

Supplementary methods

Study design

We identified pregnant women in the eastern part of Denmark by reviewing the monthly lists of reimbursements to general practitioners for first pregnancy visits. The screening and information procedure has previously been described (1). Exclusion criteria were gestational age above week 26; any endocrine, cardiovascular, or nephrological disorders; and >600IU/day vitamin D intake.

Study allocation

The allocation procedure was performed by simple randomization procedures using a computer-generated list of random numbers prepared by an external investigator with no other involvement in the trial. Capsules were consecutively numbered and kept in closed containers. The fatty acid content and the oxidation levels in both kinds of oil capsules were analyzed by the manufacturer at two time-points during the study, showing levels within the expected.

Maternal fatty acid desaturase (FADS) genotype

rs1535 was chosen because this SNP, and its proxies in close linkage disequilibrium, has been associated with n-3 LCPUFA levels in a genome-wide association study (2) and with blood levels of EPA and DHA during pregnancy (3). The risk genotype (GG) has been associated with lower n-3 LCPUFA levels compared to the non-risk genotypes (AA/AG).

Baseline characteristics

Information on the baseline characteristics race, sex, gestational age, maternal age at birth, parity, older siblings, maternal asthma, smoking during pregnancy, mode of delivery, antibiotics during pregnancy, preeclampsia and diabetes in pregnancy was obtained by personal interviews and when possible validated with registry data.

The social circumstances in the household were defined as the first component of a principal component analysis (PCA) on household income, maternal age and maternal level of education at 2 years with a mean value of zero and standard deviation of one (explained 55% of the variance) (4).

Information on breastfeeding was collected prospectively including duration of exclusive and total breastfeeding period and the use of infant formula. As soon as the child's diet was supplemented or replaced by continual use (>7 days) of infant formula/complementary foods,

we considered the exclusive breastfeeding period terminated. Information on pre-pregnancy weight of the mother was collected from pregnancy records and BMI was calculated from the height measured in the clinic.

The participants completed a validated 360-item food frequency questionnaire assessing dietary intake in the 4 weeks prior to randomization (5–7). Maternal whole blood EPA+DHA levels were assessed at the time of randomization (8,9).

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5. Olsen SF, Mikkelsen TB, Knudsen VK, Orozova-Bekkevold I, Halldórsson TI, Strøm M, et al. Data collected on maternal dietary exposures in the Danish National Birth Cohort. *Paediatric and Perinatal Epidemiology*. 2007;21(1):76–86.
6. Mikkelsen TB, Olsen SF, Rasmussen SE, Osler M. Relative validity of fruit and vegetable intake estimated by the food frequency questionnaire used in the Danish National Birth Cohort. *Scand J Public Health*. 2007;35(2):172–9.
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8. Armstrong JM, Metherel AH, Stark KD. Direct microwave transesterification of fingertip prick blood samples for fatty acid determinations. *Lipids*. 2008 Feb;43(2):187–96.
9. Metherel AH, Taha AY, Izadi H, Stark KD. The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed). *Prostaglandins Leukot Essent Fatty Acids*. 2009 Dec;81(5–6):417–23.

Supplementary Figures

Figure A: Flow chart of enrolment and allocation of the COPSAC₂₀₁₀ pregnancy cohort and follow-up of the COPSAC₂₀₁₀ birth cohort.

