

## Maternal plasma polyunsaturated fatty acid levels during pregnancy and childhood lipid and insulin levels

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**Abstract** *Background and aims:* Maternal polyunsaturated fatty acid (PUFA) levels are associated with cord blood lipid and insulin levels. Not much is known about the influence of maternal PUFAs during pregnancy on long-term offspring lipid and insulin metabolism. We examined the associations of maternal plasma n-3 and n-6 PUFA levels during pregnancy with childhood lipid and insulin levels.

*Methods and results:* In a population-based prospective cohort study, among 3230 mothers and their children, we measured maternal second trimester n-3 and n-6 PUFA plasma levels. At the median age of 6.0 years (95% range, 5.6–7.9), we measured childhood total-cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride, insulin and c-peptide levels. Higher maternal total n-3 PUFA levels, and specifically DHA levels, were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels (p-values <0.05), but not with LDL-cholesterol and triglycerides. Maternal total n-6 PUFA levels were not associated with childhood outcomes, but higher levels of the individual n-6 PUFAs, EDA and DGLA were associated with a lower childhood HDL-cholesterol, and higher AA levels with higher childhood total-cholesterol and HDL-cholesterol levels (all p-values <0.05). A higher maternal n-6/n-3 PUFA ratio was only associated with lower childhood HDL-cholesterol and insulin levels (p-values <0.05). These associations were not explained by childhood body mass index.

*Conclusions:* Higher maternal total n-3 PUFAs and specifically DHA levels during pregnancy are associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels. Only individual maternal n-6 PUFAs, not total maternal n-6 PUFA levels, tended to be associated with childhood lipid and insulin levels.

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## Introduction

Suboptimal maternal nutritional status during pregnancy is associated with an increased risk of cardio-metabolic diseases in the offspring [1]. Fatty acids are required in adequate amounts for fetal growth and development [2]. During pregnancy, maternal fatty acids, mainly polyunsaturated fatty acids (PUFAs), act as precursors of eicosanoid synthesis and lipid messengers for fetal development [3]. Animal studies have shown that a maternal diet containing higher levels of PUFA during pregnancy improves the offspring's lipid profile in the postnatal period [4,5]. Among humans, studies in non-pregnant adults, showed that higher levels of docosahexaenoic acid (DHA), a n-3 PUFA, were associated with higher high-density lipoprotein (HDL)-cholesterol, lower triglycerides, and a lower risk of the metabolic syndrome, whereas higher levels of  $\alpha$ -linolenic acid (ALA), a n-6 PUFA, seem to be associated with a higher risk of an adverse metabolic profile [6,7]. A small study among 242 Dutch mother–child pairs showed that higher levels of maternal docosapentaenoic acid (DPA), a n-3 PUFA, during pregnancy were associated with higher total-cholesterol and low-density-lipoprotein (LDL)-cholesterol levels in the offspring. In this study, higher levels of maternal linoleic acid (LA), a n-6 PUFA, were associated with higher childhood proinsulin levels [8]. However, results from randomized controlled trials that assessed the influence of fish oil supplementation during pregnancy on offspring lipids and risk of diabetes mellitus, showed inconsistent results [9–11]. Thus, the influence of different maternal n-3 and n-6 PUFAs during pregnancy on long-term offspring lipid and insulin metabolism remains unclear [12].

Therefore, we examined, in a population-based prospective cohort study from early pregnancy onwards among 3230 mothers and their children, the associations of maternal plasma n-3 and n-6 PUFA levels during pregnancy with childhood total-cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, insulin and c-peptide levels. We further explored whether these associations are independent of maternal and childhood socio-demographic and lifestyle-related characteristics and childhood body mass index.

## Methods

### Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life to adulthood in Rotterdam, the Netherlands [13]. The study has been approved by the Medical Ethical Committee of Erasmus Medical Center in Rotterdam (MEC 198.782/2001/31). All mothers gave written consent. Pregnant women with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 8,879 mothers were enrolled during pregnancy, of whom 7,072 had information about PUFA levels available and 6,925 gave birth to singleton live born children. Childhood lipid, insulin, or

c-peptide levels were available in 3,230 of these children (a flow chart is given in [Supplemental Fig. S1](#)). Missing blood samples were mainly due to non-consent for venous puncture or crying of the child.

