AJCN. First published ahead of print April 6, 2016 as doi: 10.3945/ajcn.115.128520.

# Reduction of the n–6:n–3 long-chain PUFA ratio during pregnancy and lactation on offspring body composition: follow-up results from a randomized controlled trial up to 5 y of age[1–3]

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## ABSTRACT

**Background:** It has been hypothesized that the n–6:n–3 (ω-6:ω-3) long-chain polyunsaturated fatty acid (LCPUFA) ratio in the maternal diet during the prenatal and early postnatal phase positively affects the body composition of the offspring. However, only limited data from prospective human intervention studies with long-term follow-up are available.

**Objective:** We assessed the long-term effects of a reduced n–6:n–3 LCPUFA ratio in the diets of pregnant and lactating women [1020 mg docosahexaenoic acid (DHA) plus 180 mg eicosapentaenoic acid (EPA)/d together with an arachidonic acid–balanced diet compared with a control diet] on the body weights and compositions of their offspring from 2 to 5 y of age with a focus on the 5-y results.

**Design:** Participants in the randomized controlled trial received follow-up assessments with annual body-composition measurements including skinfold thickness (SFT) measurements (primary outcome), a sonographic assessment of abdominal subcutaneous and preperitoneal fat, and child growth. In addition, abdominal MRI was performed in a subgroup of 5-y-old children. For the statistical analysis, mixed models for repeated measures (MMRMs) were fit with the use of data from each visit since birth (except for MRI).

**Results:** Maternal LCPUFA supplementation did not significantly influence the children's sum of 4 SFTs [means ± SDs at 5 y of age: intervention, 23.9 ± 4.7 mm ($n$ = 57); control, 24.5 ± 5.0 mm ($n$ = 55); adjusted mean difference, −0.5 (95% CI: −2.2, 1.2)], growth, or ultrasonography measures at any time point in the adjusted MMRM model (all $P$ values $< 0.05$). Results were consistent with abdominal MRI measurements ($n$ = 44) at 5 y of age, which showed no significant differences in subcutaneous and visceral adipose tissue volumes and ratios.

**Conclusion:** The current study provides no evidence that a dietary reduction of the n–6:n–3 LCPUFA ratio in the maternal diet during pregnancy and lactation is a useful early preventive strategy against obesity at preschool age. This trial was registered at clinicaltrials.gov as NCT00362089. *Am J Clin Nutr* doi: 10.3945/ajcn.115.128520.

**Keywords:** body composition, LCPUFA, obesity, preschool age, prevention

## INTRODUCTION

The increasing prevalence of overweight and obesity, particularly in early life stages, has negative implications for individuals and society as a whole (1). To prevent and overcome this problem, different approaches are being pursued on a global level (2). These methods include approaches that are based on the concept of fetal programming, which hypothesizes that the intrauterine environment during the prenatal period is associated with lifelong, adverse health-related outcomes in the offspring. One such outcome is risk of obesity and its associated diseases (3). Specific emphasis has been placed on intake and the ratio of essential n–3 and n–6 long-chain PUFAs (LCPUFAs)[9] because they may be involved in early adipocyte differentiation (4). On the basis of in vitro and animal studies (5), it was hypothesized that the ratio of n–6:n–3 LCPUFA intake during the prenatal and early postnatal period influences the development of fat mass in offspring, with a lower ratio preventing excess adipose tissue development (6).

[1] Supported by grants from the Else Kröner-Fresenius Foundation, the International Unilever Foundation, the European Union–funded Early Nutrition Programming Project consortium (FOOD-CT-2005-007036), the German Ministry of Education and Research via the Competence Network Obesity (Kompetenznetz Adipositas, 01GI0842), and Danone Research-Centre for Specialised Nutrition.

[2] There was no intervention from any sponsor in any of the research aspects of the study including the study design, intervention, data collection, analysis and interpretation, or writing of the manuscript.

[3] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[9] Abbreviations used: AA, arachidonic acid; INFAT, Impact of Nutritional Fatty Acids during Pregnancy and Lactation on Early Human Adipose Tissue Development; LCPUFA, long-chain PUFA; MMRM, mixed model for repeated measures; NAT, nonadipose tissue; PA, physical activity; RCT, randomized controlled trial; SAT, subcutaneous adipose tissue; SFT, skinfold thickness; VAT, visceral adipose tissue.

Received December 9, 2015. Accepted for publication March 10, 2016.
doi: 10.3945/ajcn.115.128520.

On the basis of this consideration, several prospective human cohort studies and randomized controlled trials (RCTs) have been performed that investigated the impact observed on altering the n–6:n–3 fatty acid ratio. These studies showed conflicting results (7–12). However, they were mostly post hoc analyses in trials that were primarily designed to investigate other outcomes such as infant neurodevelopment. To our knowledge, the INFAT (Impact of Nutritional Fatty Acids during Pregnancy and Lactation on Early Human Adipose Tissue Development) study was the first human RCT to focus on the impact of a dietary change or modification of the n–6:n–3 LCPUFA ratio in pregnant and lactating women on infant adipose tissue growth as the primary outcome. Supplementation with fish-oil capsules (1020 mg DHA plus 180 mg EPA/d) together with an arachidonic acid (AA)–balanced diet provided no evidence that this dietary intervention could prevent excess adipose tissue growth in infants ≤1 y of age (13, 14). According to a recent meta-analysis by Stratakis et al. (15), there is currently no conclusive evidence to support a favorable programming effect of n–3 LCPUFA supplementation during pregnancy or lactation on BMI of preschool children. However, because of between-study heterogeneity and methodologic limitations, such as small sample sizes or selective attrition rates, the authors claimed that additional high-quality studies are required (15). Furthermore, the need to assess the long-term effects has also been highlighted (15–18). To assess the long-term effects of the intervention, the infants in the INFAT study were followed up until they reached preschool age. In the current article, we present the findings of the follow-up study with a primary focus on the 5-y results.

## METHODS

The INFAT study was conducted as an open-label, monocenter, randomized, controlled dietary intervention trial with 2 parallel groups, with each group consisting of 104 pregnant women. Originally, the study was designed to investigate the effect of a reduction in the n–6:n–3 LCPUFA ratio in the diets of pregnant women and breastfeeding mothers on adipose tissue growth in their infants aged ≤1 y. To investigate the long-term effects, the infants were followed up until the age of 5 y. Details of the rationale, study design (including the sample-size determination, eligibility criteria, and process of random assignment), participant characteristics, and clinical results on the fat mass of infants ≤1 y old together with maternal and fetal fatty acid profiles have been previously described (13, 14, 19).

