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DHA supplementation during pregnancy does not reduce BMI or body fat mass in children: follow-up of the DOMInO randomized controlled trial

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RM, AM and LNY have no conflicts to declare.

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Running Title: Prenatal DHA and childhood growth

Abbreviations:

BMI: Body Mass Index

BIS: Bioelectrical Impedance

DHA: Docosahexaenoic acid

DOMInO Trial: DHA to Optimize Mother Infant Outcomes Trial

LCPUFA: Long Chain Polyunsaturated Fatty Acid

RCT: Randomized Controlled Trial

SNP: Single Nucleotide Polymorphism

Trial Registration: Australian New Zealand Clinical Trials Registry: www.anzctr.org.au ACTRN1260500056906 &ACTRN12611001127998

Abstract

Background: The omega-3 long chain polyunsaturated fatty acid, docosahexaenoic acid (DHA), has proven effective at reducing fat storage in animal studies. However, a systematic review of human trials found a lack of quality data to support or refute this hypothesis.

Objective: To determine whether maternal DHA supplementation during the last half of pregnancy results in a lower body mass index (BMI) and percentage body fat mass (%BF) of children.

Design: A follow-up of children at 3 and 5 years of age born to mothers enrolled in the DOMInO (DHA to Optimize Mother Infant Outcome) double-blind randomized controlled trial, in which women with a singleton pregnancy were provided with DHA-rich fish oil capsules (providing 800 mg DHA/d) or vegetable oil capsules (control group) in the second half of pregnancy. Primary outcomes were BMI z-score and %BF at 3 and 5 years of age. Potential interactions between prenatal DHA and PPARγ genotype, as a measure of genetic predisposition to obesity, were investigated.

Results: 1614 children were eligible for the follow-up and 1531 (95%) consented and are included in the analysis. BMI z-scores and %BF of children in the DHA group did not differ from children in the control group at either 3 years (BMI z-score, adjusted mean difference 0.03, 95% CI -0.07 to 0.13, p=0.61; %BF, adjusted mean difference -0.26, 95% CI -0.99 to 0.46, p=0.47) or 5 years (BMI z-score, adjusted mean difference 0.02, 95% CI -0.08 to 0.12, p=0.66; %BF, adjusted mean difference 0.11, 95% confidence interval -0.60 to 0.82, p=0.75). No treatment effects were modified by the PPARγ genotype of the child.

Conclusions: Maternal intake of DHA-rich fish oil during pregnancy does not affect the growth or body composition of children at 3 or 5 years, independent of genetic predisposition to obesity.

Introduction

The prevalence of overweight and obesity has reached epidemic proportions in many Western countries, and there is an urgent need for effective intervention strategies. Compelling epidemiological and experimental animal data has indicated that overweight and obesity have early life origins, and that exposure to an inappropriate balance of nutrients during fetal life and/or in early infancy can permanently alter the properties of fat cells and predispose an individual to fatness (1,2). This has led to suggestions that nutritional interventions during the perinatal period are likely to be more effective than those later in life in producing lifelong reductions in body fat mass and improvements to metabolic health (3).

In this context, there has been growing interest in an increased supply of omega-3 (n-3) long chain polyunsaturated fatty acids (LCPUFA) during the perinatal period as a potential means to limit fat deposition and improve metabolic health outcomes in children (4,5). This is based on results from studies conducted *in vitro* and in adult humans and rodents which have demonstrated that the n-3 LCPUFA, in particular docosahexaenoic acid (DHA), inhibit the hyperplastic and hypertrophic expansion of fat depots and improve insulin sensitivity (6-11).

Despite these apparent benefits of n-3 LCPUFA, clinical studies designed to evaluate the effect of maternal DHA supplementation on body fat mass in children have produced mixed results (12). However, these studies have had a number of methodological limitations, including high rates of attrition, lack of statistical power and absence of appropriately sensitive measures of body composition (12). In addition, the potential impact of a genetic predisposition to obesity/type 2 diabetes on

the relationship between metabolic outcomes and maternal DHA supplementation has not yet been investigated.