### Maternal fatty acid status

Maternal non-fasting venous samples were drawn at a median gestational age of 20.5 weeks (95% range: 17.1–24.9). To analyze PUFA levels, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of plasma glycerophospholipid PUFA composition was performed using gas chromatography by a sensitive and precise high-throughput method, suitable for large epidemiological studies, as previously described [14]. Based on findings from previous studies, for our analyses we selected maternal PUFA that have been associated with the risk of cardiovascular and metabolic outcomes in adults, and with pregnancy and fetal outcomes [8,15]. Selected total maternal PUFA were total n-3 PUFA, which included:  $\alpha$ -linolenic acid (ALA, C18:3n3), eicosapentaenoic acid (EPA, C20:5n3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n3). Total n-6 PUFA included: linoleic acid (LA, C18:2n6),  $\gamma$ -linolenic acid (GLA, C18:3n-6), eicosadienoic acid (EDA, C20:2n-6), dihomogamma linolenic acid (DGLA, C20:3n6), arachidonic acid (AA, C20:4n6) and docosatetraenoic acid (DTA, C22:4n-6). PUFA levels were expressed as proportion of total fatty acids present in the chromatogram (weight percentage, wt%) to express the relative importance of a fatty acids set against the total fatty acids concentrations [16]. We also calculated the ratio of total n-6/n-3 PUFA.

### Childhood lipid and insulin measurements

At the age of 6 years (95% range, 5.6–7.9), total-cholesterol, HDL-cholesterol, and LDL-cholesterol, triglyceride, insulin and c-peptide levels were obtained enzymatically from venous blood samples 30 min after the last meal using a Cobas 8000 analyzer (Roche, Almere, The Netherlands) [17]. Quality control samples demonstrated intra- and interassay coefficients of variation ranging from 0.77 to 1.39%, and 0.87–2.40%, respectively.

### Covariates

Information on maternal age, educational level, ethnicity, and parity, was obtained with questionnaires at enrolment [13]. We calculated body mass index. Information on maternal smoking habits during pregnancy and folic acid supplement use was assessed by self-reported questionnaires during pregnancy. Weight gain up to 30 weeks of gestation was calculated as the difference between maternal weight measured at 30 weeks of gestation and self-reported weight before pregnancy, as described previously [18]. We used a 293-item food frequency

questionnaire covering the first trimester to assess maternal dietary intake during pregnancy. Information about pregnancy complications, child's sex, gestational age at birth, and weight at birth was obtained from medical records [19,20]. Information about breastfeeding, timing of introduction of solid foods and average TV watching time was obtained by questionnaires in infancy [21]. Information about infant PUFA intake at 13 months, measured with a 211-item food frequency questionnaire was available in a subgroup of the study ( $n = 1566$ ) [22].

### Statistical analysis

We used linear regression analyses to assess the associations of maternal total and individual plasma n-3 and n-6 PUFA levels with the childhood outcomes. The regression models were adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use, total caloric intake during pregnancy, pregnancy complications, child's sex, gestational age-adjusted birth weight standard-deviation scores (SDS), breastfeeding duration, timing of introduction of solid foods, and TV watching time. We additionally adjusted these analyses for children's body mass index at the age of 6 years to explore if these associations were explained by childhood body mass index. For all analyses, we log-transformed not normally distributed childhood outcomes (triglycerides and insulin). We constructed SDS [(observed value - mean)/SD] for all PUFAs and childhood outcomes measures to enable comparison of effect estimates. We tested for interaction terms between maternal PUFA levels and child's sex in relation to the childhood outcomes, but since no significant interactions were present no further stratified analyses were performed. In order to reduce potential bias associated with missing data and to maintain statistical power, we performed multiple imputations of missing covariates by generating 5 independent datasets using the Markov Chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed using Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (IBM Corp, Chicago, IL, Armonk, NY, USA).

## Results

### Subject characteristics

Table 1 shows the maternal and childhood characteristics. Mean (SD) second trimester maternal plasma levels of n-3 and n-6 PUFA were 105.4 mg/L (27.7) and 602.8 mg/L (89.0), respectively (Table 2). Results from the non-response analysis are given in Supplemental Table S1 and show that mothers who were not included in the analyses smoked more often during pregnancy and developed more often gestational hypertensive disorders as compared to those who were included ( $p$ -values < 0.05). Mothers not included in the analyses had lower total n-3 PUFA levels compared to those included (Supplemental Table S2).

