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Maternal but Not Fetal *FADS* Gene Variants Modify the Association between Maternal Long-Chain PUFA Intake in Pregnancy and Birth Weight[1–3]

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Abstract

Several studies have shown a positive association between maternal fish intake in pregnancy and pregnancy duration and child birth weight (BW), probably due to fish n–3 (ω-3) long-chain polyunsaturated fatty acids (LC-PUFAs). n–3 LC-PUFAs can also be synthesized endogenously, and their synthesis depends on single nucleotide polymorphisms (SNPs) in the fatty acid desaturase (*FADS*) gene encoding for FADS. We assessed the associations of maternal docosahexaenoic acid (DHA) intake in pregnancy with pregnancy duration and BW and investigated whether these associations are modified by maternal or fetal *FADS* SNP genotypes. We hypothesized that we would find stronger associations in minor allele homozygous mothers or fetuses due to their lower n–3 LC-PUFA endogenous synthesis and hence higher dependence on dietary supply. Data on maternal diet, pregnancy duration, and BW were available for 2622 mother-child pairs from the KOALA (Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg) Birth Cohort Study. The rs174556 *FADS* SNP was genotyped in 1516 mothers and 1515 children. Associations and gene-diet interactions were tested with linear regression adjusting for potential confounders, including intake of other PUFAs. Women at the 75th percentile of DHA intake had 0.7-d longer pregnancies ($P = 0.016$) and 28-g heavier infants ($P = 0.039$) than did women at the 25th percentile of intake. Associations with arachidonic acid intake were of the same order but in the opposite direction. Mothers who were homozygous for the minor allele had 2-d shorter pregnancies ($P = 0.035$) and infants who were nearly 140 g lighter ($P = 0.006$) than did mothers who were major allele homozygotes. Post hoc analyses revealed that they had higher prepregnancy BMI ($P = 0.020$). Among the women homozygous for the minor allele, those at the 75th percentile of DHA intake had 226-g heavier infants than those at the 25th percentile of intake ($P = 0.030$), whereas DHA intake was not significantly associated with BW in major allele carriers. These findings suggest that maternal and fetal fatty acid requirements during pregnancy depend on maternal genetic variation in LC-PUFA synthesis. J. Nutr. 144: 1430–1437, 2014.

Introduction

Increasing evidence suggests that alterations in intrauterine growth leading to reduced birth weight (BW)[8] can have long-term effects on the offspring and increase their risk of cardiovascular disease later in life (1). Several observational studies investigated the association between maternal fish intake during pregnancy and BW. Some of these studies showed a positive association (2–4), whereas others found a negative or no association (5–10). The positive association is thought to be due mainly to an effect of n–3 long-chain PUFAs (LC-PUFAs) from fish on the duration of pregnancy and/or fetal growth (11,12), whereas the lack of association often has been explained by confounding by fish pollutants, which are associated with a

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[3] Supplemental Methods and Supplemental Tables 1–4 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://jn.nutrition.org.
[8] Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; BW, birth weight; FADS, fatty acid desaturase; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg; LA, linoleic acid; LC-PUFA, long-chain PUFA; RCT, randomized controlled trial; SNP, single nucleotide polymorphism.

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lower BW. To prove causality, randomized controlled trials (RCTs) supplementing pregnant women with n–3 LC-PUFAs have been performed. Results have been mixed; most of these RCTs showed that supplemented women had newborns with a higher BW (13–17), but others have not (18,19). Differences in study results were attributed to differences in dosage, timing, and duration of the supplementation [reviewed in (20)].

We believe that differences in the efficiency of n–3 LC-PUFA endogenous synthesis in both mothers and fetuses within and between populations may also contribute to the differences in results between studies. Indeed, LC-PUFA synthesis occurs in humans, including in women of reproductive age (21), children (22–25), and fetuses (26,27). This synthesis involves a series of desaturation and elongation steps catalyzed by the enzymes fatty acid desaturases (FADS) and elongases (for a representation of the metabolic pathway see reference 28). Single nucleotide polymorphisms (SNPs) in the gene encoding for FADS 5 and 6 (*FADS* gene cluster) are associated with proportions of n–3 and n–6 LC-PUFAs such as DHA (22:6n–3) and arachidonic acid (AA; 20:4n–6), respectively, in several kind of biologic samples, including plasma and breast milk (28–36). The direction of the association is consistent for most of the studied *FADS* SNPs: compared with individuals who are homozygous for the major allele, those carrying the minor allele generally have lower concentrations of LC-PUFAs (i.e., DHA and AA) and higher concentrations of the precursor FAs linoleic acid (LA; 18:2n–6) and α-linolenic acid (ALA; 18:3n–3) (i.e., there is substrate accumulation), probably due to lower expression or activity of desaturases. We hypothesized that the association between maternal DHA intake and pregnancy duration and BW differs between maternal or fetal *FADS* SNP genotype groups. We foresaw the association to be stronger in individuals who are homozygous for the minor allele because they have lower endogenous LC-PUFA synthesis and therefore may be more dependent on the dietary supply of preformed DHA.