We here report on the follow-up of children of Adelaide mothers who participated in the DOMInO (DHA to Optimize Mother Infant Outcome) trial (13) at 3 and 5 years of age. The primary objective of this study was to determine the effect of increased prenatal DHA on body mass index (BMI) z-score and percentage body fat (%BF) in children. A secondary objective was to determine whether the effects of maternal DHA supplementation on these outcomes were dependent on the child's genotype for the Pro/Ala single nucleotide polymorphism (SNP) in the PPARγ gene, which has been strongly associated with genetic predisposition to obesity and type 2 diabetes(14).

Subjects and Methods

Study design

This study involved a follow-up of children born to mothers enrolled in a registered, multi-center, double-blind, RCT called the DOMInO trial (ACTRN12605000569606, 3 and 5 year follow-up: ACTRN12611001127998). The DOMInO trial methods have been published previously (13). Briefly, women with singleton pregnancies <21 weeks' gestation were randomized to the treatment or control group using a computer driven service, stratified by center and parity. Women allocated to the treatment group received three 500 mg capsules per day of DHA-rich fish oil (~800 mg/d DHA and 100 mg/d eicosapentaenoic acid; Incromega 500 TG, Croda Chemicals, East Yorkshire, England) and women in the control group received three 500 mg vegetable oil capsules (without DHA) per day. Women were asked to take the capsules from

study entry until birth. The eligible children (n=1614, 97%) for the 3 and 5 year follow-up were born to all 1660 women enrolled in Adelaide centers (Flinders Medical Centre or Women's and Children's Hospital) and had not withdrawn from the study or died. All procedures were conducted in accordance with the study protocol and approved by the local institutional boards of each center. Written informed consent was obtained from the guardian of each child.

Outcome Assessments

Anthropometric assessments, BMI z-score and %BF, were conducted at the hospital study centers between 25 March 2009 and 4 October 2013. Assessments were administered by trained research staff blinded to treatment group allocation.

Anthropometric assessments

Body weight was measured without shoes and in underwear to the nearest 100g using electronic scales. Height without shoes was measured using a stadiometer. Waist-, head- and hip-circumferences were measured using a non-stretch tape. All measures were recorded in duplicate, or triplicate if the first 2 measures differed by >0.1kg (weight) or >0.5cm (height/girths), and averaged for analysis. Weight and height measurements were used to calculate BMI (weight in kg/height in metres^2). The measures for each child were compared with standardized reference charts for the child's age and sex to calculate their z-scores (15,16). Corrected ages were used for children born preterm (<37 weeks' gestation). The number of children classified as underweight (BMI<10th percentile), overweight (BMI>85th percentile) and obese (BMI> 90th percentile) was determined at each age.

Total fat and fat-free mass were assessed using Bioelectrical Impedance Spectroscopy (BIS) (17). Fat-free mass was derived from the measure of total body water using an equation previously validated for use in paediatric populations (18,19). %BF was then determined by subtracting the fat-free mass from the body weight, dividing by body weight and multiplying by 100.

Systolic, diastolic and mean arterial blood pressure at 5 years of age were assessed in duplicate using a DINAMAP Procare V100 monitor (GE Health Care) with an appropriate sized cuff.

Blood sample collection and processing

Children were instructed to fast for at least 4 hours prior to their 5 year clinic appointment and blood samples (~5ml) were collected into tubes treated with EDTA, kept on ice until transfer to the laboratory and processed (centrifugation at 1,500 x *g* for 30 minutes at 4°C) within 24 hours. The plasma and buffy coat fractions were separated into aliquots and frozen at –80°C, and the red blood cells washed in sterile saline, lipids extracted into chloroform and used to assess fatty acid composition of the phospholipids as previously described (20).

