**FADS1 FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk**

Carolina Moltó-Puigmartí, Jogchum Plat, Ronald P Mensink, André Müller, Eugène Jansen, Maurice P Zeegers, and Carel Thijs

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**ABSTRACT**

**Background:** The genes encoding Δ5- and Δ6-desaturases (FADS1 FADS2 gene cluster) were reported to be associated with n−3 (omega-3) and n−6 (omega-6) fatty acid proportions in human plasma, tissues, and milk. Docosahexaenoic acid (DHA) can be supplied especially by dietary fish or fish oil and synthesized from ω-3-linolenic acid through a pathway involving these desaturases.

**Objective:** We evaluated whether FADS gene variants modify the effect of maternal fish and fish-oil intake on plasma and milk DHA proportions.

**Design:** FADS1 rs174561, FADS2 rs174575, and intergenic rs3834458 single nucleotide polymorphisms were genotyped in 309 women from the KOALA Birth Cohort Study in the Netherlands. Plasma was collected at 36 wk of pregnancy, and milk was collected at 1 mo postpartum. Fish and fish-oil intake was assessed by using a food-frequency questionnaire at 34 wk of pregnancy and updated for the week of milk collection. Gene-diet interactions were tested by linear regression analysis.

**Results:** DHA proportions were lower in women homozygous for the minor allele than in women who were homozygous for the major allele (DHA proportions in plasma phospholipids: \( P < 0.01 \); DHA proportions in milk: \( P < 0.05 \)). Fish intake ranged from 0 to 2.5 portions of fatty fish/wk, and 12 women took fish-oil supplements during pregnancy. DHA proportions in plasma phospholipids increased with increasing fish and fish-oil intake, irrespective of the genotype. DHA proportions in milk increased only with fish and fish-oil intake in the major-allele carriers.

**Conclusion:** Lower proportions of DHA in milk from women who were homozygous for the minor allele could not be compensated for by increasing fish and fish-oil intake, possibly because of limited incorporation into milk. *Am J Clin Nutr* 2010;91:1368–76.

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**INTRODUCTION**

The supply of n−3 (omega-3) and n−6 (omega-6) long-chain polyunsaturated fatty acids (LC-PUFAs) during gestation to the fetus and postpartum to the newborn is very important because of the involvement of these LC-PUFAs in many regulatory functions (1) and because LC-PUFA arachidonic acid (AA; 20:4n−6) and docosahexaenoic acid (DHA; 22:6n−3) are constituents of the neural tissue, favoring the cognitive and visual development of children (2).

Human milk contains AA and DHA in proportions that significantly correlate with those in plasma or erythrocyte-membrane phospholipids (3–5). Both fatty acids derive mainly from maternal stores. Therefore, maternal (prolonged) supplementation with preformed AA and DHA (3, 4, 6) and habitual dietary intake of foods rich in these 2 fatty acids will influence maternal stores and, in turn, the proportions of milk AA and DHA. Red meat, chicken, and eggs are the main dietary sources of AA, whereas fish (especially oily fish) is the main dietary source of DHA (7). The maternal AA and DHA status can also depend on the endogenous synthesis of these 2 fatty acids from the essential fatty acids, linoleic acid (LA) and ω-3-linolenic acid (ALA), respectively. This conversion is catalyzed by the enzymes Δ5- and Δ6-desaturases (D5D and D6D), as well as elongases, through the pathway shown in Figure 1. Both desaturases are expressed in a majority of human tissues, with highest concentrations shown in liver but also in adipose tissue (8), brain, heart, and lung, whereas minor amounts of the desaturases are shown in the placenta, skeletal muscle, kidney, pancreas, and pregnant uterus (9, 10). A study in rats (11) indicated that both desaturases were also present in the mammary gland, which suggests the possibility that the human mammary gland participates in the synthesis of LC-PUFAs for their subsequent incorporation into human milk.