**Table 1** Characteristics of mothers and their children ( $N = 3230$ ).<sup>a</sup>

	Value
<b>Maternal characteristics</b>	
Age, (y), median (95% range)	31.0 (20.1, 39.4)
Gestational age at fatty acid measures, (weeks), median (95% range)	20.5 (17.1, 24.9)
Pre-pregnancy body mass index, (kg/m <sup>2</sup> ), mean (SD)	23.6 (4.2)
Weight gain, (kg), mean (SD)	10.4 (4.9)
Education, N. higher education (%)	1455 (47.7)
Ethnicity, European N. (%)	1963 (61.9)
Parity, N. nulliparous (%)	1808 (56.3)
Smoking during pregnancy, N. yes (%)	736 (25.6)
Folic acid supplement use, N. yes (%)	1867 (75.7)
Gestational diabetes, N. (%)	27 (0.9)
Gestational hypertensive disorders, N. (%)	176 (5.6)
<b>Birth and infant characteristics</b>	
Males, N. (%)	1650 (51.1)
Gestational age at birth (weeks), median (95% range)	40.1 (35.8, 42.3)
Breastfeeding duration (months), mean (SD)	4.5 (3.9)
Introduction of solid foods N. (%) >6 months	208 (10.1)
N-3 PUFA intake (g/d)	0.6 (0.4)
N-6 PUFA intake (g/d)	4.7 (3.0)
<b>Childhood outcomes at 6 years</b>	
Body mass index at 6 years (kg/m <sup>2</sup> ), mean (SD)	16.2 (1.8)
TV watching time, n (%)	
<2 h/day	2059 (80.6)
≥2 h/day	496 (19.4)
Total-cholesterol (mmol/L), mean (SD)	4.2 (0.6)
HDL-cholesterol (mmol/L), mean (SD)	1.3 (0.3)
LDL-cholesterol (mmol/L), mean (SD)	2.4 (0.6)
Triglycerides (mmol/L), median (95% range)	1.0 (0.4–2.5)
Insulin (pmol/L), median (95% range)	141 (17–421)
C-peptide (ng/mL), median (95% range)	1.0 (0.3–2.1)

<sup>a</sup> Values represent means  $\pm$  SDs, median (95% range) or number of subjects (valid %).

**Table 2** Second trimester maternal PUFA levels in plasma ( $N = 3230$ ).<sup>a</sup>

	Absolute values (mg/L)	Relative values (wt%)
Total PUFA	708.3 $\pm$ 98.8	43.1 $\pm$ 2.0
Total n-3 PUFA	105.4 $\pm$ 27.7	6.5 $\pm$ 1.5
ALA	5.1 $\pm$ 1.8	0.3 $\pm$ 0.1
EPA	8.8 $\pm$ 5.5	0.5 $\pm$ 0.3
DPA	12.1 $\pm$ 4.4	0.7 $\pm$ 0.2
DHA	77.9 $\pm$ 20.5	4.8 $\pm$ 1.1
Total n-6 PUFA	602.8 $\pm$ 89.0	37.2 $\pm$ 2.5
LA	361.1 $\pm$ 63.5	22.3 $\pm$ 2.8
GLA	1.5 $\pm$ 0.7	0.1 $\pm$ 0.1
EDA	8.5 $\pm$ 1.9	0.5 $\pm$ 0.1
DGLA	61.0 $\pm$ 16.5	3.7 $\pm$ 0.7
AA	156.4 $\pm$ 32.6	9.6 $\pm$ 1.6
DTA	6.9 $\pm$ 2.2	0.4 $\pm$ 1.1
Total n-6/n-3 PUFAs ratio	6.1 $\pm$ 1.7	—

Abbreviations: AA: arachidonic acid; ALA:  $\alpha$ -linolenic acid; DGLA dihomogamma-linolenic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; DTA: docosatetraenoic acid; EDA: eicosadienoic acid; EPA: eicosapentaenoic acid; GLA:  $\gamma$ -linolenic acid; LA: linoleic acid; PUFA: polyunsaturated fatty acid.