### Subjects

In total, 208 healthy, pregnant women with a mean age of 32 y and a mean prepregnancy BMI (in kg/m$^2$) of 22 were recruited before the 15th wk of gestation between July 2006 and May 2009. From enrollment until 4 mo postpartum, women in the intervention group received 1200 mg LCPUFAs (1020 mg DHA plus 180 mg EPA plus 9 mg vitamin E) as fish-oil capsules daily. In addition, the dietary n–6:n–3 LCPUFA ratio was further reduced through specific, individualized dietary counseling aimed at lowering AA intake. The control group received general recommendations regarding healthy nutrition during pregnancy. Baseline maternal clinical characteristics, dietary habits, lifestyle factors, and sociodemographic variables did not differ significantly between the 2 study groups. Within the study, 188 women gave birth to healthy infants ($n$ = 90 girls; $n$ = 98 boys) with a mean difference in pregnancy duration of 4.8 d (95% CI: 1.19, 7.67) between study groups. For the initial study, infants were assessed at ≤1 y of age (13, 14). Follow-up assessments were performed between February 2008 and November 2014 to detect the possible long-term effects of the intervention in the offspring at 2, 3, 4, and 5 y of age. All follow-up assessments included the same measurements of infant growth and fat mass that were performed during the first year of life with skinfold thickness (SFT) measurements remaining as the primary outcome measurement. Participant data were collected at the study center or were assessed by a study team member during a home visit. Furthermore, an abdominal MRI at 5 y of age (Department of Radiology, Klinikum rechts der Isar, Munich) and the assessment of the children's diet and physical activity (PA) at 3 respective time points (3, 4, and 5 y of age) were investigated. The ethical committee of the Technische Universität München approved the study protocol (1479/06/2009/10/26). Written informed consent for follow-up was obtained from both parents of each child.

### Child growth and development

For infants ≤2 y of age, weight was measured to the nearest 10 g with the use of a standard infant scale (Babywaage Ultra MBSC-55; myweight), and length was measured with the use of a measuring stick (Säuglingsmessstab seca 207; seca) to the nearest 0.5 cm while the infant was supine with stretched legs. At later time points, a standard flat scale (Seca Clara 803; seca) was used to determine weight to the nearest 100 g. In addition, a stadiometer (Stadiometer seca 214; seca) was used to measure the child height to the nearest 0.5 cm with both measures performed with the child in a standing position. BMI percentiles were determined with the use of the German reference group according to Kromeyer-Hauschild et al. (20).

### Fat mass and fat distribution of the children

#### *SFT*

The infant's SFTs as a primary outcome was measured in triplicate with the use of a Holtain caliper (Holtain Ltd.) at 4 different body sites on the left body axis (triceps, biceps, subscapular, and suprailiac). Measurements were performed at 2, 3, 4, and 5 y of age at the study center or at the family's home. For each site, the mean of the 3 measurements was used for the SFT value, and the sum of the 4 SFTs was calculated. Fat mass and the percentage of body fat were calculated with the use of predictive skinfold regression equations according to Weststrate and Deurenberg (21).

#### *Ultrasound*

In addition, ultrasonography was performed to determine abdominal subcutaneous and preperitoneal fat areas with the use of a high-resolution ultrasonographic system (Siemens Acuson ×150 Premium; Siemens) at 2, 3, 4, and 5 y of age. The originally described method from Holzhauer et al. (22) was slightly modified for our purposes and has been described in detail (23). Three trained research assistants (CB, SB, and K Pusch) collected data on infant growth, SFT measurements, and ultrasounds.

*MRI measurement*

In addition, abdominal MRI in a subgroup of 5-y old children was performed to quantify abdominal adipose tissue. Although all participating families were approached, data for the analysis were only available from 44 children. Before the examination, additional written informed consent was obtained from the accompanying parent. An MRI examination was performed without sedation on a clinical whole body scanner at 1.5 T (Magnetom Avanto; Siemens Medical Solutions). The children were positioned supine (feet first) with arms next to their bodies and with spine array receive coils included in the patient table. In addition, a body matrix coil was applied ventrally, which covered the entire abdominal region. To mitigate the possible degradation of image quality because of the limited ability to comply with the MRI scan, the imaging protocol had to be kept as short as possible. The quantitative scans were planned on localizer images, the latter of which were obtained under free-breathing conditions (field of view: 500 mm; 3 orientations; duration: 13 s). Water and fat separation was based on a 4-point (echo time: 2.38, 4.76, 7.15, and 9.53 ms) Dixon technique as described by Glover (24). The total measurement consisted of a series of blocks of 4 transverse spoiled gradient echo images, which were acquired in inspiration (breath hold of 3.9 s). The resolution was $2.34 \times 2.34$ mm$^2$ in plane and 10 mm in the head-feet direction (80 mm slice thickness and an additional gap of 2 mm). Additional imaging variables included repetition time of 50 ms, flip angle of 55°, and a monopolar readout gradient to avoid phase shifts between in-phase and opposed-phase images.

*Postprocessing of MRI data*

The acquired data were exported to a remote workstation and analyzed with self-written software in the MatLab program (R2014b; MathWorks) according to the procedure outlined by Glover (24). On the basis of the phase images, a (smoothed) B0 map was calculated that included a local off resonance and a hardware-related offset (coil phase) with the exclusion of the chemical shift between water and fat. Subsequently, the complex images were B0 corrected and transformed into real-value images. The ratio of the in-phase and opposed-phase magnitude images (evaluated independently and subsequently averaged) allowed for an estimate of the T2* decay, which further improved the water and fat separation (24). Finally, the water and fat images were calculated with the use of the Dixon approach. Images were further used for subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) segmentation.

*MRI analysis: SAT and VAT segmentation*

For image analyses, single slices that were bounded by the head of the liver to the iliac crest were manually identified from 2 individuals (C Cordes and CB). Selected slice images were automatically analyzed with a segmentation algorithm written in MatLab software (R2014b) according to the method of Cordes et al. (25). This algorithm was used to obtain an initial fat classification of the following 3 compartments: *1*) SAT, which is the fraction between the dermis and external fascia of the abdominal muscle wall; *2*) VAT, which is within the inner contour of the SAT compartment; and *3*) nonadipose tissue (NAT), which is the remaining fraction (mostly water). Image processing required manual correction and was conducted by a single person (CB). Volumes of SAT, VAT, and NAT were quantified by summing the individual slices. Ratios of SAT, VAT, and NAT volumes to total volume were also generated.

**Dietary intake**

The children's diets were assessed with the use of 3-d estimated food records at 3, 4, and 5 y of age and were completed by their parents or the daycare personnel. Data were entered in a standardized manner by one person (M-TK) with the use of OptiDiet Plus software (version 5.1.2.065; GOE mbH), which is a program that is based on a German nutrient database (Bundeslebensmittelschlüssel). Energy and macronutrient intake were analyzed.