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The genes that encode for the D5D and D6D are FADS1 (GeneID 3992; www.ncbi.nlm.nih.gov/gene) and FADS2 (GeneID 9415), respectively, both located on the desaturase gene cluster on chromosome 11q12-q13 with a head-to-head orientation (12, 13). There is evidence about the existence of a third desaturase gene, FADS3 (GeneID 3995), but its implication for the metabolism of fatty acids is still not known (12).

Several single nucleotide polymorphisms (SNPs) in the FADS genes were reported in humans, and some studies showed associations between FADS SNPs and fatty acids in serum or plasma phospholipids (14–18), plasma (19, 20), erythrocyte membranes (15, 17, 18, 20), erythrocyte-membrane phospholipids (16), and adipose tissue (19). In addition, a recent study by Xie and Innis (16) showed that the fatty acid composition of human milk is also related to these FADS SNPs. However, most of the studies did not find a significant association between DHA proportions and FADS SNPs. The only exceptions are the studies of Xie and Innis (16) and Tanaka et al (20), which reported the subjects homozygous for the minor allele for the SNPs rs154575 and rs174537, respectively, to have significantly lower proportions of DHA in milk or erythrocyte-membrane phospholipids.

Therefore, in the present study we wanted to confirm previously reported associations between FADS SNPs and fatty acid proportions, in particular those of DHA, in plasma phospholipids and human milk. The final aim was to investigate whether lower DHA proportions in women homozygous for the minor allele, if observed, could be compensated for by diet and, if so, to what extent.

SUBJECTS AND METHODS

Study design

This study is part of the KOALA Birth Cohort Study, a prospective birth cohort study in the south of the Netherlands described previously in detail (21). KOALA is an acronym for Kind, Ouders en gezondheid: Aandacht voor Leefstijl en Aanleg (Child, Parents and Health: Lifestyle and Genetic Constitution). Briefly, participants consisted of pregnant women who were recruited between 2000 and 2002 at 34 wk of gestation. At 36 wk of pregnancy, they were visited at home for collection of venous blood. At 36 wk of pregnancy, they were visited at home for collection of venous blood. In addition, from October 2002 participants were asked to consent to breast-milk sampling at 1 mo postpartum. For the present study, we used a group of 309 women, for which we had plasma and breast-milk samples taken between December 2002 and September 2003. Besides these samples, we also monitored participants’ dietary habits since they had filled in a food-frequency questionnaire (FFQ) at 34 wk of gestation and a short questionnaire about the intake of fatty fish and fish-oil supplements during the week previous to breast milk sampling (22). The study was approved by the Ethics Committee of the Maastricht University/University Hospital Maastricht, and all participants gave written informed consent. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Plasma sampling and measurement of fatty acids in plasma phospholipids

Blood was collected into a 10-mL EDTA-coated tube (VT-100STK; TERUMO, Leuven, Belgium). Plasma was separated from the buffy coat and red blood cells after centrifugation of the EDTA-coated tube at 3000 rpm (1500 × gmax) for 10 min at room temperature with no brake. The buffy coat was prepared by recentrifuging the EDTA-coated tube at 3000 rpm (1580 × gmax) for 5 min at 4°C with no brake and separating it from red blood cells with a sterile Pasteur capillary pipette of glass. EDTA-plasma and buffy coat vials were stored at −80°C until use.

Analyses of fatty acids in plasma phospholipids were performed according to the method described in Mamalakis et al (23). Briefly, plasma lipids were extracted with a mixture of chloroform:methanol (1:1; vol:vol). Different lipid classes were separated by solid-phase extraction with an amino propyl solid-phase column (Bond-Elut NH2 200 mg; Varian Inc, Middelburg, Netherlands). Phospholipid fatty acid methyl esters (FAMEs)
were prepared with a mixture of 100 μL toluene and 0.5 mL BF₃/MeOH (60 min at 100°C in a heating block) and extracted into hexane. FAMEs were separated on a 100 × 0.25-mm inside diameter WCOT (Wall Coated Open Tubular) fused silica capillary column coated with 0.25 μm CP-Select CB (Varian Inc) with a GC-3900 gas chromatograph (Varian Inc) equipped with a CP 8400 autoinjector. A baseline separation of >50 FAMEs was accomplished by using mixed FAME standards (Sigma, St Louis, MO), and fatty acids were expressed as the proportion of the total fatty acids present in the chromatogram.