<sup>a</sup> Values represent means  $\pm$  SDs.

Correlation coefficients between all maternal PUFA levels are shown in [Supplemental Table S3](#).

### Maternal n-3 PUFAs with childhood lipid and insulin levels

**Table 3** shows that, after adjustment for potential confounders, higher maternal total n-3 PUFA levels were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels (differences: 0.04 (95% CI: 0.01, 0.08), 0.06 (95% CI: 0.02, 0.09) and 0.05 (95% CI: 0.01, 0.08, respectively) per SD increase of maternal total n-3 PUFA levels), but not with childhood LDL-cholesterol and triglycerides. Among the individual n-3 PUFA levels, we observed that higher maternal EPA levels were only associated with a higher childhood HDL-cholesterol, whereas higher levels of maternal DPA were only associated with higher childhood insulin levels (all p-values<0.05). Higher maternal DHA levels were associated with higher childhood total-cholesterol, HDL-cholesterol and insulin levels (differences: 0.04 (95% CI: 0.01, 0.08), 0.06 (95% CI: 0.02, 0.09) and 0.04 (95% CI: 0.01, 0.08, respectively) per SD increase of maternal DHA levels), but not with childhood LDL-cholesterol and triglycerides. We observed similar results when we used childhood c-peptide levels instead of childhood insulin levels ([Supplemental Table S4](#)). Results did not materially change after additional adjustment for childhood concurrent body mass index.

### Maternal n-6 PUFAs with childhood lipid and insulin levels

**Table 4** shows that, after adjustment for potential confounders, higher maternal total n-6 PUFA, LA and GLA

levels were not associated with any of the childhood lipid and insulin outcomes. Higher maternal EDA and DGLA levels were only associated with a lower childhood HDL-cholesterol (p-value<0.05). Higher maternal AA levels were associated with higher childhood total-cholesterol and HDL-cholesterol levels (differences: 0.04 (95% CI: 0.01, 0.08) and 0.05 (95% CI: 0.01, 0.08, respectively) per SD increase of maternal AA levels), but not with childhood LDL-cholesterol, triglycerides or insulin levels. We observed similar results when we used childhood c-peptide levels instead of childhood insulin levels ([Supplemental Table S5](#)). These results were not materially affected by additional adjustment for childhood body mass index.

### Maternal n-6/n-3 PUFAs ratio with childhood lipid and insulin levels

**Figure 1** shows that a higher maternal n-6/n-3 PUFA ratio was not associated with childhood total-cholesterol and LDL-cholesterol levels. A higher maternal n-6/n-3 PUFA ratio was associated with lower childhood HDL-cholesterol and insulin levels (differences: −0.05 (95% CI: −0.08, −0.01) and −0.05 (95% CI: −0.09, −0.01) per SD increase of maternal n-6/n-3 PUFA ratio), but with higher childhood triglycerides levels (difference: 0.04 (95% CI: 0, 0.08) per SD increase of maternal n-6/n-3 PUFA ratio). These associations were not explained by additional adjustment for childhood body mass index. Additional adjustment for infants' PUFA intakes at 13 months did not affect the observed associations (data not shown). Replacing child body mass index by fat mass index did not change the results (results not shown).