Determination of insulin sensitivity

Glucose and insulin concentrations in the 5 year plasma samples were determined using an enzymatic assay (Thermo Electron, Pittsburgh, PA) and human ultrasensitive insulin ELISA kit (ALPCO Diagnostics, Salem, NH) respectively. The intra- and inter-assay coefficients of variation for both assays were <10%. The fasting glucose and insulin measures were used to calculate the HOMA-IR index for each child according to the equation [glucose (mmol/l) x insulin (mU/l)]/22.5.

PPARγ genotyping

DNA was extracted from 200µl of the 5 year buffy coat samples using the Qiagen DNA extraction kit (Qiagen Pty Ltd, Doncaster, Australia). PPARγ genotyping of each child was undertaken by the Australian Genome Facility (AGRF) using TaqMan technology (Applied Biosystems, Foster City, CA, USA).

Other measures

In the DOMInO trial, maternal weight, height and BMI, parity, education and smoking status were collected at enrolment. Weight and height of the biological mother of the child were re-measured by clinic staff at the time of the 5year assessments. Questions on home environment, education and employment of the primary carer and whether the participant had requested to be unblinded were also re-asked at the time of the 3 and 5 year assessments.

At both the 3 and 5 year follow-up, detailed information on care outside the home and general health of the child was collected at the clinic appointment. Information on feeding practices in the first 6-12 months, family food environment and the child's habitual dietary intake, physical activity and screen time was collected using a structured questionnaire completed by the primary carer.

Sample size and statistical analysis

Follow-up of the 1660 children born to women enrolled in Adelaide-based centers would provide over 90% power to detect a 3% relative reduction in the mean BMI (16kg/m^2 to 15.52kg/m^2, standard deviation 1.6kg/m^2), and a 2% absolute reduction in the mean %BF (25% to 23% at 3 years and 21% to 19% at 5 years, standard deviation 5%), in boys and girls separately, allowing for 10% loss to follow-up (alpha=0.05).

All analyses were performed on an intention-to-treat basis, according to the treatment group allocated at randomization. Multiple imputation was performed separately by treatment group using chained equations to create 100 complete datasets for analysis, assuming that data were missing at random. The effect estimates from the imputed datasets were combined using Rubin's rules (21). The primary analysis was based on imputed data and included all participants who consented to the follow-up study. Sensitivity analyses were performed on the available data and on imputed data for all 1660 children born to women enrolled in Adelaide-based centers. All analyses produced similar results and only the results of the primary analysis are presented.

Continuous outcomes were analyzed using linear regression models, with treatment effects expressed as differences in means. For continuous outcomes that were log transformed prior to analysis, treatment effects are expressed as ratios of geometric means on the original scale. Binary outcomes were analyzed using log binomial regression models, with treatment effects expressed as relative risks (RRs). For outcomes measured at both 3 and 5 years, the repeated measurements were taken into account using generalized estimating equations, with treatment effects estimated at each time point separately. *A priori* secondary analyses were performed to test for effect measure modification by sex and PPARγ genotype.

Both unadjusted and adjusted analyses were performed, with adjustment for the stratification variables, center and parity, as well as pre-specified variables depending on the outcome that included the child's sex and PPARγ genotype and the mother's secondary education, further education, smoking status and BMI at enrolment. Statistical significance was assessed at the 2-sided $P<0.05$ level. No

adjustment was made for multiple comparisons and results of secondary analyses should be interpreted with caution unless highly significant.

Post-randomization child demographics and clinical characteristics were compared between treatment groups based on the available data using chi-square tests for categorical variables, Mann-Whitney U tests for continuous variables and log Poisson regression for count variables. All analyses followed a pre-specified statistical analysis plan and were performed using SAS version 9.3 (Cary, NC, USA).

Results

Participant flow and baseline characteristics

Participant flow is shown in **Figure 1**. A total of 1531 families consented to the 3 and 5 year follow-up (92.2% of the 1660 originally enrolled in Adelaide centers and 94.9% of the 1614 invited to participate). BMI z-scores and %BF were determined for 1468/1531 (95.9%) and 1269/1531 (82.9%) children respectively at 3 years and 1352/1531 (88.3%) and 1120/1531 (73.2%) children respectively at 5 years. The amount of missing data requiring imputation was similar between the treatment groups.