The aims of our study were to investigate within the KOALA (Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg) Birth Cohort Study the following: *1*) the association of maternal DHA intake during pregnancy and maternal and fetal *FADS* SNP genotypes with pregnancy duration and BW and *2*) whether the dietary association is modified by maternal or fetal *FADS* SNPs genotypes (i.e., gene-diet interaction).

Participants and Methods

Cohort

The details of the cohort are described elsewhere (37). In summary, the KOALA Birth Cohort Study examines a prospective birth cohort in the center and south of The Netherlands, set up to identify factors that influence the clinical expression of atopic diseases and growth and development. The main focus is on lifestyle (e.g., conventional vs. alternative lifestyles, dietary habits, breastfeeding, intestinal microflora composition) and gene-environment interactions. Between 2000 and 2002, healthy pregnant women participating in the Pregnancy-related Pelvic Girdle Pain Study (n = 7526) (38) were invited to participate with their child in the KOALA study. Most women recruited by this means had a conventional lifestyle in terms of diet and child-rearing practices. Additional pregnant women with alternative lifestyles were recruited from 2001 to 2002 through organic food shops, anthroposophic doctors and midwives, and Steiner schools. In total, 2993 pregnant women were recruited (n = 2487 in the conventional recruitment group, n = 506 in the alternative recruitment group).

The study was approved by the Ethics Committee of the Maastricht University/University Hospital Maastricht. All parents gave written informed consent.

Participant selection

The flowchart of participant selection is depicted in **Figure 1**. Exclusion criteria for the present study were as follows: withdrawal from the study before delivery, perinatal death, newborn with congenital disease or disease affecting growth, twins, unavailability of BW measurement, and children of non-Caucasian ethnicity (<3 grandparents born in countries of predominantly European ancestry) or without available information on ethnicity. The inclusion cohort consisted of 2669 mother-child pairs eligible for our study. We defined 3 data sets depending on the availability of data (flowchart in Fig. 1): data on maternal diet in pregnancy and pregnancy duration (data set 1, n = 2622) and data on maternal diet in pregnancy and pregnancy duration plus *FADS* SNP genotypes in mothers (data set 2, n = 1516) or in children (data set 3, n = 1515). The overlap between data sets 2 and 3 consisted of 1128 mother-child pairs.

DNA isolation and SNP genotyping

Mothers. Detailed information on maternal DNA extraction and SNP genotyping is given in the **Supplemental Methods**. In short, genomic DNA was extracted from buffy coats or buccal swabs by using standard methods. Genotyping was performed by Sequenom with a multiplex assay (Sequenom) including 28 SNPs, 14 of which were in the region encompassing the *FADS1*, *FADS2*, and *FADS3* genes. The selected *FADS* SNPs were either tagSNPs or very highly correlated with tagSNPs. For quality control we included blind duplicates and negative samples. One of the 28 SNPs failed at the PCR level (not genotyped in any of the samples), resulting in valid data for 27 SNPs. We distrusted the genotyping results of individual samples when ≥7 of the 27 SNPs in the assay failed (i.e., call rate <80%). This happened in 15% of the maternal samples included in this study. After excluding these samples, duplicate concordance was 100% for all SNPs.

Children. DNA isolation and SNP selection and genotyping methods were described in detail elsewhere (39). In short, genomic DNA was extracted from buccal swabs by using standard methods (40) and afterward amplified by using REPLI-g UltraFast technology (Qiagen), as reported previously (41). Five variants of the *FADS1 FADS2* gene cluster (rs174545, rs174546, rs174556, rs174561, and rs3834458) were typed. These variants were chosen because they were previously shown to be associated with the proportions of PUFAs in serum and plasma phospholipids and erythrocyte membranes from adults (29,31,32) and children (39). Genotyping was performed with the iPLEX method (Sequenom) by using matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS (Mass Array; Sequenom). Standard genotyping

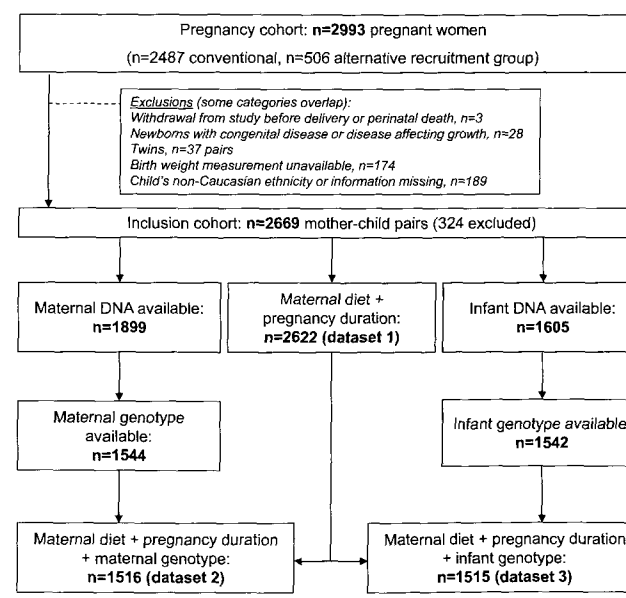

FIGURE 1 Flowchart of study design and participant selection from the KOALA Birth Cohort Study population. KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg.

quality control included 10% duplicate and negative samples. Genotyping discordance rate was <0.3%.