**PA questionnaires**

We assessed the PA and inactivity of the children with the use of a basic questionnaire from the German Health Interview and Examination Survey for children and adolescents at 3 respective time points (3, 4, and 5 y of age) (26, 27). One of the parents of each child filled out the questionnaire during the follow-up visits or at home. The protocol contained questions on play, exercise, television viewing, and computer consumption and was evaluated by a single person (M-TK).

**Statistical analysis**

All analyses were based on children who actively participated at the follow-up. For each group, means ± SDs of anthropometric data, SFT, and ultrasound data are presented for children at 2, 3, 4, and 5 y of age; dietary values are presented for children at 3, 4, and 5 y of age; and MRI analyses are presented for children at 5 y of age. For outcomes measured more than once (all except MRI), likelihood-based mixed models for repeated measures (MMRMs) according to Bell et al. (28) were fit with the use of data from each visit (birth and 6 wk, 4 mo, and 1, 2, 3, 4, and 5 y of age). The independent variables included were the visit number as a factor variable and indicator variables for the group assignment at each visit. In adjusted analyses, sex and pregnancy duration were also included as independent variables. Unstructured covariance matrices were used to model the within-subject error. Although the difference in groups at 5 y of age was the focus of this study, estimated mean differences between the groups are presented for each measure at each time point together with 95% CIs. For the MRI data, differences between groups were analyzed with the use of simple and multiple linear regression models that were adjusted for sex and pregnancy duration. The binary outcomes from the PA questionnaire were compared between groups, at each time point separately, with the use of chi-square tests. All statistical analyses were performed with the use of the statistical program R (version R 3.1.3; R Foundation for Statistical Computing); in particular, the mixed models were fit with the use of the gls function within the nlme library and with PASW software (version 21.0; SPSS Inc.). A 2-sided $P$ value $<0.05$ was considered statistically significant, and no correction was made for multiple comparisons.

## RESULTS

### Participants

Of the 208 women included at the beginning of the study, data for the body composition of 170 children (81.7%) were available at 1 y of age (13). For the follow-up study, we analyzed the data from 118 children at 2 y of age (56.7%), 120 children at 3 y of age (57.7%), 107 children at 4 y of age (51.4%), and 114 children at 5 y of age (54.8%) with similar numbers between study groups (at 5 y: intervention group, $n = 58$; control group, $n = 56$). Data from all children who participated at the follow-up are presented in **Figure 1**. The most common reasons for dropout were a lack of time or relocation.

### Child growth and development

Growth patterns, including weight, height, and head, arm, and waist circumferences for children aged between 2 and 5 y are summarized in **Table 1**. These outcome variables did not significantly differ between the intervention and control group in the unadjusted and adjusted MMRM analyses at any time point except for weight and BMI at 4 y of life. These 2 variables showed significantly higher values for the intervention group in the unadjusted analysis but not in the adjusted analysis. BMI percentiles, which were estimated with the use of German reference data (20), showed values in the recommended range for most but not all children. For example, 51 of 58 children aged

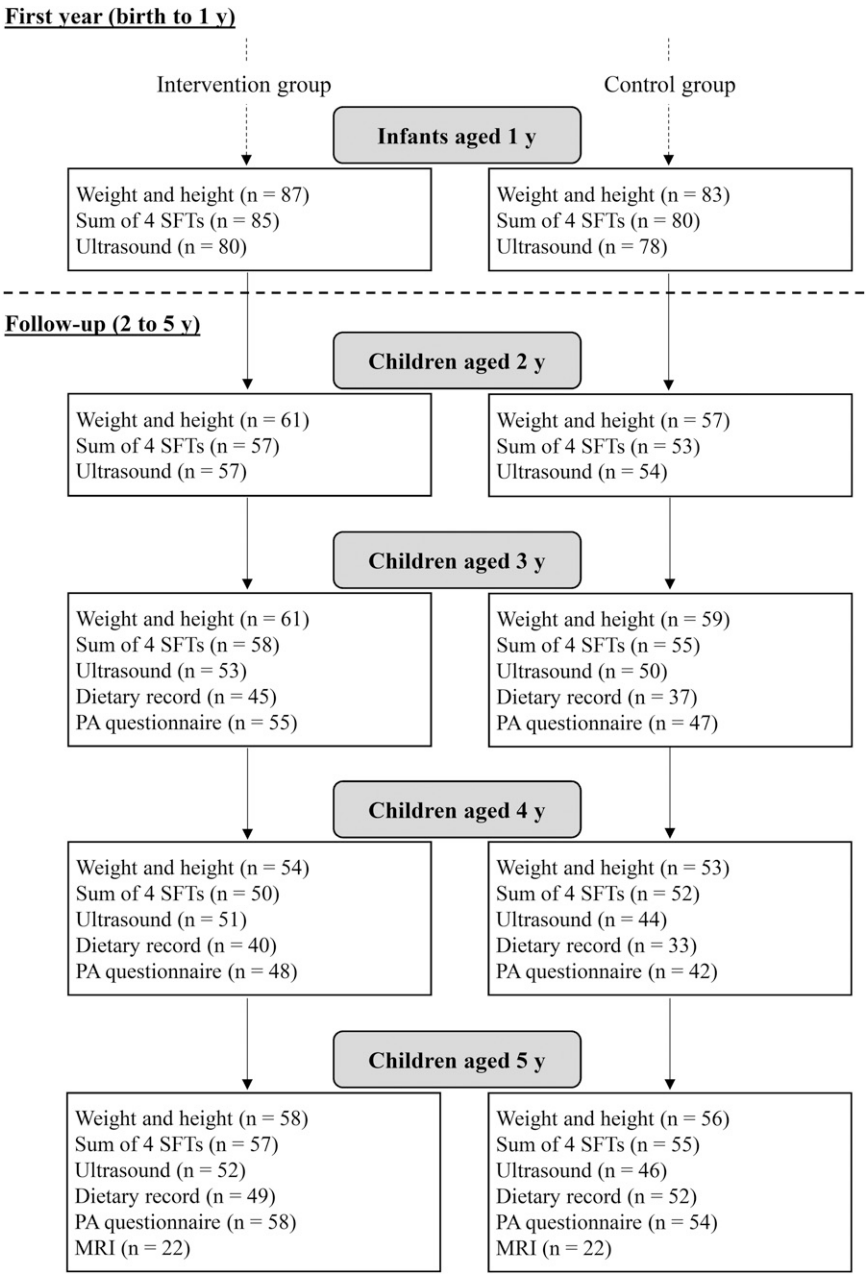


**FIGURE 1** Flowchart of follow-up data of participants in the INFAT study. The flowchart of the first year of life has been published elsewhere (13). As indicated in parentheses, data for the sum of 4 SFTs, ultrasounds, dietary records, and PA questionnaires were not available for all children. INFAT, Impact of Nutritional Fatty Acids during Pregnancy and Lactation on Early Human Adipose Tissue Development; PA, physical activity; SFT, skinfold thickness.