The sociodemographic characteristics of the families in the subset consenting to follow-up were comparable between the treatment groups at baseline **(Table 1)** and at 3 and 5 years **(Supplementary Table 1)**. The distribution of PPARγ genotypes in the children was similar between groups **(Table 1)**.

BMI z-score and %BF

The BMI z-scores of children in the DHA group did not differ from the control group at either 3 years (**Table 2**; adjusted mean difference 0.03, 95% CI -0.07 to 0.13, P=.61) or 5 years (**Table 2**; adjusted mean difference 0.02, 95% CI -0.08 to 0.12, P=.66). The %BF was also not different between children in the DHA and control groups at either 3 or 5 years (**Table 2**, 3 years, adjusted mean difference -0.26, 95% CI -0.99 to 0.46, P=.47; 5 years, adjusted mean difference 0.11, 95% CI -0.60 to 0.82, P=.75). There were no significant interactions between treatment group and either sex or PPARγ genotype in relation to BMI z-score or %BF at 3 or 5 years of age (data not shown).There was no difference in the proportion of children classified as overweight or obese between the treatment groups at either 3 or 5 years (**Table 2**).

Other anthropometric outcomes

Bodyweight and height z-scores were similar between groups, as was the average weight gain between 3 and 5 years of age (**Table 2**). Hip and waist circumferences and waist circumference z-scores were also not different between the treatment groups at either 3 or 5 years (**Table 2**). The waist:hip ratio was slightly higher in the DHA group compared to the control group at 3 years (adjusted mean difference, 0.00, 95% CI 0.00 to 0.01, P=.04), but was not different between groups at 5 years (P=.43). Total and percentage fat-free mass, total body water and the impedance index was not different between groups at either 3 or 5 years (**Table 2**). Head circumference, head circumference z-score and the change in head circumference between 3 and 5 years were also similar between groups **(Table 2).**

Insulin sensitivity at 5 years of age

In both adjusted and unadjusted analyses, insulin resistance at 5 years of age, as assessed by HOMA-IR, was higher in children in the DHA group compared to controls (**Table 3**;adjusted ratio of geometric means, 1.20, 95% CI 1.04 to 1.39, P=.01). Fasting insulin levels were also higher in the DHA group (adjusted ratio of geometric means, 1.17, 95% CI 1.03 to 1.33, P=.02). There was an interaction between treatment group and sex for fasting glucose concentrations (P=.03), such that boys in the DHA group had higher fasting glucose concentrations than boys in the control group (adjusted mean difference 0.21, 95% CI 0.01 to 0.42, P=.04), however there were no differences between groups in girls. Similar effects were observed for both fasting insulin concentrations and HOMA-IR. Boys in the DHA group had significantly higher mean HOMA-IR (adjusted ratio of geometric means 1.35, 95% CI 1.11 to 1.65, P=.003) and fasting insulin levels (adjusted ratio of geometric means 1.26, 95% CI 1.05 to 1.51, P=.01) compared with the control group, while no differences were seen for girls, however, the interactions between treatment and sex were not significant for HOMA-IR (P=.13) or fasting insulin (P=.28). All results were independent of the PPARγ genotype of the child.

Other post-randomization variables

More families from the control group had requested to be un-blinded compared with the DHA group at both the 3 and 5 year time-points, but these represented <10% of the cohort. Maternal and paternal BMI at baseline and at the time of the 3 and 5 year follow-up was also similar between groups **(Supplementary Table 1)**.

There were no significant differences between groups in frequency of hospitalizations or diagnosis of any medical conditions between birth and 5 years **(Supplementary**

Table 2) or habitual dietary intake, family food environment or reported levels of physical activity or screen time at either 3 or 5 years **(Supplementary Table 3)**. Systolic, diastolic and mean arterial blood pressure and fatty acid composition of red blood cell phospholipids at 5 years of age were also similar between groups **(Supplementary Table 4)**.