SNP selection for the present study. We made the a priori decision to restrict our analyses to SNPs already known to be associated with PUFA concentrations and available both in mothers and children. These were the SNPs rs174556 and rs174561. Maternal and fetal genotype frequencies for these SNPs did not deviate from Hardy-Weinberg equilibrium. Both SNPs are highly correlated with each other; hence, we would expect similar results irrespective of the SNP analyzed. We selected the SNP rs174556 given that we obtained slightly better genotyping clustering (i.e., the spectra for the different genotypes were unambiguous). By using the Tagger tool (42) with HapMap release 21 data, we estimated the rs174556 SNP to tag ($r^2 \geq 0.8$) up to 15 SNPs between base-pair positions 61300075 and 61379716 of *FADS1* and *FADS2*.

Data on main outcomes and covariates

Data on BW were obtained from obstetric reports. Information on pregnancy duration was obtained from a questionnaire filled out by the mothers at 2 wk postpartum. Information on maternal height and prepregnancy weight was obtained from maternal self-reported height and weight on the basis of questionnaires filled out at 14 and 30 wk of pregnancy; prepregnancy BMI was calculated as weight/height squared.

Information on potential confounding variables was retrieved from obstetric reports and questionnaires filled out at different time points during pregnancy and the first 2 wk postpartum. These variables were as follows: recruitment group (conventional vs. alternative lifestyle), level of maternal education, parity before index pregnancy, smoking habits during pregnancy, alcohol use during pregnancy, maternal age at delivery, and child gender.

Maternal dietary information

At 34 wk of pregnancy, mothers filled out an FFQ, which was an extended version that included food supplements of an existing, validated FFQ (43). It consisted of ~198 food items, for which the consumption frequency and portion size over the previous month had to be recorded by the mothers.

The average daily dietary intake of energy (in kcal), fat (in g), and FAs (in mg or g) was calculated by combining information about each FFQ item (frequency of consumption and amount consumed per day) with nutrient composition as reported in the Dutch Food Composition Table (NEVO Table 2010) (44). A separate question assessed the intake of fish oil supplements during pregnancy, including trimester of use and supplement brand. However, fish oil supplements were not accounted for in our nutrient calculations because exact data on dose and frequency of use were not reported and only 1% of the study population reported fish oil supplement use.

Statistical analyses

All analyses were performed with Predictive Analytics SoftWare Statistics for Windows (version 18.0; SPSS) with untransformed variables. *P* values were based on 2-sided tests, and significance was set at $P < 0.05$. Agreement of genotype frequencies with Hardy-Weinberg equilibrium expectations was tested by chi-square test. Normality of outcomes was confirmed by means of histograms and Q-Q plots. Maternal fat and FA intake, age at delivery, prepregnancy BMI, and pregnancy duration were analyzed as continuous variables. Maternal education, parity, smoking, alcohol use, maternal and fetal genotypes, child gender, and recruitment group were treated as categorical variables and recoded into dummy variables if there were >2 categories. Cases with missing values were excluded from the corresponding analysis. Only for alcohol use in pregnancy, given the relatively high amount of missing data, we coded missing values as a new category to avoid losing cases in the analyses.

The association between maternal DHA intake and pregnancy duration was examined with multivariable linear regression. In a first model we adjusted only for the intake of total fat and other PUFAs (model 1). In a second model (model 2) we adjusted for several additional potential confounders: child gender, recruitment group, maternal education, parity, maternal smoking status during pregnancy, maternal alcohol use during pregnancy, and maternal age at delivery. Subsequently, we extended model 2 by adding the maternal genotypes (model 3) and the interaction terms between maternal genotype and FA intake (model 4) to assess the main effects of maternal genotype on pregnancy duration as well as gene-diet interactions. Finally, the previous 2 models were repeated including the fetal (instead of the maternal) genotype (models 5 and 6). These 6 models were then repeated with BW as outcome (models 1b–6b).