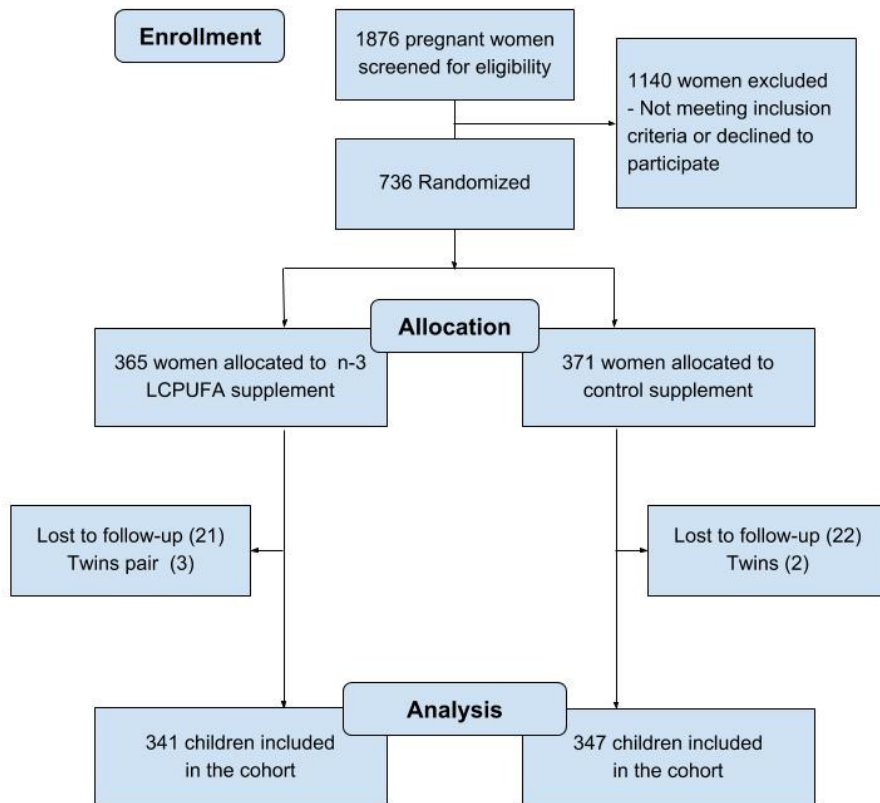


Figure B: Curves showing mean BMI with standard errors according to visit age for children in the n-3 LCPUFA supplementation group and control group until 6 years of age, stratified by sex.