**Table 3** Maternal plasma n-3 PUFA levels and childhood lipid and insulin levels (N = 3230).<sup>a</sup>

Maternal n-3 PUFA in SDS	Differences in childhood lipid and insulin outcomes (95% confidence interval) in SDS				
	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
Total n-3 PUFA					
Adjusted model <sup>b</sup>	<b>0.04 (0.01, 0.08)*</b>	<b>0.06 (0.02, 0.09)*</b>	0.02 (−0.02, 0.05)	−0.03 (−0.07, 0.01)	<b>0.05 (0.01, 0.08)*</b>
Childhood BMI model <sup>c</sup>	<b>0.04 (0.01, 0.08)*</b>	<b>0.06 (0.02, 0.09)*</b>	0.02 (−0.02, 0.05)	−0.03 (−0.07, 0.01)	<b>0.05 (0.01, 0.08)*</b>
ALA					
Adjusted model	−0.02 (−0.06, 0.02)	−0.02 (−0.06, 0.01)	−0.03 (−0.07, 0.01)	0.01 (−0.02, 0.05)	−0.01 (−0.05, 0.03)
Childhood BMI model	−0.02 (−0.06, 0.02)	−0.03 (−0.06, 0.01)	−0.03 (−0.07, 0.01)	0.01 (−0.02, 0.05)	−0.01 (−0.04, 0.03)
EPA					
Adjusted model	0.03 (−0.01, 0.07)	<b>0.07 (0.03, 0.10)*</b>	0.01 (−0.03, 0.04)	−0.03 (−0.07, 0.01)	0.04 (−0.01, 0.07)
Childhood BMI model	0.03 (−0.01, 0.07)	<b>0.07 (0.03, 0.10)*</b>	0.01 (−0.03, 0.04)	−0.03 (−0.07, 0.01)	0.04 (−0.01, 0.07)
DPA					
Adjusted model	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	−0.01 (−0.04, 0.04)	−0.01 (−0.05, 0.02)	<b>0.04 (0, 0.07)*</b>
Childhood BMI model	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)	−0.01 (−0.05, 0.02)	<b>0.04 (0.01, 0.08)*</b>
DHA					
Adjusted model	<b>0.04 (0.01, 0.08)*</b>	<b>0.06 (0.02, 0.09)*</b>	0.02 (−0.02, 0.06)	−0.03 (−0.06, 0.01)	<b>0.04 (0.01, 0.08)</b>
Childhood BMI model	<b>0.04 (0.01, 0.08)*</b>	<b>0.06 (0.02, 0.09)*</b>	0.02 (−0.02, 0.06)	−0.03 (−0.06, 0.01)	<b>0.04 (0.01, 0.08)*</b>

\*P-value<0.05.

<sup>a</sup> Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood lipid and insulin levels per SD change in maternal n-3 PUFA levels, respectively.

<sup>b</sup> Adjusted model is adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time.

<sup>c</sup> Childhood BMI model is additionally adjusted for body mass index standard-deviation scores at 6 years.



**Table 4** Maternal plasma n-6 PUFA levels and childhood lipid and insulin levels (N = 3230).<sup>a</sup>

Maternal n-6 PUFA in SDS	Differences in childhood lipid and insulin outcomes (95% confidence interval) in SDS				
	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
Total n-6 PUFA					
Adjusted model <sup>b</sup>	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.05)	0.02 (−0.02, 0.06)	0.02 (−0.02, 0.06)	−0.02 (−0.06, 0.02)
Childhood BMI model <sup>c</sup>	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.05)	0.02 (−0.02, 0.06)	−0.02 (0.06, 0.02)
LA					
Adjusted model	−0.01 (−0.05, 0.02)	0.01 (−0.03, 0.04)	−0.01 (−0.04, 0.03)	0.01 (−0.03, 0.05)	−0.02 (−0.05, 0.02)
Childhood BMI model	−0.01 (−0.05, 0.03)	0.01 (−0.03, 0.04)	−0.01 (−0.04, 0.03)	0.01 (−0.03, 0.05)	−0.01 (−0.05, 0.02)
GLA					
Adjusted model	−0.02 (−0.06, 0.02)	−0.02 (−0.06, 0.01)	−0.02 (−0.06, 0.02)	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)
Childhood BMI model	−0.02 (−0.05, 0.02)	−0.02 (−0.06, 0.01)	−0.02 (−0.05, 0.02)	0.01 (−0.03, 0.04)	0.01 (−0.03, 0.04)
EDA					
Adjusted model	−0.02 (−0.06, 0.01)	−0.04 (−0.07, −0.01)*	−0.01 (−0.05, 0.02)	0.01 (−0.03, 0.05)	−0.02 (−0.05, 0.02)
Childhood BMI model	−0.02 (−0.06, 0.01)	−0.04 (−0.07, −0.01)*	−0.01 (−0.05, 0.02)	0.01 (−0.03, 0.04)	−0.02 (−0.05, 0.02)
DGLA					
Adjusted model	−0.01 (−0.05, 0.03)	−0.07 (−0.10, −0.03)*	0.01 (−0.04, 0.04)	0.06 (0.02, 0.10)*	−0.03 (−0.06, 0.01)
Childhood BMI model	−0.01 (−0.05, 0.03)	−0.07 (−0.10, −0.03)*	0.01 (−0.04, 0.04)	0.06 (0.02, 0.09)*	−0.03 (−0.06, 0.01)
AA					
Adjusted model	0.04 (0.01, 0.08)*	0.05 (0.01, 0.08)*	0.03 (−0.01, 0.07)	−0.02 (−0.06, 0.02)	0.02 (−0.01, 0.06)
Childhood BMI model	0.04 (0.01, 0.07)*	0.05 (0.01, 0.09)*	0.03 (−0.01, 0.06)	−0.02 (−0.06, 0.01)	0.02 (−0.02, 0.05)
DTA					
Adjusted model	−0.02 (−0.06, 0.02)	−0.03 (−0.07, 0.01)	0.01 (−0.03, 0.04)	−0.01 (−0.04, 0.03)	−0.02 (−0.05, 0.02)
Childhood BMI model	−0.02 (−0.06, 0.02)	−0.03 (−0.07, 0.01)	0.01 (−0.03, 0.04)	−0.01 (−0.04, 0.03)	−0.02 (−0.06, 0.02)