To explore possible mechanisms for the associations that we found, we performed the following additional analyses: *1*) we adjusted model 2b for pregnancy duration to assess whether the association between DHA intake and BW was explained by pregnancy duration and *2*) we studied the association between maternal genotype and prepregnancy BMI and adjusted model 3b for prepregnancy BMI to rule out the possibility that the association between maternal *FADS* genotype and BW was explained by maternal prepregnancy BMI. In sensitivity analyses we evaluated whether results differed when *1*) we excluded mothers with premature delivery (<37 wk of gestation), *2*) we excluded mothers who used fish oil supplements during pregnancy, and *3*) we imputed missing values in covariates by using the multiple imputation procedure in PASW Statistics 18.

Results

The population characteristics of each study data set (data set 1, initial cohort; data set 2, subgroup with maternal genotype data

TABLE 1 Characteristics (continuous variables) of mothers and children in data sets 1, 2, and 3 from the KOALA Birth Cohort Study[1]

	Data set 1		Data set 2		Data set 3	
Variable	Participants	Mean ± SD	Participants	Mean ± SD	Participants	Mean ± SD
	n		*n*		*n*	
Birth weight, *g*	2622	3519 ± 500	1516	3527 ± 494	1515	3528 ± 503
Pregnancy duration, *d*	2622	280 ± 10	1516	280 ± 9	1515	280 ± 10
Birth length, *cm*	2438	51 ± 2	1404	51 ± 2	1414	51 ± 3
Prepregnancy BMI, kg/m^2	2610	24 ± 4	1512	24 ± 4	1511	24 ± 4
Pregnancy weight gain, *kg*	2458	14.1 ± 5.2	1417	14.1 ± 5.0	1429	14.0 ± 4.8
Maternal age at delivery, *y*	2622	32.0 ± 3.8	1516	32.4 ± 3.7	1515	32.3 ± 3.7
Maternal energy intake in pregnancy, *kcal/d*	2622	2500 ± 590	1516	2472 ± 573	1515	2496 ± 568
Maternal fat intake in pregnancy, *g/d*	2622	98 ± 27	1516	97 ± 26	1515	98 ± 26
Linoleic acid intake in pregnancy, *g/d*	2622	17 ± 6	1516	17 ± 6	1515	17 ± 6
α-Linolenic acid intake in pregnancy, *g/d*	2622	1.9 ± 0.6	1516	1.9 ± 0.5	1515	1.9 ± 0.5
Arachidonic acid intake in pregnancy, *mg/d*	2622	36 ± 20	1516	35 ± 20	1515	35 ± 19
DHA intake in pregnancy, *mg/d*	2622	150 ± 162	1516	148 ± 155	1515	150 ± 158

[1] Data sets were defined according to availability of data as follows: data set 1, data on maternal diet in pregnancy and pregnancy duration available ($n = 2622$); data set 2, same as in data set 1 plus available genotype data in mothers ($n = 1516$); data set 3, same as in data set 1 plus available genotype data in children ($n = 1515$). KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg.

available; data set 3, subgroup with fetal genotype data available) are shown in **Table 1** (for continuous variables) and in **Table 2** (for categorical variables). There were no apparent differences between data sets. Information regarding maternal PUFA status was previously published for a subset of this study population (28).

Associations with pregnancy duration. Maternal DHA intake was significantly and positively associated with pregnancy duration, both in the partly adjusted model (model 1; **Supplemental Table 1**) and in the fully adjusted one (model 2; **Table 3**). LA and AA intakes were negatively associated with pregnancy duration.

Maternal genotype was significantly associated with pregnancy duration; women who were homozygous for the minor allele had significantly shorter pregnancies (model 3; **Table 4**). No association was found between fetal genotype and pregnancy duration (model 5; Table 4). Neither the maternal nor the fetal genotypes modified the association between maternal PUFA intake and pregnancy duration (models 4 and 6; data not shown).

Associations with BW. Maternal DHA intake was significantly and positively associated with BW, both in the partly adjusted model (model 1b; Supplemental Table 1) and in the fully adjusted one (model 2b; Table 3). Moreover, we identified a significant positive association between ALA intake and BW and a negative association between AA intake and BW. We adjusted model 2b by pregnancy duration to investigate whether the association between DHA intake and BW could be mediated by pregnancy duration. Adjustment for pregnancy duration attenuated the strength of the association with DHA (β = 0.067 g; 95% CI: −0.065, 0.199 g; P = 0.32), ALA (β = 0.074 g; 95% CI: 0.004, 0.144 g; P = 0.037), and AA (β = −1.177 g; 95% CI: −2.312, 0.042 g; P = 0.042).

Maternal genotype was significantly associated with BW: mothers who were homozygous for the minor allele had lighter-weight children than did mothers who were homozygous for the major allele (model 3b; Table 4). Fetal genotype was not significantly associated with BW (model 5b; Table 4). We considered the possibility that the association between maternal genotype and BW was mediated by maternal prepregnancy BMI. Hence, we tested the association between maternal genotype and prepregnancy BMI. Mothers who were homozygous for the minor allele had a higher prepregnancy BMI compared with major allele homozygotes (β = 1.0 kg/m^2; 95% CI: 0.2, 1.7 kg/m^2; P = 0.020). The adjustment of model 3b for prepregnancy BMI did not decrease the strength of the genetic effect (data not shown), ruling out the possibility that the association between maternal *FADS* genotype and lower BW was explained by higher prepregnancy BMI.