Discussion

The results of this study do not support the hypothesis that increasing maternal DHA intake by 800mg/day during the second half of pregnancy can influence body weight, BMI z-score or body fat mass of the children either positively or negatively. We have many reasons to have a high degree of confidence in our findings. The DOMInO trial is the largest RCT of DHA supplementation during pregnancy, and has high retention and long-term follow-up rates of the children. It is also the first study to include two measures of obesity/body fat mass, i.e. BIS and BMI z-score, at two ages and to investigate the potential impact of child genotype on their response to the prenatal DHA intervention.

The percentage of DOMInO children classified as overweight or obese, >30% at 3 years and >25% at 5 years, is similar to figures reported in previous studies of pre-school children in South Australia by us (22) and others (23), indicating that this study population is representative of the general Australian pediatric population. Our new data confirm that the percentage of overweight and obese children in Australia remains high at 5 years of age, despite the fact that this is considered to be a period of increased physical activity and lower BMI/fat mass which precedes the adiposity rebound (24).

Our study suggests a possible negative effect of prenatal DHA supplementation on waist:hip ratio and insulin sensitivity. An increased waist circumference has previously been reported in children at 2.5 years whose mothers were supplemented with DHA during lactation (5). While ours is the first study to determine the effect of prenatal DHA supplementation on insulin sensitivity, our findings are unexpected given existing data from *in vitro* and experimental animal studies suggesting that DHA increases insulin sensitivity (25,26). While it is possible that the observed differences in insulin sensitivity and waist:hip ratio may indicate a true underlying adverse effect of DHA supplementation, these were secondary outcomes and as such require confirmation. It is also important to note that the differences between groups were small and that the measures in both groups fell within the normal range.

The PPARγ Pro12Ala SNP is present in ~20% of Caucasian populations and has been consistently associated with a reduced risk of obesity and type 2 diabetes in epidemiological studies (14,27). While there were no significant interactions between PPARγ genotype and treatment in our study, we were likely underpowered to detect such interaction effects, and further studies will be needed to explore possible interactions.

In light of the fact that ~70% of pregnant women in Adelaide are now consuming nutritional supplements which provide at least some DHA, it is encouraging that this long-term follow-up of the DOMInO trial showed no detrimental effects of maternal DHA supplementation on childhood growth or body composition. These data, together with the absence of significant effects on development in this same study

population at 4 years(28), support the safety of high-dose DHA supplements in pregnancy for the long term health of the child.

Conclusion

The results of this follow-up study provide no evidence to support the hypothesis that increasing maternal DHA intake during the second half of pregnancy influences body weight, BMI or body fat mass of the children, at least up to 5 years of age. We cannot extend our conclusion to suggest that maternal DHA intake does not influence later fat deposition in the child, however, it seems that any effects on growth are likely small, and are far outweighed by the influence of other factors, such as genetics and environment, experienced by the child after birth. This trial therefore provides the most robust data to date that maternal DHA supplementation during pregnancy is not an effective strategy by which to reduce the population burden of childhood obesity.

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406 **Original DOMInO trial Steering Committee:** Maria Makrides (chair); Robert A
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408 Ryan.

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410 **Author Contributions:** BSM, RG, MM, AM, LT and RM designed the research;
411 BSM and MM conducted research; LY performed the statistical analysis; BM, RG
412 and MM wrote the paper; BSM had primary responsibility for final content. All
413 authors read and approved the final manuscript.