**TABLE 1**
Growth patterns from 2 to 5 y of life

| Variable and age | Intervention group | Control group | Unadjusted difference[1] | *P* | Adjusted difference[2] | *P* |
|---|---|---|---|---|---|---|
| Weight, kg | | | | | | |
| 2 y | 12.5 ± 1.4 [61][3] | 12.3 ± 1.3 [57] | 0.4 (−0.1, 0.9)[4] | 0.082 | 0.2 (−0.2, 0.6) | 0.372 |
| 3 y | 14.8 ± 1.9 [61] | 14.3 ± 1.5 [59] | 0.5 (−0.1, 1.1) | 0.122 | 0.2 (−0.3, 0.8) | 0.452 |
| 4 y | 17.0 ± 2.2 [54] | 16.2 ± 1.7 [53] | 0.8 (0.1, 1.5) | 0.032 | 0.5 (−0.2, 1.1) | 0.146 |
| 5 y | 19.2 ± 3.0 [58] | 18.4 ± 3.2 [56] | 0.7 (−0.3, 1.6) | 0.174 | 0.3 (−0.6, 1.1) | 0.548 |
| Height, cm | | | | | | |
| 2 y | 87.1 ± 2.9 [61] | 87.0 ± 2.7 [57] | 0.3 (−0.6, 1.3) | 0.492 | −0.2 (−1.1, 0.8) | 0.758 |
| 3 y | 96.4 ± 3.8 [61] | 96.0 ± 3.5 [59] | 0.3 (−0.8, 1.5) | 0.580 | −0.2 (−1.3, 1.0) | 0.804 |
| 4 y | 104.0 ± 4.3 [54] | 103.3 ± 3.6 [53] | 0.7 (−0.6, 1.9) | 0.312 | 0.1 (−1.2, 1.4) | 0.829 |
| 5 y | 112.2 ± 4.8 [58] | 110.7 ± 4.0 [56] | 0.5 (−0.9, 2.0) | 0.465 | −0.0 (−1.5, 1.4) | 0.975 |
| BMI percentile[5] | | | | | | |
| 2 y | 56.7 ± 27.5 [61] | 52.2 ± 27.9 [57] | 4.6 (−4.5, 13.7) | 0.325 | 2.3 (−6.9, 11.6) | 0.621 |
| 3 y | 53.9 ± 27.6 [61] | 45.9 ± 23.1 [59] | 5.7 (−2.7, 14.1) | 0.186 | 3.4 (−5.2, 12.0) | 0.437 |
| 4 y | 54.3 ± 24.6 [54] | 41.3 ± 27.1 [53] | 10.0 (1.2, 18.8) | 0.026 | 7.8 (−1.0, 16.6) | 0.081 |
| 5 y | 49.8 ± 27.4 [58] | 45.0 ± 24.3 [56] | 3.8 (−4.9, 12.5) | 0.394 | 1.6 (−7.2, 10.3) | 0.722 |
| Head circumference, cm | | | | | | |
| 2 y | 48.7 ± 1.3 [61] | 48.6 ± 1.3 [57] | 0.2 (−0.2, 0.6) | 0.305 | −0.0 (−0.4, 0.4) | 0.983 |
| 3 y | 49.9 ± 1.4 [61] | 49.8 ± 1.3 [59] | 0.2 (−0.2, 0.6) | 0.326 | −0.0 (−0.4, 0.4) | 0.985 |
| 4 y | 50.6 ± 1.3 [54] | 50.6 ± 1.2 [53] | 0.1 (−0.3, 0.5) | 0.486 | −0.1 (−0.5, 0.3) | 0.715 |
| 5 y | 51.2 ± 1.3 [58] | 51.2 ± 1.3 [56] | 0.1 (−0.3, 0.6) | 0.549 | −0.1 (−0.5, 0.3) | 0.679 |
| Arm circumference, cm | | | | | | |
| 2 y | 15.7 ± 1.1 [61] | 15.5 ± 1.1 [57] | 0.2 (−0.1, 0.6) | 0.222 | 0.2 (−0.2, 0.6) | 0.323 |
| 3 y | 16.1 ± 1.3 [61] | 15.9 ± 1.1 [59] | 0.2 (−0.2, 0.6) | 0.417 | 0.1 (−0.3, 0.5) | 0.545 |
| 4 y | 16.5 ± 1.3 [53] | 16.3 ± 1.1 [52] | 0.1 (−0.3, 0.5) | 0.557 | 0.1 (−0.3, 0.5) | 0.710 |
| 5 y | 16.9 ± 1.4 [58] | 16.8 ± 1.0 [56] | 0.0 (−0.4, 0.5) | 0.837 | 0.0 (−0.4, 0.4) | 0.987 |
| Waist circumference, cm | | | | | | |
| 2 y | 47.8 ± 2.9 [50] | 48.0 ± 2.7 [51] | 0.2 (−0.9, 1.2) | 0.767 | 0.1 (−1.0, 1.1) | 0.877 |
| 3 y | 50.0 ± 3.0 [61] | 49.4 ± 2.2 [59] | 0.5 (−0.4, 1.4) | 0.316 | 0.4 (−0.6, 1.3) | 0.417 |
| 4 y | 52.0 ± 2.9 [54] | 51.3 ± 2.9 [52] | 0.4 (−0.6, 1.5) | 0.410 | 0.4 (−0.7, 1.4) | 0.502 |
| 5 y | 53.4 ± 5.2 [58] | 52.8 ± 2.9 [56] | 0.5 (−1.0, 2.0) | 0.501 | 0.4 (−1.1, 1.9) | 0.568 |

[1]From mixed models for repeated measures with the use of data from each visit since birth.
[2]From mixed models for repeated measures with the use of data from each visit since birth and controlled for sex and pregnancy duration for all variables except BMI percentiles (controlled for pregnancy duration).
[3]Mean ± SD; *n* in brackets (all such values). Values were calculated from the observed data.
[4]Mean; 95% CI in parentheses (all such values).
[5]Calculated according to Kromeyer-Hauschild et al. (20).

5 y (87.9%) from the intervention group and 49 of 56 children aged 5 y (87.5%) from the control group were within the recommended range. There was no significant evidence of a difference in BMI percentiles between groups at 5 y of age (adjusted mean difference at 5 y: 1.6; 95% CI: −7.2, 10.3). At 5 y of age, 5.2% of the children in the intervention group were defined as overweight (including obesity), and 1.7% of the children in the intervention group were defined as obese compared with 3.6% of children who were overweight and 0% of children who were obese in the control group.