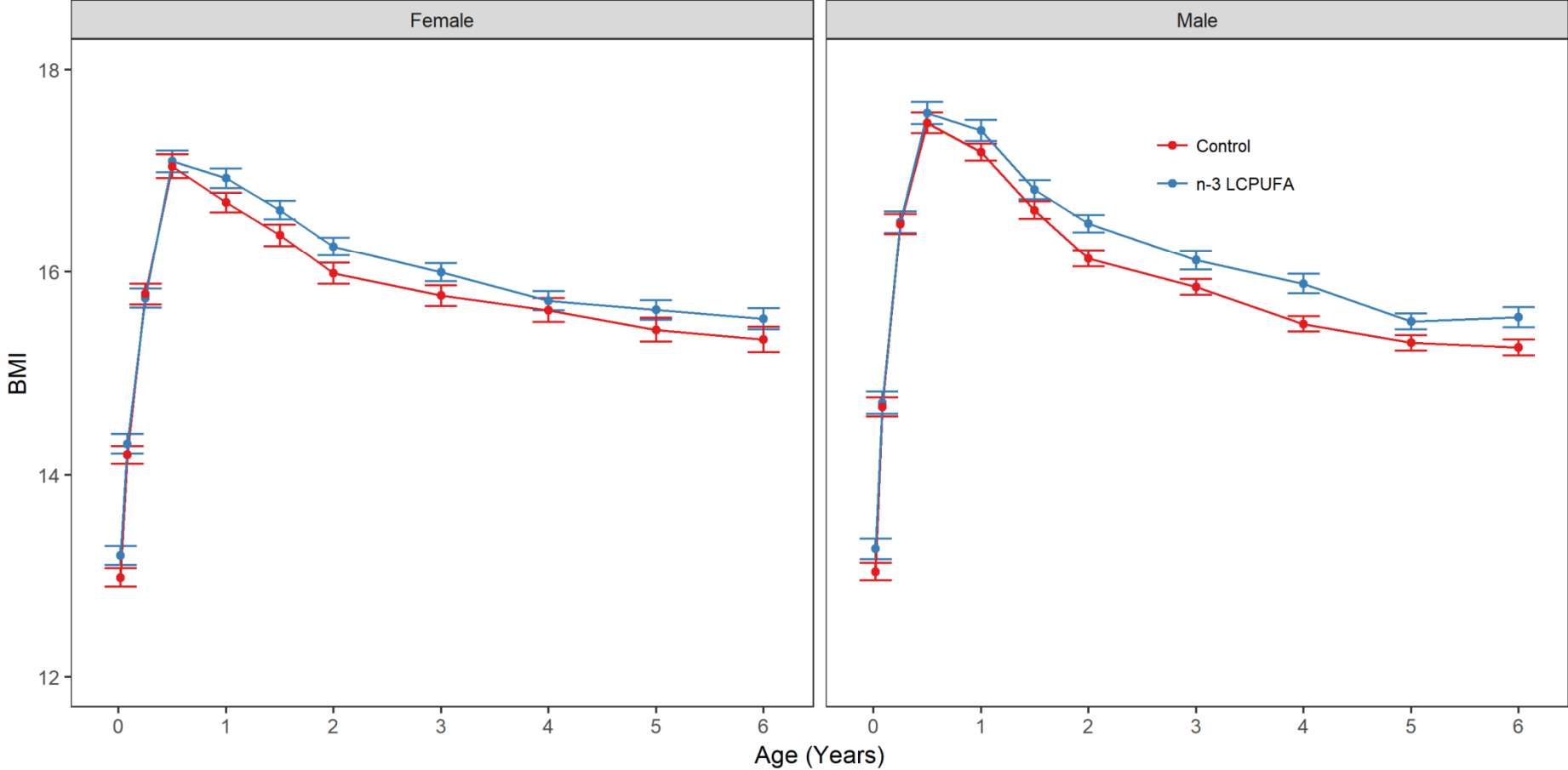
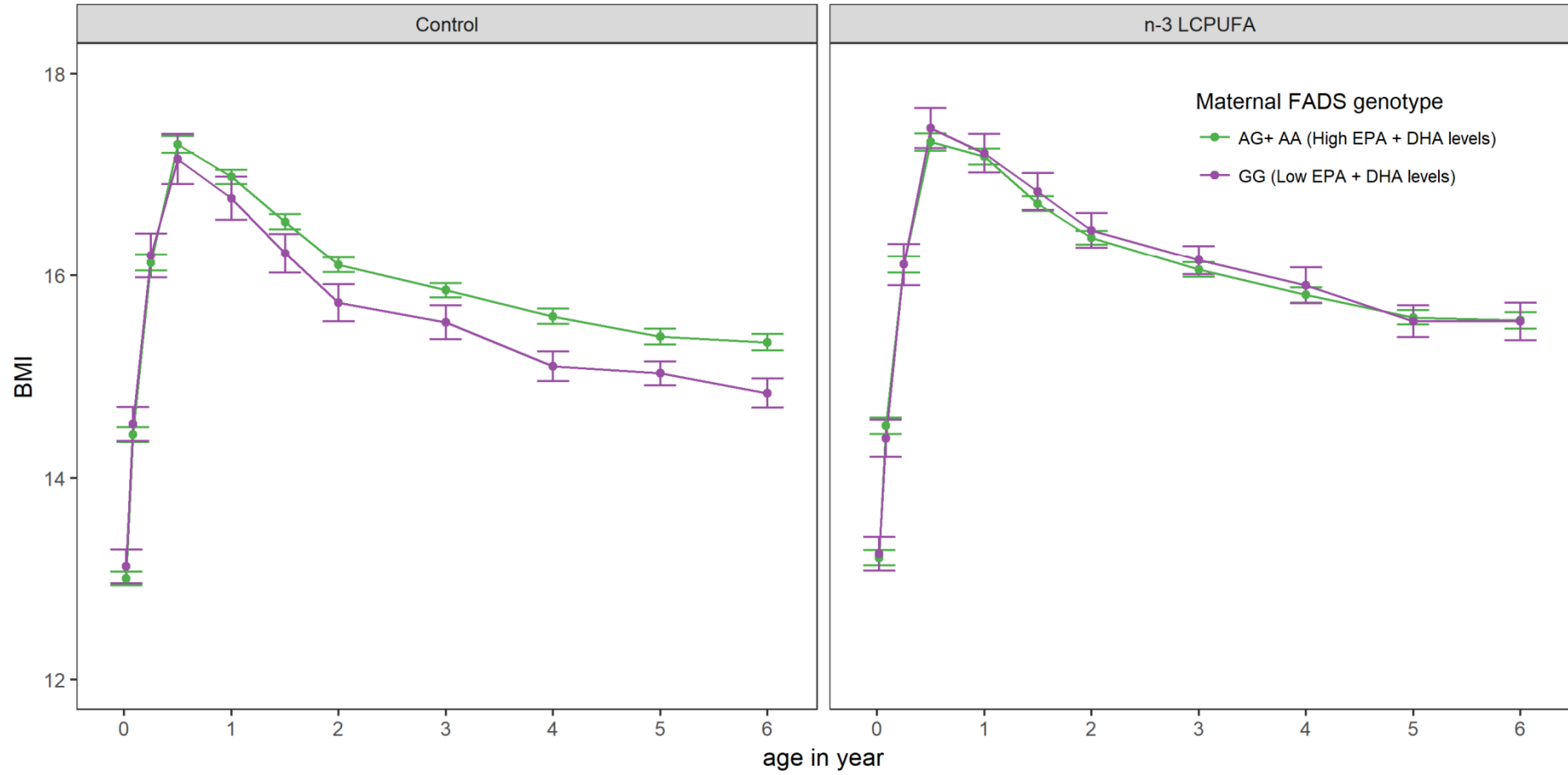


