either obesity or under-nutrition in children can alter fatty acid composition in serum and erythrocyte membrane lipids [21,40,41]. Nevertheless, our results were consistent after controlling for dietary sources, yet caution should be paid because of the instrument used to assess dietary intake. Moreover, we do not know whether serum LCPUFA levels will maintain stable over and after the sexual development

and how changes in physical activity and dietary patterns could affect this apparent programming effect of BW.

In conclusion, type of dietary fatty acid intake plays an important role in modulating LCPUFA metabolism. It is also possible, however, that early metabolic changes, such as prenatal environmental influences, affect LCPUFA metabolism later in life. The relationship between BW and serum EPA and DHA levels may contribute to explain the relationship between low BW and later metabolic disorders such as atherosclerosis or CVD.

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