## Fat mass and fat distribution of the children

### *SFT*

Results for the sums of the 4 SFTs from 2, 3, 4, and 5 y are provided in **Table 2**. At 5 y of age, the sum of 4 SFTs (sum of the 4 individual SFTs) was 23.9 ± 4.7 mm in the intervention group ($n = 57$) and 24.5 ± 5.0 mm in the control group ($n = 55$), with no significant evidence of a difference between groups provided by the unadjusted or adjusted analyses. Similar results were observed when the 4 individual SFTs were analyzed separately (**Supplemental Table 1**). Likewise, the percentage of body fat and body fat mass (kg) as estimated by the SFT equations (21) did not significantly differ between groups during the follow-up period. The same results applied to lean body mass (kg) and the percentage of lean body mass (Table 2).

### *Ultrasound*

**Table 3** presents results from the adipose tissue growth and abdominal fat distribution at 2, 3, 4, and 5 y of age as assessed with the use of ultrasonography. Consistent with the SFT measurements, the unadjusted and adjusted analyses showed comparable abdominal subcutaneous and preperitoneal fat areas with increasing mean values observed over time. At 5 y of age, the adjusted mean difference between the groups was 0.28 $mm^2$ (95% CI: −4.75, 5.31 $mm^2$) in the preperitoneal area, −2.29 $mm^2$ (95% CI: −6.91, 2.34 $mm^2$) in the subcutaneous $area_{sagittal}$ and −3.88 $mm^2$ (95% CI: −11.22, 3.46 $mm^2$) in the subcutaneous $area_{axial}$. There was also no evidence of a difference in the fat distribution between groups on the basis of the preperitoneal: subcutaneous ratio in the sagittal plane (adjusted mean difference at 5 y of age: 0.49; 95% CI: −0.25, 1.24).

**TABLE 2**
Adipose tissue development, subcutaneous fat, and lean body mass distribution from 2 to 5 y of life assessed with the use of SFT measurements[1]

| Variable and age | Intervention group | Control group | Unadjusted difference[2] | *P* | Adjusted difference[3] | *P* |
|---|---|---|---|---|---|---|
| Sum of 4 SFTs,[4] mm | | | | | | |
| 2 y | 23.8 ± 3.3 [57][5] | 23.5 ± 3.5 [53] | 0.5 (−0.7, 1.7)[6] | 0.398 | 0.6 (−0.6, 1.8) | 0.307 |
| 3 y | 23.4 ± 3.7 [58] | 23.3 ± 3.6 [55] | 0.4 (−0.9, 1.6) | 0.543 | 0.5 (−0.7, 1.7) | 0.455 |
| 4 y | 23.6 ± 3.5 [50] | 23.4 ± 3.8 [52] | 0.2 (−1.1, 1.6) | 0.762 | 0.3 (−1.0, 1.6) | 0.648 |
| 5 y | 23.9 ± 4.7 [57] | 24.5 ± 5.0 [55] | −0.6 (−2.3, 1.1) | 0.493 | −0.5 (−2.2, 1.2) | 0.549 |
| Fat mass,[7] kg | | | | | | |
| 2 y | 2.4 ± 0.5 [57] | 2.3 ± 0.5 [53] | 0.1 (−0.1, 0.3) | 0.211 | 0.1 (−0.1, 0.3) | 0.246 |
| 3 y | 2.7 ± 0.7 [58] | 2.6 ± 0.5 [55] | 0.1 (−0.1, 0.3) | 0.286 | 0.1 (−0.1, 0.3) | 0.321 |
| 4 y | 3.1 ± 0.7 [50] | 2.9 ± 0.6 [52] | 0.2 (−0.1, 0.4) | 0.183 | 0.1 (−0.1, 0.4) | 0.211 |
| 5 y | 3.5 ± 1.1 [57] | 3.4 ± 0.8 [55] | 0.1 (−0.3, 0.4) | 0.734 | 0.1 (−0.3, 0.4) | 0.770 |
| Lean body mass,[8] kg | | | | | | |
| 2 y | 10.1 ± 1.0 [57] | 9.9 ± 0.9 [53] | 0.2 (−0.1, 0.5) | 0.252 | 0.1 (−0.2, 0.4) | 0.439 |
| 3 y | 12.0 ± 1.3 [58] | 11.7 ± 1.2 [55] | 0.1 (−0.3, 0.5) | 0.480 | 0.1 (−0.3, 0.5) | 0.702 |
| 4 y | 13.9 ± 1.8 [50] | 13.3 ± 1.4 [52] | 0.4 (−0.1, 0.9) | 0.095 | 0.3 (−0.1, 0.8) | 0.165 |
| 5 y | 15.8 ± 2.1 [57] | 15.3 ± 1.7 [55] | 0.3 (−0.3, 0.9) | 0.375 | 0.2 (−0.4, 0.8) | 0.527 |
| Fat mass,[7] % | | | | | | |
| 2 y | 19.2 ± 2.3 [57] | 19.0 ± 2.4 [53] | 0.4 (−0.5, 1.2) | 0.400 | 0.4 (−0.4, 1.3) | 0.274 |
| 3 y | 18.4 ± 2.6 [58] | 18.3 ± 2.6 [55] | 0.3 (−0.6, 1.2) | 0.501 | 0.4 (−0.5, 1.2) | 0.378 |
| 4 y | 18.2 ± 2.6 [50] | 17.9 ± 3.0 [52] | 0.3 (−0.8, 1.3) | 0.627 | 0.3 (−0.6, 1.3) | 0.495 |
| 5 y | 17.9 ± 3.4 [57] | 18.1 ± 3.6 [55] | −0.2 (−1.4, 1.0) | 0.766 | −0.1 (−1.3, 1.0) | 0.840 |
| Lean body mass,[9] % | | | | | | |
| 2 y | 80.8 ± 2.3 [57] | 81.0 ± 2.4 [53] | −0.4 (−1.2, 0.5) | 0.402 | −0.5 (−1.3, 0.4) | 0.276 |
| 3 y | 81.6 ± 2.6 [58] | 81.7 ± 2.6 [55] | −0.3 (−1.2, 0.6) | 0.502 | −0.4 (−1.2, 0.5) | 0.380 |
| 4 y | 81.8 ± 2.6 [50] | 82.1 ± 3.0 [52] | −0.3 (−1.3, 0.8) | 0.627 | −0.3 (−1.3, 0.6) | 0.496 |
| 5 y | 82.1 ± 3.4 [57] | 81.9 ± 3.6 [55] | 0.2 (−1.0, 1.4) | 0.766 | 0.1 (−1.1, 1.3) | 0.840 |

[1]SFT, skinfold thickness.
[2]From mixed models for repeated measures with the use of data from each visit since birth.
[3]From mixed models for repeated measures with the use of data from each visit since birth and controlled for sex and pregnancy duration.
[4]Sum of 4 SFTs was calculated as biceps + triceps + subscapular + suprailiac SFTs.
[5]Mean ± SD; *n* in brackets (all such values). Values were calculated from the observed data.
[6]Mean; 95% CI in parentheses (all such values).
[7]Calculated according to Weststrate and Deurenberg (21).
[8]Lean body mass (kg) was calculated as body weight (kg) − fat mass (kg).
[9]Percentage of lean body mass was calculated as 100 – the percentage of fat mass.