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TABLE 1. Baseline Characteristics by Treatment Group

Characteristic	DHA Supplement n=770	Control Supplement n=761
Maternal data collected at enrolment		
Primiparous, n (%)	319 (41.4)	321 (42.2)
Mother completed secondary education, n(%)	485 (63.0)	495 (65.0)
Mother completed further education, n (%)[a]	515 (66.9)	533 (70.0)
Non-smoker before and during early pregnancy, n(%)	556 (72.2)	512 (67.3)
Maternal BMI, median (IQR)	26.2 (23.5-30.1)	26.3 (23.2-30.5)
Infant pre-randomization characteristics		
Infant female sex, n(%)	384 (49.9)	382 (50.2)
PPARγ Pro12Ala genotype, n(%)[b]		
Pro/Pro	260 (77.6)	245 (77.3)
Pro/Ala	66 (19.7)	66 (20.8)
Ala/Ala	9 (2.7)	6 (1.9)

[a] Degree, diploma, certificate, trade

[b] Numbers do not add up to total in each group due to missing data. Percentages calculated based on participants with available data.

TABLE 2 . Primary and Secondary Anthropometric Outcomes at 3 and 5 years of age

	DHA Supplement n=770	Control Supplement n=761	Unadjusted		Adjusted[b]	
			Effect (95% CI)	*P* Value	Effect (95% CI)	*P* Value
3-years						
BMI z-score	0.72 (0.97)	0.70 (1.06)	0.02 (-0.08,0.12)	0.73	0.03 (-0.07,0.13)	0.61
Percent Body Fat [c]	24.54 (7.07)	24.87 (6.69)	-0.32 (-1.08,0.43)	0.40	-0.26 (-0.99,0.46)	0.47
Body Weight (kg)	15.40 (2.02)	15.34 (2.01)	0.07 (-0.14,0.27)	0.53	0.08 (-0.12,0.27)	0.43
Body Weight z-score	0.51 (0.97)	0.48 (0.95)	0.04 (-0.06,0.13)	0.43	0.04 (-0.05,0.14)	0.38
BMI (kg/m^2)	16.52 (1.41)	16.51 (1.54)	0.01 (-0.14,0.16)	0.92	0.02 (-0.13,0.16)	0.81
BMI >85th percentile [a]	256 (33.2%)	287 (37.7%)	0.88 (0.77,1.01)	0.07	0.89 (0.78,1.02)	0.10
BMI >90th percentile [a]	195 (25.4%)	216 (28.4%)	0.89 (0.76,1.06)	0.19	0.91 (0.77,1.07)	0.26
BMI <10th percentile [a]	11 (1.4%)	21 (2.8%)	0.51 (0.24,1.07)	0.07	0.51 (0.24,1.07)	0.07
Total Fat Mass (kg)	3.79 (1.26)	3.84 (1.25)	-0.05 (-0.18, 0.09)	0.48	-0.03 (-0.17, 0.10)	0.62
Total Fat-Free Mass (kg)	11.61 (1.76)	11.50 (1.68)	0.11 (-0.07, 0.20)	0.24	0.11 (-0.06, 0.27)	0.20
Percent Fat-Free Mass [c]	75.43 (7.03)	75.13 (6.64)	0.30 (-0.44, 1.05)	0.43	0.24 (-0.47, 0.95)	0.51
Total Body Water (kg)	8.68 (1.25)	8.59 (1.19)	0.09 (-0.04,0.22)	0.20	0.09 (-0.04, 0.21)	0.17
Impedance Index	13.00 (2.02)	12.88 (1.91)	0.12 (-0.08,0.33)	0.23	0.12 (-0.07,0.32)	0.22
Height (cm)	96.43 (4.21)	96.27 (4.04)	0.16 (-0.26,0.57)	0.45	0.16 (-0.23,0.55)	0.41
Height z-score [c]	0.03 (1.04)	-0.01 (0.97)	0.05 (-0.05,0.15)	0.36	0.05 (-0.06,0.15)	0.38
Head Circumference (cm) [c]	50.04 (1.57)	50.06 (1.55)	-0.02 (-0.17, 0.14)	0.84	-0.02 (-0.16, 0.13)	0.81
Head Circumference z-score	0.69 (1.02)	0.69 (1.00)	0.00 (-0.11,0.10)	0.96	0.00 (-0.11,0.10)	0.96
Waist Circumference (cm) [c]	50.73 (3.53)	50.50 (3.48)	0.23 (-0.12,0.59)	0.20	0.25 (-0.10,0.60)	0.17
Waist Circumference z-score	0.47 (0.88)	0.40 (0.91)	0.07 (-0.02,0.16)	0.11	0.08 (-0.01,0.17)	0.09
Hip Circumference (cm) [c]	53.65 (3.54)	53.66 (3.67)	-0.01 (-0.38,0.35)	0.95	0.03 (-0.33,0.39)	0.87
Waist:Hip ratio [c]	0.95 (0.05)	0.94 (0.04)	0.00 (0.00,0.01)	0.04	0.00 (0.00,0.01)	0.04
5-years						
BMI z-score	0.56 (0.97)	0.54 (1.03)	0.01 (-0.09,0.12)	0.78	0.02 (-0.08,0.12)	0.66
Percent Body Fat [c]	23.46 (6.82)	23.42 (6.59)	0.05 (-0.72,0.81)	0.91	0.11 (-0.60,0.82)	0.75
Body Weight (kg)	19.95 (3.00)	19.87 (3.07)	0.09 (-0.22,0.39)	0.58	0.06 (-0.23,0.36)	0.68
Body Weight z-score	0.45 (0.98)	0.42 (0.97)	0.03 (-0.06,0.13)	0.49	0.04 (-0.06,0.14)	0.43
Body weight increase 3-5 years (kg)	4.51 (1.60)	4.47 (1.71)	0.04 (-0.13,0.22)	0.65	0.02 (-0.15,0.18)	0.85
BMI (kg/m^2)	16.19 (1.61)	16.20 (1.73)	-0.01 (-0.18,0.16)	0.90	0.00 (-0.17, 0.17)	0.99
BMI >85th percentile [a]	221 (28.7%)	223 (29.4%)	0.98 (0.83,1.15)	0.78	0.99 (0.84,1.16)	0.90
BMI >90th percentile [a]	165 (21/5%)	168 (22.1%)	0.97 (0.80,1.19)	0.78	0.99 (0.81,1.20)	0.91
BMI <10th percentile [a]	13 (1.7%)	19 (2.5%)	0.66 (0.31,1.40)	0.28	0.66 (0.31,1.40)	0.28