Figure C: Curves showing mean BMI with standard errors according to visit age for children born by mothers with GG risk allele and non-risk alleles (AA+AG) until 6 years of age stratified by the intervention.



Supplementary Tables

Table A: Effects of n-3 LCPUFA on the anthropometric measurements at 6 years of age; sex stratified

| | Female | | | Male | | | Interaction |
|----------------------------------|---------------------|------------------|-------------|---------------------|------------------|-------------|-------------|
| | n-3 LCPUFA N=159 | Control N=144 | P- value | n-3 LCPUFA N=147 | Control N=157 | P- value | p-value |
| Z-score BMI, Mean (SD) | 0.1 (0.8) | -0.1 (0.9) | 0.07 | 0.1 (0.8) | -0.1 (0.8) | 0.03 | 0.85 |
| Waist, Mean (SD), cm | 55.5 (3.9) | 54.9 (4.1) | 0.19 | 55.4 (3.6) | 54.7 (3.3) | 0.10 | 0.93 |
| Weight, Mean (SD), Kg | 21.7 (3.1) | 21.3 (3.0) | 0.19 | 21.8 (2.8) | 21.5 (2.8) | 0.32 | 0.70 |
| Height, Mean (SD), cm | 118.0 (4.8) | 117.8 (5.0) | 0.63 | 118.3 (4.39) | 118.6 (5.27) | 0.64 | 0.53 |
| Weight/height, Mean (SD) g/cm | 183.6 (19.8) | 180.1 (20.4) | 0.15 | 183.9 (18.3) | 181.2 (17.0) | 0.18 | 0.85 |
| Head, Mean (SD), cm | 51.8 (1.4) | 51.8 (1.3) | 0.84 | 52.5 (1.4) | 52.4 (1.5) | 0.83 | 0.55 |
| ZBMI <10 perc. % (N) | 9 (15) | 11 (16) | 0.77 | 10 (15) | 12 (18) | 0.80 | 0.47 |
| ZBMI <25 perc. % (N) | 19 (30) | 32 (47) | 0.009 | 23 (34) | 28 (43) | 0.39 | 0.29 |
| ZBMI > 75 perc. % (N) | 28 (45) | 23 (33) | 0.35 | 31 (46) | 21 (32) | 0.06 | 0.44 |
| ZBMI > 90 perc. % (N) | 9 (15) | 11 (16) | 0.77 | 11 (17) | 9 (14) | 0.62 | 0.41 |
| IOTF* >0, % (N) | 6 (10) | 7 (10) | 0.98 | 4 (6) | 2 (4) | 0.66 | 0.52 |
| IOTF** <0, % (N) | 10 (16) | 12 (18) | 0.60 | 7 (10) | 7 (12) | 0.96 | 0.71 |

ZBMI= Z-score Body Mass Index, IOTF= International Obesity Task Force, N=Number, SD – Standard Deviation

*IOTF above grade zero means the child are in risk of adulthood overweight and obesity.