*MRI*

In a subgroup of 44 children, an additional abdominal MRI was performed at 5 y of age (**Table 4**). The mean number of analyzed slices was 16.6 ± 1.2 in the intervention group ($n = 22$) compared with 16.7 ± 1.3 in the control group ($n = 22$). Mean SAT, VAT, and NAT volumes did not differ between control and intervention groups [SAT-volume adjusted mean difference: −8.84 $cm^3$ (95% CI: −105.51, 87.83 $cm^3$); VAT-volume adjusted mean difference: −7.18 $cm^3$ (95% CI: −28.65, 14.29 $cm^3$); NAT-volume adjusted mean difference: 160.44 $cm^3$ (95% CI: −62.48, 383.37 $cm^3$)]. Similarly, the calculated percentages (SAT, VAT, and NAT ratio) were not significantly different between groups after adjustment for sex and pregnancy duration. Therefore, these findings were consistent with the results of the other methods.

## Dietary intake

Mean energy in kcal and MJ and macronutrient intakes in grams and percentages of energy are provided in **Table 5**. There was a gradual increase in the mean energy intake in both groups. However, the mean proportion of caloric intake from carbohydrates, fat, and protein did not change notably over time. In the investigated period, the analysis of the mean daily energy and macronutrient intakes showed no significant evidence of a group difference in daily energy and macronutrient intakes at any time point.

## PA

The results of the PA questionnaires provided no evidence regarding differences between groups in active or sedentary behaviors (**Supplemental Table 2**). At 5 y of age, 53 of 58 children (91%) in the intervention group and 50 of 54 children (93%) in the control group regularly participated in a sport activity (≥1 time/wk). Furthermore, all children in both groups played outside ≥3 times/wk. As regards sedentary behavior, 19 of 54 children (35%) in the control group and 16 of 58 children (28%) in the intervention group regularly watched television or played on a computer ≥1 h/d [number of children who played on a computer ≥1 h/d: intervention group: one of 58 (2%); control group: one of 54 (2%)].

## DISCUSSION

Our previous analysis did not provide any evidence that a dietary intervention with fish-oil capsules (1020 mg DHA

**TABLE 3**
Adipose tissue growth and abdominal fat distribution from 2 to 5 y of life assessed by ultrasonography

| Variable and age | Intervention group | Control group | Unadjusted difference[1] | *P* | Adjusted difference[2] | *P* |
|---|---|---|---|---|---|---|
| Preperitoneal $area_{sagittal}$,[3] $mm^2$ | | | | | | |
| 2 y | 22.91 ± 7.04 [57][4] | 24.48 ± 7.97 [54] | −1.71 (−4.30, 0.89)[5] | 0.196 | −1.61 (−4.22, 0.99) | 0.223 |
| 3 y | 32.87 ± 11.05 [52] | 32.34 ± 11.43 [50] | 1.07 (−2.89, 5.03) | 0.594 | 1.16 (−2.80, 5.12) | 0.564 |
| 4 y | 41.10 ± 13.26 [51] | 39.99 ± 14.83 [43] | 1.02 (−3.94, 5.98) | 0.683 | 1.13 (−3.84, 6.09) | 0.654 |
| 5 y | 48.89 ± 12.32 [51] | 47.77 ± 16.18 [45] | 0.18 (4.85, 5.20) | 0.944 | 0.28 (−4.75, 5.31) | 0.913 |
| Subcutaneous $area_{sagittal}$,[3] $mm^2$ | | | | | | |
| 2 y | 16.80 ± 9.59 [57] | 20.74 ± 11.93 [54] | −1.93 (−5.70, 1.84) | 0.313 | −2.41 (−6.18, 1.37) | 0.210 |
| 3 y | 18.86 ± 12.73 [53] | 20.43 ± 11.25 [50] | −0.82 (−4.83, 3.18) | 0.685 | −1.32 (−5.19, 2.54) | 0.499 |
| 4 y | 19.21 ± 11.48 [51] | 20.84 ± 13.15 [42] | −1.06 (−5.57, 3.44) | 0.641 | −1.57 (−5.83, 2.68) | 0.466 |
| 5 y | 20.23 ± 13.76 [52] | 21.24 ± 11.92 [45] | −1.78 (−6.58, 3.01) | 0.463 | −2.29 (−6.91, 2.34) | 0.330 |
| Subcutaneous $area_{axial}$,[6] $mm^2$ | | | | | | |
| 2 y | 22.00 ± 9.55 [57] | 26.96 ± 14.36 [54] | −3.31 (−7.61, 1.00) | 0.131 | −3.88 (−8.14, 0.38) | 0.074 |
| 3 y | 26.36 ± 19.35 [52] | 28.10 ± 16.28 [50] | −0.29 (−6.25, 5.68) | 0.925 | −0.75 (−6.59, 5.09) | 0.800 |
| 4 y | 26.44 ± 17.18 [51] | 29.77 ± 21.69 [44] | −3.40 (−10.68, 3.88) | 0.357 | −3.81 (−10.91, 3.29) | 0.290 |
| 5 y | 28.53 ± 20.68 [52] | 30.08 ± 19.39 [46] | −3.46 (−10.95, 4.04) | 0.363 | −3.88 (−11.22, 3.46) | 0.298 |
| Preperitoneal:subcutaneous ratio[7] | | | | | | |
| 2 y | 1.79 ± 1.31 [57] | 1.49 ± 0.82 [54] | 0.20 (−0.17, 0.58) | 0.279 | 0.21 (−0.16, 0.58) | 0.270 |
| 3 y | 2.48 ± 1.77 [52] | 1.95 ± 1.03 [50] | 0.37 (−0.10, 0.85) | 0.124 | 0.38 (−0.10, 0.86) | 0.121 |
| 4 y | 2.82 ± 1.86 [51] | 2.69 ± 1.79 [42] | −0.02 (−0.65, 0.62) | 0.958 | −0.01 (−0.65, 0.62) | 0.969 |
| 5 y | 3.42 ± 2.42 [51] | 2.94 ± 1.73 [45] | 0.48 (−0.26, 1.23) | 0.201 | 0.49 (−0.25, 1.24) | 0.194 |