In line with our hypothesis, we found that maternal DHA and AA intake interacted with maternal genotype (model 4b; **Table 5**, **Fig. 2**): DHA intake was associated with higher BW and AA intake was associated with lower BW only in women who were homozygous for the minor allele. There was no interaction between maternal PUFA intake and fetal genotype (model 6b; data not shown).

Sensitivity analyses. Approximately 1% of women in data sets 1, 2, and 3 reported to take fish oil supplements during pregnancy and between 2% and 3% of women had a premature delivery (Table 2). The exclusion of mothers who took fish oil supplements in pregnancy or the exclusion of mothers with premature delivery did not essentially change the results presented above. The results after imputation of missing values in covariates were also consistent with those reported here (**Supplemental Tables 2–4**).

TABLE 2 Characteristics (categorical variables) of mothers and children in data sets 1, 2, and 3 from the KOALA Birth Cohort Study[1]

Variable and category	Data set 1 (n = 2622)	Data set 2 (n = 1516)	Data set 3 (n = 1515)
Recruitment group			
Alternative	439 (16.7)	307 (20.3)	303 (20.0)
Conventional	2183 (83.3)	1209 (79.7)	1212 (80.0)
Infant's gender			
M	1341 (51.1)	766 (50.5)	754 (49.8)
F	1281 (48.9)	750 (49.5)	761 (50.2)
Maternal educational level			
High	1252 (47.7)	783 (51.6)	795 (52.5)
Middle	1236 (47.1)	676 (44.6)	662 (43.7)
Low	126 (4.8)	55 (3.6)	57 (3.8)
Missing	8 (0.3)	2 (0.1)	1 (0.1)
Parity before index pregnancy			
≥2	375 (14.3)	238 (15.7)	217 (14.3)
1	1092 (41.6)	655 (43.2)	670 (44.2)
0	1155 (44.1)	623 (41.1)	628 (41.5)
Smoking during pregnancy			
Smoking early + late pregnancy	165 (6.3)	70 (4.6)	53 (3.5)
Smoking late pregnancy	19 (0.7)	9 (0.6)	12 (0.8)
Smoking early pregnancy	11 (0.4)	4 (0.3)	7 (0.5)
No smoking during pregnancy	2419 (92.3)	1430 (94.3)	1437 (94.9)
Missing	8 (0.3)	3 (0.2)	6 (0.4)
Alcohol intake during pregnancy			
Alcohol intake early + late pregnancy	221 (8.4)	133 (8.8)	134 (8.8)
Alcohol intake late pregnancy	194 (7.4)	120 (7.9)	97 (6.4)
Alcohol intake early pregnancy	49 (1.9)	31 (2.0)	26 (1.7)
No alcohol intake during pregnancy	1446 (55.1)	900 (59.4)	890 (58.7)
Missing	712 (27.2)	332 (21.9)	368 (24.3)
rs174556 Maternal genotype			
TT (minor allele homozygotes)	—	105 (6.9)	—
CT (heterozygotes)	—	636 (42.0)	—
CC (major allele homozygotes)	—	775 (51.1)	—
rs174556 Child's genotype			
TT (minor allele homozygotes)	—	—	122 (8.1)
CT (heterozygotes)	—	—	645 (42.6)
CC (major allele homozygotes)	—	—	748 (49.4)
Intake of fish oil supplements			
No	1900 (72.5)	1108 (73.1)	1118 (73.8)
Yes	26 (1.0)	15 (1.0)	16 (1.1)
No answer	696 (26.5)	393 (25.9)	381 (25.1)
Premature delivery (<37 wk)			
No	2547 (97.1)	1488 (98.2)	1468 (96.9)
Yes	75 (2.9)	28 (1.8)	47 (3.1)
Low birth weight (<2500 g)			
No	2564 (97.8)	1481 (97.7)	1478 (97.6)
Yes	58 (2.2)	35 (2.3)	37 (2.4)

[1] Values are n (%). Data sets were defined according to availability of data as follows: data set 1, data on maternal diet in pregnancy and pregnancy duration available; data set 2, same as in data set 1 plus available genotype data in mothers; data set 3, same as in data set 1 plus available genotype data in children. KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg.