**IOTF below grade zero means the child are in risk of adulthood underweight.

Table B: Effects of n-3 LCPUFA on the dual-energy X-ray absorptiometry measurements at 3.5 years of age.

| | Crude | | Adjusted difference | |
|---|------------------------|------------------|------------------------------|-----------------|
| | n-3 LCPUFA N=176 | Control N=180 | Estimate [95 CI-interval] | P- valu e |
| Fat (TBLH), Mean (SD), kg | 3.77 (0.84) | 3.69 (0.81) | 0.05 [0.08;0.17] | 0.49 |
| Fat % (TBLH),Mean (SD) | 28.7 (4.6) | 28.6 (4.3) | 0.1 [-0.6;0.7] | 0.87 |
| Fat (Trunk), Mean (SD), kg* | 1.40 (0.41) | 1.39 (0.42) | 0.003 [-0.06;0.07] | 0.92 |
| Fat % (Trunk), Mean (SD) | 20.3 (4.7) | 20.4 (4.6) | -0.1 [-0.8;0.7] | 0.81 |
| Fat (Android), Mean (SD), kg* | 0.17 (0.06) | 0.017 (0.06) | 0.00 [0.01;0.00] | 0.93 |
| Fat % (Android), Mean (SD) | 17.0 (4.8) | 17.1 (4.8) | -0.1 [-0.8;0.7] | 0.86 |
| Lean mass (TBLH), Mean (SD), kg * | 9.04 (1.27) | 8.89 (1.28) | 0.09 [-0.05;0.23] | 0.19 |
| Lean mass % (TBLH), Mean (SD) | 69.0 (4.5) | 69.1 (4.3) | 0.0 [-0.7;0.6] | 0.90 |
| Lean mass (Trunk), Mean (SD), kg* | 5.31 (0.70) | 5.24 (0.86) | 0.05 [-0.04;0.15] | 0.27 |
| Lean mass % (Trunk), Mean (SD) | 77.6 (4.7) | 77.6 (4.6) | 0.1 [-0.7;0.8] | 0.81 |
| Total BMC (TBLH), Mean (SD), kg ** | 0.31 (0.05) | 0.30 (0.05) | 0.003 [-0.002;0.008] | 0.31 |
| Total BMD (TBLH), Mean (SD), g/cm ² ** | 0.45 (0.03) | 0.45 (0.03) | 0.005 [-0.001;0.01] | 0.09 |

Adjusted for age and sex

**Additional adjusted for height and height², ** Additional adjusted for height,*

BMC: Bone Mineral Content, BMD: Bone Mineral Density, CI: Confidence Interval, SE: Standard Error, TBLH: Total Body Less Head

Table C: Effects of maternal FADS genotype, GG risk allele and non-risk alleles (AA+AG), stratified by the intervention, on the anthropometric measurements at 6 years of age.

| | n-3 LCPUFA | | | Control | | | Interaction |
|------------------------|----------------|----------------|-------------|-----------------|-----------------|-------------|-------------|
| | GG N=40 | AA+AG N=257 | P- value | GG N=36 | AA+AG N=252 | P- value | P-value |
| Z-score BMI, Mean (SD) | 0.11 (0.82) | 0.11 (0.82) | 0.98 | -0.36 (0.62) | -0.05 (0.84) | 0.01 | 0.13 |
| Waist, Mean (SD), cm | 55.6 (3.9) | 55.4 (4.1) | 0.81 | 54.0 (2.9) | 54.9 (3.8) | 0.10 | 0.25 |
| Weight, Mean (SD), Kg | 21.5 (2.8) | 21.8 (2.9) | 0.49 | 20.61 (2.2) | 21.49 (3.0) | 0.04 | 0.45 |
| Height, Mean (SD), cm | 117.5 (4.9) | 118.2 (4.6) | 0.36 | 117.8 (5.6) | 118.2 (5.1) | 0.66 | 0.78 |

ZBMI= Z-score Body Mass Index, N=Number, SD – Standard Deviation