\*P-value &lt; 0.05.

<sup>a</sup> Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood lipid and insulin levels per SD change in maternal n-6 PUFA levels, respectively.<sup>b</sup> Adjusted model is adjusted for maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time.<sup>c</sup> Childhood BMI model is additionally adjusted for body mass index standard-deviation scores at 6 years.

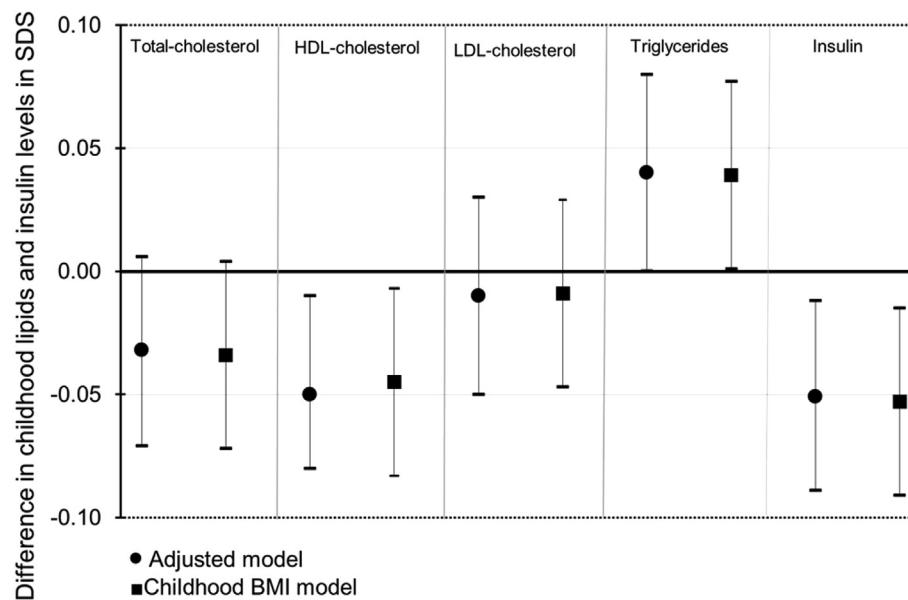
## Discussion

We observed that higher maternal total n-3 PUFA and specifically DHA levels during pregnancy were associated with higher childhood total-cholesterol, HDL-cholesterol, and insulin levels. The individual maternal n-6 PUFAs, EDA, DGLA and AA, but not total maternal n-6 PUFA levels during pregnancy, were associated with childhood total and HDL-cholesterol levels in different directions. These associations were not explained by childhood concurrent body mass index.