Discussion

Our study confirms that maternal DHA intake is positively associated with pregnancy duration and BW and shows for the first time to our knowledge that maternal *FADS* rs174556 SNP genotype is associated with both outcomes. Fetal genotype does not seem to be involved. In addition, we found that maternal

TABLE 3 Association of maternal fat and PUFA intake with pregnancy duration and birth weight in the KOALA Birth Cohort Study[1]

	Outcome = pregnancy duration (d) Model 2 (fully adjusted)		Outcome = birth weight (g) Model 2b (fully adjusted)	
	β (95% CI)	*P*	β (95% CI)	*P*
Maternal fat intake (g/d)	0.01 (−0.02, 0.04)	0.42	−0.44 (−1.66, 0.79)	0.49
Maternal DHA intake (mg/d)	0.004 (0.001, 0.007)	0.016	0.160 (0.008, 0.313)	0.039
Maternal ALA intake (mg/d)	0.001 (0.000, 0.003)	0.11	0.106 (0.026, 0.186)	0.009
Maternal AA intake (mg/d)	−0.029 (−0.056, −0.002)	0.036	−1.871 (−3.178, −0.564)	0.005
Maternal LA intake (g/d)	−0.19 (−0.35, −0.029)	0.021	−5.22 (−13.06, 2.63)	0.19

[1] Results from linear regression analysis adjusted for child gender, study recruitment group, maternal education, parity, maternal smoking status during pregnancy, maternal alcohol use in pregnancy, and maternal age at delivery. Analyses were performed in data set 1 (see *flowchart in Fig. 1*) with $n = 2606$ cases (differs from sample size in data set 1 due to missing values in covariates). AA, arachidonic acid; ALA, α-linolenic acid; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg; LA, linoleic acid.

genotype modifies the association between maternal DHA intake and BW, which supports our hypothesis, but not the association with pregnancy duration.

Strengths of the present study include its prospective design, which allowed collection of dietary data and covariates before measurement of the outcomes, and the availability of a very detailed FFQ, which allowed the estimation of FA intake. Limitations include the fact that we could not study the risk of preterm delivery or low BW as a separate outcome because of the low prevalence within our study population. In addition, because the intake of DHA is very highly correlated with that of other n–3 LC-PUFAs, such as EPA (20:5n–3), we could not check whether the associations with DHA intake are independent from those with EPA intake. In fact, replacing DHA by EPA in the models led to essentially the same results, as expected. Lastly, the SNP we used in our analyses does not cover the whole genetic variation in the *FADS1 FADS2* gene cluster.

Consistency with previous studies and novel gene-diet interaction

Recently, Leventakou et al. (45) analyzed harmonized individual data from 19 population-based European birth cohorts and combined the results in a meta-analysis. They found that fish intake in pregnancy was positively associated with BW: women in the highest tertile of intake (≥3 times/wk) were found to give birth to infants who were 15.2 g (95% CI: 8.9, 21.5 g) heavier than those of women in the lowest tertile (≤1 times/wk). Fish intake was also associated with higher gestational age (0.2-d longer gestation for fish intake ≥3 times/wk vs. ≤1 time/wk). A Cochrane review of RCTs with marine oil supplementation during pregnancy concluded that women allocated to the intervention group had a longer gestation (2.6 d) than did women allocated to placebo or no treatment and had newborns with slightly greater BW (47 g) (46). In the recent RCT by Carlson et al. (17), women supplemented with high doses of DHA during pregnancy (600 mg/d) had heavier newborns (172 g) and longer gestation (2.9 d) compared with controls. The direct comparison of the effect sizes observed in our and previous studies is not straightforward. On the one hand, most observational studies looked at associations with fish intake, not specifically with intake of n–3 LC-PUFAs, and RCTs have used different types and doses of supplements. On the other hand, we accounted for the intake of other PUFAs in our statistical analysis. This was important, because we found that the associations of DHA and AA intakes with BW and pregnancy duration went in opposite directions; it also suggests that differences in AA intake within populations may contribute to the variability of results among previous studies. Despite the differences, our results are consistent with observational studies in that the effect size at the

TABLE 4 Associations of maternal and fetal *FADS1* rs174556 SNP genotype with pregnancy duration and birth weight in the KOALA Birth Cohort Study[1]

	Outcome = pregnancy duration (d)				Outcome = birth weight (g)			
	Model 3		Model 5		Model 3b		Model 5b	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Maternal rs174556 genotype, TT vs. CC	−2.1 (−4.0, −0.1)	0.035	—	—	−137 (−235, −39)	0.006	—	—
Maternal rs174556 genotype, CT vs. CC	0.3 (−0.7, 1.3)	0.54	—	—	−13 (−63, 37)	0.61	—	—
Fetal rs174556 genotype, TT vs. CC	—	—	−0.2 (−2.1, 1.8)	0.87	—	—	−15 (−109, 79)	0.76
Fetal rs174556 genotype, CT vs. CC	—	—	−0.5 (−1.5, 0.6)	0.39	—	—	−29 (−81, 22)	0.27

[1] *Results are derived from linear regression analysis. Models include the following covariates: intakes of fat, α-linolenic acid, arachidonic acid,* DHA, and linoleic acid; child gender; study recruitment group; maternal education; parity; maternal smoking status during pregnancy; maternal alcohol use in pregnancy; and maternal age at delivery. Models 3 and 3b were studied in data set 2; models 5 and 5b were studied in data set 3 (see flowchart in Fig. 1). Analyses were performed with $n = 1511$ cases in models 3 and 3b and with $n = 1508$ cases in models 5 and 5b; these numbers differ from sample sizes in data sets 2 and 3, respectively, due to missing values in covariates. *FADS1*, fatty acid desaturase 1; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg; SNP, single nucleotide polymorphism.