Total Fat Mass (kg)	4.75 (1.78)	4.74 (1.85)	0.01 (-0.18, 0.20)	0.92	0.02 (-0.17, 0.20)	0.86
Total Fat-Free Mass (kg) [c]	15.25 (2.36)	15.15 (2.22)	0.11 (-0.14, 0.35)	0.40	0.08 (-0.15, 0.32)	0.48
Percent Fat-Free Mass [c]	76.52 (6.80)	76.61 (6.52)	-0.09 (-0.84, 0.67)	0.82	-0.15 (-0.85, 0.55)	0.67
Total Body Water (kg)	11.32 (1.67)	11.24 (1.58)	0.08 (-0.10,0.26)	0.39	0.06 (-0.11,0.24)	0.48
Impedance Index	16.98 (2.67)	16.88 (2.50)	0.10 (-0.17,0.38)	0.45	0.08 (-0.19,0.34)	0.56
Height (cm)	110.82 (5.06)	110.58 (4.93)	0.24 (-0.27,0.75)	0.35	0.16 (-0.32, 0.65)	0.51
Height z-score	0.12 (1.03)	0.08 (0.98)	0.04 (-0.06,0.14)	0.46	0.04 (-0.07,0.14)	0.48
Height increase between 3 and 5 years (cm)	14.36 (2.66)	14.28 (2.75)	0.07 (-0.22,0.37)	0.61	-0.02 (-0.26,0.22)	0.86
Head Circumference (cm) [c]	51.35 (1.53)	51.33 (1.56)	0.02 (-0.14, 0.18)	0.80	0.01 (-0.14, 0.16)	0.86
Head Circumference z-score	0.66 (0.98)	0.64 (0.98)	0.02 (-0.09,0.13)	0.71	0.02 (-0.09,0.13)	0.71
Head Circumference increase between 3 and 5 years (cm)	1.30 (0.86)	1.25 (0.91)	0.04 (-0.06,0.14)	0.40	0.03 (-0.07,0.13)	0.53
Waist Circumference (cm) [c]	53.69 (3.88)	53.57 (4.24)	0.11 (-0.31,0.54)	0.60	0.10 (-0.31,0.51)	0.62
Waist Circumference z-score	0.24 (0.74)	0.20 (0.79)	0.04 (-0.04,0.12)	0.34	0.04 (-0.04,0.12)	0.29
Hip Circumference (cm) [c]	59.34 (4.16)	59.31 (4.55)	0.03 (-0.41,0.47)	0.90	0.04 (-0.40,0.48)	0.87
Waist:Hip ratio [c]	0.91 (0.04)	0.90 (0.04)	0.00 (0.00,0.01)	0.47	0.00 (0.00,0.01)	0.43
BMI z-score	0.72 (0.97)	0.70 (1.06)	0.02 (-0.08,0.12)	0.73	0.03 (-0.07,0.13)	0.61