## Methodological considerations

We used a population-based prospective cohort study design with a large number of subjects. Of all children whose maternal PUFA levels were available, 64% participated in the follow-up studies at the age of 6 years. The non-response could lead to biased effect estimates if the associations of maternal PUFA levels with childhood lipid and insulin levels would be different between children included and not included in the analyses. Non-response analysis showed that mothers included in the analyses had higher total n-3 PUFA levels and a lower n-6/n-3 PUFAs ratio compared to those not included. It is hard to speculate whether these differences would have affected the observed associations materially. We measured a large number of maternal PUFA levels in plasma samples once during pregnancy. No information was available about

PUFA levels earlier or later in pregnancy. Nevertheless, PUFAs measured in plasma reflect a time frame of dietary intake of approximately 2 weeks and seem to be reasonable indicators for the recent intake [23]. The fasting time before venous punctures was limited. Due to the young age of the children and the structure of the follow-up visits, it was ethically not possible to obtain 3-hours fasting blood samples. We used blood samples obtained 30-minutes after the last meal to measure childhood lipid, insulin and c-peptide levels. This may have led to non-differential misclassification and an underestimation of the associations with maternal PUFAs. It has only been shown among adult populations that also non-fasting lipid and insulin levels are associated with increased risks of cardiovascular events [24–26]. Further studies are needed with more detailed fasting measurements of offspring lipids and insulin metabolism to assess the associations of maternal PUFA status during pregnancy with both fasting and non-fasting offspring lipid and insulin metabolism throughout the life course. The percentages of women with gestational diabetes were relatively low within our study cohort. Accurate diagnosis of gestational diabetes is difficult. Information on gestational diabetes was obtained from the medical records. Unfortunately, in our study, no data were available on glucose tolerance before and during pregnancy, which would have allowed a better identification of women at risk of development of gestational diabetes. The prevalence in our study was lower than those in previous studies, which may be explained by use of different criteria



**Figure 1** Maternal plasma n-6/n-3 PUFA ratio and childhood lipids and insulin levels (N = 3320). Values are regression coefficients (95% CIs) that reflect the difference in SDS of childhood lipids and insulin levels per SD change in maternal n-6/n-3 PUFAs ratio, respectively. Models are adjusted for gestational age at blood sampling, maternal age, educational level, ethnicity, parity, pre-pregnancy body mass index, smoking habits during pregnancy, folic acid supplement use and total caloric intake during pregnancy and pregnancy complications, and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. \*P-value < 0.05.

for gestational diabetes and the relatively healthy study population within our study cohort [27]. Finally, although we performed adjustment for a large number of potential maternal and childhood confounders, residual confounding might still occur, as in any observational study.

### Interpretation of main findings

Suboptimal maternal nutritional status during pregnancy is associated with an increased risk of cardio-metabolic diseases in the offspring [28,29]. Previous studies have shown that maternal PUFA status during pregnancy can affect lipid and insulin metabolism, measured in cord blood [8,30,31]. However, associations with lipid and insulin metabolism in the offspring at later age are less clear.

Previous studies on different maternal PUFAs during pregnancy and offspring lipid metabolism have shown inconsistent results. A study among 965 Danish pregnant women reported no associations between dietary intake of total n-3 PUFA during the second trimester of pregnancy and total-cholesterol, HDL-cholesterol and LDL-cholesterol levels in the 19-year-old offspring [32]. In the same study, offspring triglyceride levels were higher among children whose mothers had total n-3 PUFA intake in the higher quintiles as compared to the lowest quintile. A study among 243 mothers and their offspring from Denmark showed no effect of fish oil supplementation during the third trimester of pregnancy on plasma total-cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, and lipoproteins in their 19-years-old offspring [9]. A small study in 242 mother–child pairs from the Netherlands showed that a higher maternal DPA, a n-3 PUFA, measured at the

first trimester of pregnancy was associated with higher total-cholesterol and LDL-cholesterol levels in 7-year-old children. In the same study, a higher maternal AA level, a n-6 PUFA, was associated with lower childhood triglycerides and LDL-cholesterol levels, but no associations were observed for other n-6 PUFA levels [8]. In this current study, we observed that higher maternal total n-3 PUFA and DHA levels during pregnancy were associated with higher childhood total-cholesterol and HDL-cholesterol levels. No associations of maternal total n-6 PUFAs during pregnancy with childhood lipids were present. However, among the individual n-6 PUFAs, higher maternal EDA and DGLA levels were associated with lower childhood HDL-cholesterol, whereas higher AA levels were associated with higher childhood total-cholesterol and HDL-cholesterol levels. In addition, a higher maternal n-6/n-3 PUFA ratio was associated with a lower HDL-cholesterol, but with higher childhood triglycerides levels. These associations were independent of childhood body mass index. Thus, our results suggest that a suboptimal maternal PUFA status during pregnancy is associated with childhood lipid levels, independent of childhood current body mass index. It appears that individual maternal n-3 and n-6 PUFAs may have alternating effects on offspring lipids metabolism. DHA and AA are preferentially transferred from the maternal to the fetal circulation by means of a preferential placental transfer mediated by fatty acid transport and binding proteins [33,34] which may partly explain the observed associations specifically for these PUFAs since they comprise a relative large part of the total fetal PUFA pool.