TABLE 5 Gene-diet interactions between maternal *FADS1* rs174556 SNP genotype and PUFA intake in the KOALA Birth Cohort Study[1]

	Outcome = birth weight (g) Model 4b	
	β (95% CI)	*P*
rs174556 genotype, TT vs. CC	−291 (−660, 79)	0.12
rs174556 genotype, CT vs. CC	−149 (−338, 41)	0.12
DHA intake (mg/d)	0.176 (−0.114, 0.465)	0.23
ALA intake (mg/d)	0.002 (−0.143, 0.148)	0.97
AA intake (mg/d)	−1.16 (−3.50, 1.19)	0.33
LA intake (g/d)	−2.10 (−16.10, 11.89)	0.77
DHA intake × genotype, TT vs. CC	1.118 (0.109, 2.127)	0.030
DHA intake × genotype, CT vs. CC	−0.190 (−0.589, 0.209)	0.35
ALA intake × genotype, TT vs. CC	0.299 (−0.204, 0.802)	0.24
ALA intake × genotype, CT vs. CC	0.019 (−0.195, 0.234)	0.86
AA intake × genotype, TT vs. CC	−11.48 (−19.24, −3.72)	0.004
AA intake × genotype, CT vs. CC	−1.28 (−4.63, 2.08)	0.46
LA intake × genotype, TT vs. CC	−8.19 (−51.87, 35.49)	0.71
LA intake × genotype, CT vs. CC	10.36 (−9.63, 30.34)	0.31

[1] Results from linear regression analyses adjusted for fat intake, child gender, study recruitment group, maternal education, parity, maternal smoking status during pregnancy, maternal alcohol use in pregnancy, and maternal age at delivery. Analysis was performed in data set 2 (see flowchart in Fig. 1) with $n = 1511$ cases (differs from sample size in data set 2 due to missing values in covariates). AA, arachidonic acid; ALA, α-linoleic acid; *FADS1*, fatty acid desaturase 1; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg; LA, linoleic acid; SNP, single nucleotide polymorphism.

population level is modest: we found that women with a DHA intake corresponding to the 75th percentile in the study population (209 mg/d) had pregnancies that were 0.7 d longer and infants who were 28 g heavier than did women with a DHA intake corresponding to the 25th percentile (34 mg/d); these values were obtained by multiplying the regression coefficients for DHA intake from models 2 and 2b by the IQR of DHA intake (175 mg/d). For AA intake, women at the 75th percentile of intake (43 mg/d) had 0.6-d shorter pregnancies and 37-g lighter-weight infants than did women at the 25th percentile of intake (22 mg/d).

Our most important finding is that the modest effect size observed at the population level may actually hide a much greater effect [comparable in magnitude to that of cigarette smoking in pregnancy, i.e., ~200 g lower birth weight (47)] in only 1 genotype group (Fig. 2). On the basis of model 4, women who were homozygous for the minor allele at the 75th percentile of DHA intake had 226-g heavier infants than did those at the 25th percentile of intake. BWs of infants from women carrying the major allele did not significantly vary with maternal DHA intake. A similar interaction was also found for AA intake: women who were homozygous for the minor allele at the 75th percentile of AA intake had 254-g lighter-weight infants than did women at the 25th percentile of intake, whereas no association was found in major allele carriers.

Mechanisms

Association between maternal DHA intake and pregnancy duration and BW. We found higher DHA intake to be associated with longer pregnancy and increased BW, whereas the opposite results were found for AA. Opposite effects are not surprising, given that n–3 PUFAs might delay labor by inhibiting the formation of the AA-derived prostaglandins such as prostaglandin $F_{2\alpha}$ and prostaglandin E_2, which favor uterine contractions and cervical ripening (11). As a result of a longer pregnancy, BW would increase. In addition, a direct effect of n–3 LC-PUFAs on fetal growth rate was also proposed that would work through an increase in the functional prostacyclin:thromboxane ratio, which would lower blood viscosity and ultimately increase placental blood flow (2,48).