[1]From mixed models for repeated measures with the use of data from each visit from 6 wk onward.
[2]From mixed models for repeated measures with the use of data from each visit from 6 wk onward and controlled for sex and pregnancy duration.
[3]Sagittal subcutaneous and preperitoneal fat were measured as areas of 1-cm length in the middle of the xiphoid process according to an adapted method of Holzhauer et al. (22); adapted method described in detail elsewhere (23).
[4]Mean ± SD; *n* in brackets (all such values). Values were calculated from the observed data.
[5]Mean; 95% CI in parentheses (all such values).
[6]Axial subcutaneous fat was measured between the middle of the xiphoid process and the navel directly above the linea alba.
[7]Ratio of preperitoneal to subcutaneous fat was calculated as $preperitoneal\ area_{sagittal} \div subcutaneous\ area_{sagittal}$.

plus 180 mg EPA/d) combined with an AA-balanced diet in pregnant and lactating women had an effect on adipose tissue growth in their offspring for ≤1 y of life (as indicated by SFT measurements and ultrasonography) (13). With the use of the same methods, we followed this cohort carefully up to the fifth year of life. Data from the current study revealed no evidence of any long-term effects of the intervention at 2, 3, 4, and 5 y of age, which was consistent with our previous findings (the observed significant difference in weight and BMI between the 2 groups in the unadjusted MMRM model at 4 y of age should be treated with caution because it may be an artifact of multiple testing). In addition, abdominal MRI measurements at 5 y of age in a subgroup of children showed no significant difference in abdominal fat distribution (SAT and VAT) between intervention and control groups. Furthermore, we showed no differences in dietary energy and macronutrient intakes or PA over the time period between the 2 groups.

**TABLE 4**
Abdominal subcutaneous and visceral adipose tissue volumes and ratios at 5 y of life assessed by MRI[1]

| | Intervention group (*n* = 22) | Control group (*n* = 22) | Unadjusted difference[2] | *P* | Adjusted difference[3] | *P* |
|---|---|---|---|---|---|---|
| SAT volume, $cm^3$ | 563.41 ± 154.00[4] | 563.61 ± 160.31 | −0.21 (95.85, 95.44)[5] | 0.997 | −8.84 (−105.51, 87.83) | 0.854 |
| VAT volume, $cm^3$ | 100.20 ± 35.28 | 108.17 ± 32.39 | −7.97 (−28.57, 12.64) | 0.440 | −7.18 (−28.65, 14.29) | 0.503 |
| NAT volume, $cm^3$ | 3136.01 ± 371.91 | 3056.59 ± 385.81 | 114.25 (−151.14, 309.99) | 0.491 | 160.44 (−62.48, 383.37) | 0.154 |
| SAT ratio,[6] % | 14.68 ± 2.52 | 15.04 ± 3.18 | −0.36 (−2.10, 1.39) | 0.681 | −0.85 (−2.47, 0.77) | 0.298 |
| VAT ratio,[7] % | 2.59 ± 0.65 | 2.91 ± 0.84 | −0.32 (−0.77, 0.14) | 0.170 | −0.35 (−0.82, 0.13) | 0.152 |
| NAT ratio,[8] % | 82.73 ± 2.87 | 82.05 ± 3.74 | 0.68 (−1.35, 2.70) | 0.505 | 1.19 (−0.72, 3.11) | 0.216 |

[1]NAT, nonadipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.
[2]Calculated with the use of Student's *t* test.
[3]Calculated with the use of a multiple regression analysis (*F* test; ANCOVA) and controlled for sex and pregnancy duration.
[4]Mean ± SD (all such values). Values were calculated from the observed data.
[5]Mean; 95% CI in parentheses (all such values).
[6]Ratio was calculated as [SAT volume ÷ (SAT volume + VAT volume + NAT volume)] × 100.
[7]Ratio was calculated as [VAT volume ÷ (SAT volume + VAT volume + NAT volume)] × 100.
[8]Ratio was calculated as [NAT volume ÷ (SAT volume + VAT volume + NAT volume)] × 100.

**TABLE 5**
Energy and macronutrient intakes from 3 to 5 y of life[1]

| Variable and age | Intervention group | Control group | Unadjusted difference[2] | *P* |
|---|---|---|---|---|
| Energy, kcal/d | | | | |
| 3 y | 1361.56 ± 284.45 [45][3] | 1300.51 ± 276.26 [37] | 73.74 (−49.05, 196.52)[4] | 0.236 |
| 4 y | 1546.75 ± 227.79 [40] | 1492.45 ± 344.74 [33] | 47.89 (−78.91, 174.68) | 0.455 |
| 5 y | 1557.24 ± 303.02 [49] | 1610.13 ± 328.14 [52] | −53.50 (−176.91, 69.91) | 0.392 |
| Energy, MJ/d | | | | |
| 3 y | 5.70 ± 1.19 [45] | 5.44 ± 1.16 [37] | 0.31 (−0.21, 0.82) | 0.236 |
| 4 y | 6.47 ± 0.95 [40] | 6.24 ± 1.44 [33] | 0.20 (−0.33, 0.73) | 0.456 |
| 5 y | 6.52 ± 1.27 [49] | 6.73 ± 1.37 [52] | −0.22 (−0.74, 0.29) | 0.391 |
| Protein, g/d | | | | |
| 3 y | 47.96 ± 12.61 [45] | 45.21 ± 10.75 [37] | 3.51 (−1.63, 8.65) | 0.178 |
| 4 y | 54.80 ± 11.88 [40] | 49.88 ± 13.54 [33] | 4.38 (−1.11, 9.87) | 0.117 |
| 5 y | 53.45 ± 13.79 [49] | 54.41 ± 14.19 [52] | −1.15 (−6.58, 4.29) | 0.676 |
| Fat, g/d | | | | |
| 3 y | 51.55 ± 16.68 [45] | 49.26 ± 11.72 [37] | 2.92 (−3.53, 9.37) | 0.371 |
| 4 y | 56.93 ± 12.01 [40] | 56.21 ± 15.77 [33] | −0.51 (−6.79, 5.78) | 0.873 |
| 5 y | 55.21 ± 12.78 [49] | 59.88 ± 15.30 [52] | −4.54 (−10.10, 1.01) | 0.108 |
| Carbohydrate, g/d | | | | |
| 3 y | 172.53 ± 36.92 [45] | 164.95 ± 44.12 [37] | 8.07 (−9.24, 25.38) | 0.357 |
| 4 y | 199.13 ± 31.85 [40] | 191.85 ± 49.30 [33] | 8.96 (−9.36, 27.29) | 0.333 |
| 5 y | 206.80 ± 43.82 [49] | 208.15 ± 49.44 [52] | −1.14 (−19.46, 17.18) | 0.902 |
| Protein, % of energy | | | | |
| 3 y | 14.41 ± 1.98 [45] | 14.33 ± 2.47 [37] | 0.22 (−0.75, 1.18) | 0.654 |
| 4 y | 14.55 ± 2.56 [40] | 13.64 ± 1.74 [33] | 0.86 (−0.13, 1.85) | 0.086 |
| 5 y | 14.02 ± 2.10 [49] | 13.82 ± 2.07 [52] | 0.18 (−0.63, 0.99) | 0.661 |
| Fat, % of energy | | | | |
| 3 y | 34.86 ± 5.86 [45] | 35.44 ± 5.54 [37] | −0.42 (−2.93, 2.08) | 0.738 |
| 4 y | 34.10 ± 4.10 [40] | 35.04 ± 5.95 [33] | −1.28 (−3.61, 1.04) | 0.275 |
| 5 y | 32.99 ± 4.01 [49] | 34.70 ± 5.47 [52] | −1.74 (−3.64, 0.16) | 0.073 |
| Carbohydrate, % of energy | | | | |
| 3 y | 52.31 ± 6.33 [45] | 51.74 ± 6.34 [37] | 0.29 (−2.47, 3.06) | 0.833 |
| 4 y | 52.87 ± 4.60 [40] | 52.76 ± 6.20 [33] | 0.45 (−2.02, 2.93) | 0.716 |
| 5 y | 54.48 ± 4.71 [49] | 52.96 ± 6.25 [52] | 1.60 (−0.58, 3.78) | 0.148 |

