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Cord blood *n*-3 LC-PUFA is associated with adiponectin concentrations at 10 years of age

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ABSTRACT

An elevated ratio of *n*-6 to *n*-3 long-chain (LC-) polyunsaturated fatty acids (PUFA) may be a potential risk factor for obesity development. *N*-3 LC-PUFA are thought to alter adiponectin concentrations, and thus may have a beneficial effect on weight development. We analysed the association between *n*-3 LC-PUFA concentrations in cord blood and adiponectin concentrations at 10 years.

Fatty acid composition was measured in cord blood and at 10 years of age by gas chromatography, and adiponectin concentrations were measured only at 10 years of age in 237 children from the Munich LISAplus birth cohort study. Linear regression models assessed associations between *n*-3 LC-PUFA, *n*-6 LC-PUFA and the *n*-6/*n*-3 ratio in cord blood with adiponectin concentrations at 10 years of age. LC-PUFA were presented as percentages and categorized into tertiles. Regression models were adjusted for LC-PUFA percentages at 10 years of age and other potential confounding factors.

Cord blood *n*-3 LC-PUFA tertiles were significantly associated with adiponectin concentrations in an inverse J-shaped relationship [2nd tertile versus 1st tertile: Beta=1.84 (SE=0.65), and 3rd tertile versus 1st tertile: 1.02 (0.68), *p*-value < 0.01 (ANOVA)]. Further, cord blood *n*-6/*n*-3 ratios were significantly associated with adiponectin concentrations [2nd tertile versus 1st tertile: 0.14 (0.67), and 3rd tertile versus 1st tertile: −1.37 (0.68), *p*-value=0.03 (ANOVA)]. The cord blood *n*-6 LC-PUFA tertiles were not associated with adiponectin concentrations.

Our results suggest that a higher *n*-3 LC-PUFA concentrations and a lower *n*-6/*n*-3 ratio in cord blood are associated with higher adiponectin concentrations at 10 years of age.

1. Introduction

It has been suggested that, among other factors, an elevated ratio of *n*-6 to *n*-3 long-chain polyunsaturated fatty acids (LC-PUFA) may be a potential risk factor for obesity [1,2].

N-6 LC-PUFA and *n*-3 LC-PUFA been proposed to have different effects on the development of adipose tissue. The main *n*-6 LC-PUFA, arachidonic acid (AA), is a precursor of eicosanoids, such as prostacyclin, which enhances the differentiation of adipose precursor cells to adipocytes. In contrast, *n*-3 LC-PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be less adipogenic and may inhibit adipose tissue development by attenuating the production of eicosanoids [2,3]. Moreover, *n*-3 LC-PUFA may have a regulatory effect on adiponectin [4]. Adiponectin is an anti-inflammatory hormone secreted by adipose tissue [4]. Decreased adiponectin concentrations are associated with obesity and insulin resistance [4,5]. Several studies have shown a positive association between *n*-3 LC-PUFA and adiponectin concentrations [4,6].

Adipocyte development increases exponentially with gestational age [7]. The highest increase in number and size of adipocytes occurs during the first year of life. Following this, the differentiation of precursor cells into adipocytes continues later in life [2,8]. Thus, pre- and early postnatal life are critical periods for adipose tissue development.

However, it is not clear whether there is a long lasting priming effect of *n*-3 LC-PUFA concentrations in cord blood on adiponectin concentrations later in life [7].

In a previous analysis based on the same study population, we reported a time-varying association between the *n*-6/*n*-3 ratio in

Abbreviations: AA, arachidonic acid; ANOVA, Analysis of variance; BMI, Body mass index; DHA, Docosahexaenoic acid; DPA, Docosapentaenoic acid; EPA, Eicosapentaenoic acid; (LC-)PUFA, (long-chain) polyunsaturated fatty acids

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cord blood and BMI (body mass index) up to the age of 10 years [9]. Based on these findings, we investigated the association between the *n*-3 LC-PUFA concentrations, the *n*-6 LC-PUFA concentrations and the *n*-6/*n*-3 ratio in cord blood with adiponectin concentrations at the age of 10 years. The *n*-3 LC-PUFA concentrations, the *n*-6 LC-PUFA concentrations and the *n*-6/*n*-3 ratio, measured at the 10-year follow-up examination, were included in the analysis in order to rule out confounding by life-style or dietary factors, which may be reflected in the fatty acid composition in blood. We hypothesize that higher *n*-3 LC-PUFA concentrations, lower *n*-6 LC-PUFA concentrations and a lower *n*-6/*n*-3 ratio in cord blood are associated with higher adiponectin concentrations at 10 years of age, even after accounting for later LC-PUFA concentrations in blood.

2. Materials and methods

2.1. Study population

LISAplus (Life-style Related Factors on the Immune System and the Development of Allergies in Childhood PLUS the influence of traffic emissions and genetics) is a German population based birth cohort study in which a total of 3097 neonates were recruited between 1997 and 1999 from the cities of Munich, Leipzig, Wesel and Bad Honnef. Details of the study design have been described elsewhere [10]. During the recruitment in maternity wards, cord blood samples were collected and deep frozen until the time of measurement. Questionnaires were completed by the parents at birth, 0.5, 1, 1.5, 2, 4, 6 and 10 years of age, and physical examinations took place at 2, 6 and 10 years.