The literature is inconclusive regarding whether the increase in BW is merely a reflection of a longer pregnancy. Makrides et al. (46) found that the increase in BW with marine oil supplementation was proportional to the increase in pregnancy duration. In contrast, Leventakou et al. (45) found significant associations between fish intake and BW, which were independent of pregnancy duration; and Carlson et al. (17) found that the effect of DHA supplementation on BW was greater than would be expected from the effect on pregnancy duration. Our results would suggest that pregnancy duration at least partially mediates the association between maternal DHA intake and BW,

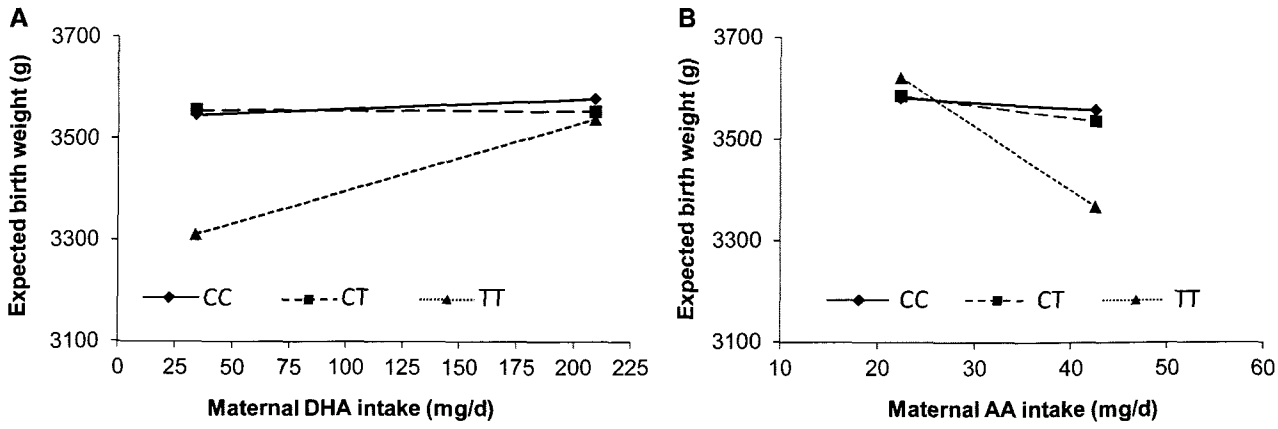

FIGURE 2 Representation of the interaction between maternal *FADS* rs174556 SNP genotype and intakes of DHA (*A*) and AA (*B*) found in the study population of the KOALA Birth Cohort Study. The origin and end of each line are set at an amount of intake corresponding to the 25th and 75th percentiles of intake, respectively, in the study population (i.e., 34 and 209 mg/d for DHA intake and 22 and 43 mg/d for AA intake). The expected birth weight was calculated from the regression coefficients obtained in model 4, assuming values of all other covariates to be equal to the mean values in the population. AA, arachidonic acid; *FADS*, fatty acid desaturase; KOALA, Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg; SNP, single nucleotide polymorphism.

because the latter association lost statistical significance when adjusting for pregnancy duration.

Novel insights. A recent genome-wide association study found major allele carriers for the *FADS1* SNP rs174550 to have higher fasting glucose concentrations (49). Maternal hyperglycemia, in turn, seems to be linked to increased insulin-mediated fetal growth (50). On the basis of this, we speculate that the fact that women who were homozygous for the minor allele had lighter-weight infants may be explained not only by differences in the mother-to-fetus LC-PUFA supply but also by differences in maternal insulin secretion or action.

To the best of our knowledge, this is the first time that a significant association between *FADS* gene variants and a measure of body size has been reported; we found that women who were homozygous for the minor allele had higher prepregnancy BMI than did major allele homozygotes. This finding is not only interesting on its own but it may also help to explain the unexpected results from 2 previous studies showing that maternal prepregnancy overweight status modified the relation between seafood consumption and BW. In the first study, Drouillet et al. (51) found a positive association between maternal seafood consumption and child BW only in overweight women. In the second, Leventakou et al. (45) observed a stronger association between fish intake during pregnancy and BW in the stratum of women who were overweight or obese before pregnancy compared with women with normal prepregnancy BMI. The authors ascribed their findings to the fact that overweight women might have an enhanced ability to release FAs (among which is DHA) from adipose tissue to sustain fetal growth. In view of our results, another possibility is that in the stratum of overweight/obese women, there is a higher proportion of minor allele homozygotes, who have the lowest endogenous DHA synthesis and therefore the highest dependence on dietary DHA supply.

In conclusion, our results suggest that increasing maternal DHA intake during pregnancy without increasing the intake of AA could contribute to an increase in the infant's BW. There seems to be a target group who could especially benefit from such dietary advice: women with low DHA intake (e.g., the lowest quartile, 25%, of the population) who are homozygous for the minor allele (6.9% in this study), representing at least 2% of the population (25% × 6.9%). This could have important public health implications, and replication studies are therefore warranted.

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