[1]Dietary records were collected at the ages of 35–39 mo (3 y), 47–52 mo (4 y), and 59–63 mo (5 y).
[2]From mixed models for repeated measures with the use of data from 3, 4, and 5 y of age.
[3]Mean ± SD (all such values). Values were calculated from the observed data.
[4]Mean; 95% CI in parentheses (all such values).

The strengths and uniqueness of the current follow-up study are that it provides one of the largest sets of combined methods for the assessment of body composition and fat distribution. Other RCTs have primarily assessed growth measures, such as weight, height (29), BMI and/or BMI *z* scores (30–33), waist and head circumferences (29, 33), and SFT measurements (33) in preschool-age children. In the current study, we combined several methods (anthropometric measures, SFT measurements, and ultrasound) in a longitudinal approach with annual assessments. With consideration of the importance of MRI measurements as a gold standard, providing one of the most precise estimates of adipose tissue deposition in children (34), this method was used to complement our 5-y results. With the use of several tools of body-composition assessments in a combinational approach (all of which indicated the same conclusion), the current study contributes strong evidence that suggests that reducing the n–6:n–3 LCPUFA ratio in the maternal diet during pregnancy and lactation does not affect adipose tissue growth in preschool-age children.

Another strength of our study was the use of 3-d estimated food records, which are a valid instrument to assess diet in toddlers and children (35). The exploration of preschool child nutrition over time revealed that energy and macronutrient intakes were not significantly different between study groups. Likewise, the concentration of PA was regularly assessed with the use of validated instruments and was also shown to be not significantly different between groups, thereby excluding relevant confounding by lifestyle influences.

Our findings are consistent with the current scientific literature. A number of reviews on this topic have emerged (15–17, 36–38). The latest review, which primarily focused on early fatty acid exposure and obesity risk in later life, concluded that current data from observational studies and RCTs have been inconsistent. However, the data available provide little evidence to support the proposed fatty acid hypothesis (16). Our results are similar to those in the meta-analysis conducted by Stratakis et al. (15) on the effect of an n–3 LCPUFA supplementation during pregnancy or lactation on adiposity status in childhood. Stratakis et al. included 6 RCTs with a total of 2847 participants in the meta-analysis. For the preschool-age category (≤5 y), they examined 4 RCTs and looked at BMI as the primary outcome. They showed no effect of a maternal n–3 LCPUFA

supplementation during pregnancy and/or lactation on childhood BMI and without an association of the supplemented n–3 LCPUFA dosage or age (15). Preschool-age (≤5-y) and school-age (6–12-y) data of 4 primary and follow-up studies each were included in the meta-analysis. However, to our knowledge, there has only been one study to date with a follow-up period that extended into adolescence (>13 y of age) that reported data on body composition at the age of 19 y with no difference in BMI and waist circumference (39).

Some limitations may have weakened our findings such as the small sample size that included only 104 pregnant women/study group. Because the study was initially planned for 1 y, there was a relatively high attrition rate after this particular time with a slight decrease that continued over the follow-up period. These factors resulted in the loss of statistical power. However, considerable efforts were made to obtain the compliance of the families, in particular for the 5-y follow-up. Because of missing outcome data, an MMRM approach was used for the analysis with the use of data from each visit (birth and 6 wk, 4 mo, and 1, 2, 3, 4, and 5 y of age). This approach has been recommended for the analysis of longitudinal data from an RCT, and it is a valid method under the assumption that data are missing at random. We considered this assumption that data were missing at random, which was conditional on the observed values for each outcome, to be reasonable for our study (28). With consideration of the open-label design of this study, both participants and investigators who performed the measurements and analysis were not blinded to the treatment, which may have introduced a potential bias. In addition, our sample was rather lean at the time of study entry (mean prepregnancy BMI: 22) and relatively well educated. These factors may have reflected a more health-conscious behavior in our study group than is present in the general population. This behavior might have resulted in lower prevalence rates in children who were overweight or obese at 5 y of age than the current German prevalence rates from school-enrollment examinations (prevalence of overweight including obesity: 8.4–11.9%; prevalence of obesity: 3.3–5.4%) (40). Furthermore, we determined energy intake and expenditure with the use of 3-d estimated food records and PA questionnaires, which may not have accurately reflected the true values in terms of underreporting and overreporting, respectively. The given information, which was provided by parents or daycare personnel, could be, for example, biased because of social desirability (41).

In conclusion, the current study does not provide evidence that a dietary reduction of the n–6:n–3 LCPUFA ratio would be a useful early preventive strategy against obesity at preschool age. One strong point of the current study is the use of several tools of body-composition assessment in a combinational approach and longitudinal manner with consistent results. However, the impact of LCPUFAs during pregnancy and lactation on offspring adipose tissue development is still a subject of interest. Data for adolescents and adults are limited.

We thank Karina Pusch for supporting the team with growth, SFT, and ultrasound measurements for the study population as well as for trial management. The authors also thank Christian Cordes for providing guidance with anatomical questions.

The authors' responsibilities were as follows—CB: analyzed the MRI data; CB and LS: performed the statistical analysis; CB and CG: wrote the manuscript; CB and SB: were responsible for the data collection and trial management; DM, UA-G, SB, and HH: designed the follow-up study; M-TK: performed the analysis of dietary records and PA questionnaires; JS and DCK: developed the algorithm for the MRI analysis and provided scientific advice regarding the analysis; CG: performed the MRI measurements and postprocessing of the data; and all authors: contributed to the critical revision of the manuscript. HH has received grants from Riemser and Weight Watchers for clinical trials and payment for lectures from Novartis, Roche Germany, and Sanofi-Aventis. The other authors reported no conflicts of interest related to the study.

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