Breast-milk sampling and measurement of fatty acids

The methods of breast-milk collection and fatty acid analyses were described in detail previously (24). Briefly, mothers were instructed to collect their milk in the morning before breastfeeding their child from the second breast after finishing the first and to keep the sample in the refrigerator (at ≈4°C) until use. FAMEs were prepared from the lipid fraction and analyzed by using gas chromatography with flame ionization detection and Ag⁺-HPLC.

Lipids were extracted from milk with a mixture of chloroform: methanol (2:1; vol:vol) containing 0.001% butyraldehyde, and the lipid fraction was stored at −80°C at the B informal University of Maastricht (Maastricht, Netherlands) until use. FAMEs were prepared by using gas chromatography with flame ionization detection and Ag⁺-HPLC with diode array detection as previously described (24). All measurements were done in duplicate and reported as averages. A total of 60 fatty acids were identified, from 4:0 to 22:6n−3, including also some branched chain fatty acids and trans fatty acids. For data analyses, we ended up with a list of 36 fatty acids. Fatty acid concentrations were expressed as the proportion of total milk fat [weight percentage (%w) in mg/100 mg].

Total fat content of human milk was estimated by calculating the creatometric of all samples following the method described by Lucas et al (25).

**FFQ**

The FFQ was included in a self-administered questionnaire in the 34th wk of pregnancy. It was based on an existing validated FFQ (26) and consisted of ≈160 food items, for which the frequency of consumption in the previous month and portion size had to be estimated by the participants. For estimation of the intake of n−3 LC-PUFAs, the sources considered were fish and seafood because they contain much higher concentrations of these fatty acids than other sources (eg, eggs and poultry) (27, 28). Of note, n−3 LC-PUFA–fortified products were not commercially available in the Netherlands at the time of the study. Items on seafood intake included different types of the fatty fish most frequently eaten in the Netherlands with cold and warm meals (ie, herring, salmon, mackerel, and blue mussels as separate items), and other (nonfatty and canned) fish types. In addition, participants were asked about the use of fish-oil supplements during pregnancy. On the day of breast-milk sampling, a short questionnaire was taken on the intake of fatty fish and fish-oil supplements during the week previous to the milk sampling.

On the basis of the content of n−3 LC-PUFAs [eicosapentaenoic acid (EPA; 20:5n−3), docosapentaenoic acid (DPA; 22:5n−3), and DHA] in seafood in the Dutch food-composition tables, we estimated that each portion of fatty fishes and blue mussels per week contributed equivalent amounts of weekly n−3 LC-PUFA intake, and that each portion of other fishes contributed one-third of that equivalent (29). From the standard dosage of fish-oil supplements (which provide ≈300 mg n−3 LC-PUFAs/1-g capsule), we estimated that they contributed an equivalent of one portion of fatty fish per week. Therefore, we estimated the total dietary n−3 LC-PUFA intake from seafood and supplements as equivalents of portions of fatty fish per week (FFEq; sum of portions of herring, salmon, mackerel, and blue mussels, plus one-third of total portions of other fishes, plus one equivalent for fish-oil supplement use).

**Selection of SNPs**

The selection of SNPs was based on 3 criteria: 1) SNPs should have been already included in previous studies (14–19, 30) regarding essential fatty acid metabolism in humans (6 SNPs in the FADS1 gene, 14 SNPs in the FADS2 gene, and 5 SNPs in intergenic regions fulfilled this requirement); 2) among these SNPs, only those for which an association with the essential fatty acid or LC-PUFA concentrations in tissues had been described were considered as candidates; and 3) the minor allele frequency had to be ≥18% so that the expected number of subjects homozygous for the rare allele in our study population was ≥10.