Data are presented as mean (SD) with effect being difference in means unless otherwise indicated. Analyses are based on 100 imputed datasets.

[a]Data are presented as number (percentage) with effect being relative risk.

[b]Adjusted for center, parity, maternal BMI at study entry, mother's secondary education, mother's further education, mother's smoking status, PPARγ genotype.

[c] Also adjusted for infant sex and actual age of child at assessment.

TABLE 3.Secondary Outcomes Related to Insulin Sensitivity at 5 years of age

	DHA Supplement n=770	Control Supplement n=761	Unadjusted		Adjusted[b]	
			Effect (95% CI)	*P* Value	Effect (95% CI)	*P* Value
HOMA-IR [a]	0.80 (0.43-1.71)	0.68 (0.38-1.31)	1.20 (1.04, 1.39)	0.01	1.20 (1.04, 1.39)	0.01
Fasting Glucose	4.07 (1.08)	4.02 (1.02)	0.05 (-0.11, 0.20)	0.56	0.05 (-0.11, 0.20)	0.56
Fasting Insulin [a]	4.63 (2.68-9.20)	4.01 (2.38-7.25)	1.17 (1.03, 1.32)	0.02	1.17 (1.03, 1.33)	0.02
Boys						
HOMA-IR [a]	0.86 (0.44-1.88)	0.62 (0.35-1.21)	1.35 (1.11, 1.65)	0.003	1.35 (1.11, 1.65)	0.003
Fasting Glucose	4.26 (1.07)	4.03 (1.00)	0.22 (0.02, 0.43)	0.03	0.21 (0.01, 0.42)	0.04
Fasting Insulin [a]	4.75 (2.70-9.63)	3.63 (2.22-6.81)	1.25 (1.04, 1.50)	0.02	1.26 (1.05, 1.51)	0.01
Girls						
HOMA-IR [a]	0.75 (0.43-1.58)	0.74 (0.41-1.41)	1.07 (0.86, 1.33)	0.55	1.07 (0.86, 1.33)	0.52
Fasting Glucose	3.87 (1.04)	4.01 (1.04)	-0.14 (-0.36, 0.09)	0.24	-0.12 (-0.35, 0.11)	0.29
Fasting Insulin [a]	4.55 (2.66-8.90)	4.40 (2.56-7.72)	1.09 (0.90, 1.31)	0.37	1.09 (0.90, 1.31)	0.37

Data are presented as mean (SD) with effect being difference in means unless otherwise indicated. Analyses are based on 100 imputed datasets.

[a] Data are presented as median (IQR) with effect being ratio of geometric means.

[b] Adjusted for center, parity, maternal BMI at study entry, infant sex, mother's secondary education, mother's further education, mother's smoking status, PPARγ genotype.