Previous studies assessing the influence of maternal PUFAs intake on offspring insulin sensitivity also reported

inconsistent results [10,11,31]. A randomized controlled trial among 533 pregnant Danish women showed no effect of n-3 PUFA supplementation during the third trimester of pregnancy on insulin levels in 19-year-old offspring [10]. A case–control study among 85 diabetic mothers and 1071 controls from Norway reported that cod liver oil supplementation, a n-3 PUFA, during pregnancy was associated with reduced risk of offspring type I diabetes mellitus [11]. Another trial among 47 women and their infants showed that mothers consuming DHA, a n-3 PUFA, during the last half of pregnancy had lower cord blood insulin levels, compared to the control group [31]. A study among 242 Dutch mother–child pairs showed that higher levels of EPA, a n-3 PUFA, were associated with lower glucose levels in 7-year-old children, whereas only higher levels of maternal LA, a n-6 PUFA, were associated with children's higher proinsulin levels [8]. Contrary to these previous studies, we observed that higher maternal total n-3 PUFA and specifically DHA levels during pregnancy were associated with higher childhood insulin and c-peptide levels, independent of childhood concurrent body mass index. No associations were observed between maternal n-6 PUFA levels and childhood insulin levels. Differences between our study and previous studies may be explained by differences in study design, age of participants and different methods to assess maternal PUFA status during pregnancy and childhood lipid and insulin/glucose metabolism [8–11,31,32]. The previous studies all used fasting blood samples, whereas in our study we used non-fasting blood samples to measure childhood lipid and insulin metabolism [8–10,32]. Thus far, it remains unclear whether maternal PUFA status has different effects on offspring fasting vs non-fasting lipid and insulin metabolism. Further studies are needed to assess the detailed associations of maternal PUFA levels during pregnancy with offspring fasting and non-fasting measures of lipid and insulin metabolism.

The mechanisms underlying these observed associations are not known. We did not observe an effect of additional adjustment for childhood body mass index, which suggests that these associations may not be explained by the influence of childhood adiposity on lipid and insulin metabolism. Maternal n-3 PUFAs may affect offspring total-cholesterol and triglycerides through a reduction of hepatic synthesis of triacylglycerols and very-low-density-lipoprotein [35]. Furthermore, as a part of the cell membrane, n-3 PUFAs may be able to regulate insulin secretion from pancreatic  $\beta$ -cells directly by altering lipid raft structure [36]. Among n-6 PUFAs, AA may play a beneficial metabolic role in the health of offspring together with EPA and DHA during critical periods of fetal development through changes in gene expression, the production of eicosanoids and inflammatory markers [37,38]. PUFAs may also affect offspring insulin levels during pregnancy through epigenetic regulation of imprinted genes, especially insulin growth factor 2 (IGF2), which is known to control fetal growth, development and insulin/glucose metabolism [39]. Future research focused on epigenetic mechanisms is needed to assess the effect of

PUFAs during pregnancy on epigenetic regulation of imprinted genes, including IGF2, which may be involved in the underlying mechanisms in the observed associations.

## Conclusion

We observed that higher maternal total n-3 PUFAs and specifically DHA levels during pregnancy were associated with higher childhood total-cholesterol, HDL-cholesterol, and insulin levels. Only specific individual maternal n-6 PUFAs during pregnancy, not total maternal n-6 PUFA levels, tended to be associated with childhood lipid and insulin levels. Further observational and experimental studies are needed for replication of our findings and to obtain further insight into the potential different role of individual maternal PUFAs on offspring lipid and insulin metabolism.

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## Conflict of interest

None of the authors had a financial or personal conflict of interest.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2016.10.001>.

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