This sub-study is restricted to children from the Munich study center. Of the 1467 successfully recruited children, 814 cord blood samples could be collected. Total immunoglobulin E concentrations were measured in these 814 cord blood samples [10]. Sufficient serum remained in 681 samples for further measurements of fatty acids. Of the 1467 children recruited at birth, 953 (65%) were followed-up until the age of 10 years. Of these 953 children, 581 children participated in the clinical examination of which 540 provided blood samples. Adiponectin concentrations and the fatty acid composition at age 10 years were measured successfully in 527 samples. Complete information on the fatty acid composition in cord blood as well as adiponectin concentrations and fatty acid composition at age 10 years was available for 249 children. After excluding 12 children with missing covariable information, 237 children were included in this analysis.

Approval by the local Ethics Committees (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from each participant's family were obtained.

2.2. Physical examination and blood tests

Blood samples were collected during the physical examination at 10 years of age. The analysis of adiponectin, estradiol and testosterone has been previously described [11]. Blood samples were centrifuged after collection and stored frozen at −80 °C until assayed for adiponectin, estradiol (females) and testosterone (males). Adiponectin concentrations in serum were measured using a commercially available radioimmunoassay (Mediagnost, Reutlingen). The samples were diluted by a factor of 1:300. The sensitivity of the test was 0.6 µg/L. The intra- and interassay coefficients of variation were between 2.35% and 8.59% for adiponectin samples ranging from 3.36 mg/L to 15.19 mg/L. Testosterone and estradiol concentrations were measured in the serum samples by the fully mechanized immunoassay system Modular (Roche, Mannheim, Germany). The analytical sensitivity was 0.087 nmol/L for testosterone and 18.4 pmol/L for estradiol. Intra- and interassay coefficients of variation were below 4.06% and 2.83% for 6.2 nmol/L and 20.2 nmol/L testosterone, respectively. For estradiol, intra- and interassay coefficients of variation were below 5.29% and 3.56% for 378 pmol/L and 1941 pmol/L, respectively.

The measurement of fatty acids has been previously described in detail for the serum from cord blood and from blood samples collected at 2, 6 and 10 years of age [9,12,13].

The analysis was performed by selective transfer of glycerophospholipid fatty acids from 100 µl serum into their methyl esters and their gas chromatographic separation and quantification [14].

The total *n*-6 LC-PUFA concentration was calculated by summing the concentrations of eicosadienoic acid (C20:2*n*-6), dihomo-gamma-linolenic acid (C20:3*n*-6), AA (C20:4*n*-6), adrenic acid (C22:4*n*-6) and docosapentaenoic acid (C22:5*n*-6). Similarly, the total *n*-3 LC-PUFA concentration was calculated by summing the concentrations of eicosatrienoic acid (C20:3*n*-3), EPA (C20:5*n*-3), docosapentaenoic acid (DPA, C22:5*n*-3) and DHA (C22:6*n*-3). Total *n*-6 LC-PUFA and total *n*-3 LC-PUFA concentrations are presented as a percentage of the concentrations of all the measured fatty acids with 14–24 carbon atoms. The *n*-6/*n*-3 ratio was calculated by dividing the total *n*-6 LC-PUFA concentration by the total *n*-3 LC-PUFA concentration.

2.3. Statistical analysis

The percentages of *n*-3 and *n*-6 LC-PUFA as well as the *n*-6/*n*-3 ratio were categorized into sex-specific tertiles because of their apparent non-linear relationship with adiponectin concentrations. The cut-off values used for the construction of tertile groups are presented in Table S1 (Supplementary data). Adiponectin concentrations are presented using their mean and standard deviation (SD) and differences in adiponectin concentrations between tertile groups were tested using ANOVA (analysis of variance).

Linear regression models were applied to model the association between adiponectin concentrations with *n*-6 and *n*-3 LC-PUFA and the *n*-6/*n*-3 ratio tertiles. The first tertile was used as the reference category. Results are presented as linear regression coefficient betas with corresponding standard errors (SE). *p*-values were derived from ANOVA. The linear regression models were adjusted for sex, total serum glycerophosphopholipid fatty acids in cord blood and at 10 years, fasting status and exact age at the 10-year examination, BMI at 10 years, maternal age at birth, maternal pre-pregnancy BMI, maternal education level (low/medium vs. high), birth weight (<3455 g vs. ≥ 3455 g), gestational age, breastfeeding (exclusive breastfeeding >4 months vs. no exclusive breastfeeding or exclusive breastfeeding ≤ 4 months), onset of puberty (females: estradiol >18.4 vs. ≤ 18.4; males: testosterone >0.09 vs. ≤ 0.09). Furthermore, models were adjusted for *n*-6 LC-PUFA, *n*-3 LC-PUFA and the *n*-6/*n*-3 ratio tertiles, respectively, at age 10 years. To investigate if a specific LC-PUFA might be causing the effect, all LC-PUFA previously combined into either *n*-3 or *n*-6 LC-PUFA, were analyzed separately. Additionally, a sensitivity analysis stratified by sex was conducted. Statistical significance was defined by a two-sided alpha level of 5%.

Statistical analyses were performed using R, version 2.15.2 (http://www.R-project.org/) [15]. The “effects” package was used for plotting [16].

3. Results

This analysis is based on 237 children (110 girls and 127 boys) with available information on cord blood fatty acid composition, fatty acid composition and adiponectin measurements at age 10 years.

Details of the study population are presented in Table 1. The mean adiponectin concentration was 9.5 ng/mL (SD = 4.2 ng/mL). The characteristics of the study population per *n*-6 and *n*-3