According to these criteria, 3 SNPs from the FADS1 FADS2 gene cluster were selected: SNP rs174561 in the FADS1 gene, which is thought to have a direct influence on the transcriptional level of D5D (14); SNP rs3834458, which is located in the intergenic region between the FADS1 and FADS2 genes and was suggested to play an important role in metabolism because of its location near potential regulatory regions (14, 19, 31); and SNP rs174575 in the FADS2 gene, which has no known function.

**SNP genotyping**

DNA was extracted from the buffy coat with the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). SNPs were genotyped with TaqMan SNP Genotyping assays (Applied Biosystems, Foster City, CA) with real-time polymerase chain reaction according to the instructions of the manufacturer.

**Statistical analyses**

Correlations between the proportions of a fatty acid in plasma phospholipids and human milk were computed by using Pearson’s correlation coefficient.

Deviations from Hardy-Weinberg proportions for the genotypes of each SNP were tested by using chi-square tests. Genetic data were analyzed for each SNP separately and categorized as homozygous for the major allele, heterozygous, and homozygous for the minor allele by using ANOVA to compare mean fatty acid proportions. An additive model was assumed so that the increase or decrease of fatty acid proportions was tested for each increment of one minor allele (ie, homozygous for the major allele: coded 1; heterozygous: coded 2, and homozygous for the minor allele: coded 3; tested as an interval variable in the ANOVA). A Bayesian statistical model for reconstructing uncertain haplotypes was applied with the PHASE program (version 2.1) (32).
We used linear regression as a formal statistical test of gene-diet interaction. The proportion of DHA in plasma phospholipids or breast milk was used as the dependent variable, and fish and fish-oil intake (equivalents), genotypes (2 dummy variables for heterozygous and homozygous for the minor allele, respectively, with the homozygous for the major allele as the reference group), and interaction terms (equivalents × dummy variables) were used as independent variables. The t statistics of the interaction terms in the linear regression analysis were used as significance tests for interactions. The same was done for the sum of EPA and DPA (EPA+DPA) as dependent variables. We considered \( P < 0.05 \) as statistically significant. DHA and EPA+DPA were log_{10}-transformed to normalize their distribution. All data were analyzed with SPSS 15.0 statistical software (SPSS, Chicago, IL).

RESULTS

A total of 309 pregnant women with a mean (±SD) age at delivery of 33.2 ± 3.9 y were included in the study. The fatty acid composition of plasma phospholipids and human milk and the creatamocrit for milk samples from all participants were measured. The SNPs rs174561, rs174575, and rs3834458 on the FADS1 FADS2 gene cluster were genotyped with a success rate of 100%. The genotype distribution for each SNP did not deviate from Hardy-Weinberg equilibrium (Hardy-Weinberg equilibrium \( P \) values > 0.05; Table 1). Minor allele frequencies ranged between 25.24% and 30.10% of the study population (Table 1). The total fat content of milk (measured as creatamocrit) did not significantly vary according to genotype.

SNP associations with fatty acids

With the use of the genotyping data, we analyzed the association of the 3 FADS SNPs with the fatty acid composition of plasma phospholipids and human milk (Table 2).

For the \( n = 6 \) family, subjects homozygous for the minor allele (for any of the 3 SNPs) had significantly higher proportions of LA, 20:2n−6 (eicosadienoic acid), and 20:3n−6 (dihomo-γ-linolenic acid, DGLA) and lower proportions of 18:3n−6 (γ-linolenic acid, GLA), 20:4n−6 (AA), and 22:4n−6 (adrenic acid) in their plasma phospholipids. For the \( n = 3 \) family, the results for ALA (18:3n−3), EPA (20:5n−3), and DPA (22:5n−3) in plasma phospholipids were fairly consistent with their \( n = 6 \) counterparts with respect to the direction of the differences by genotype (increased ALA and decreased EPA and DPA proportions in subjects homozygous for the minor allele compared with the carriers of the major allele) but did not reach the same level of significance. The proportions of DHA, the end product of the \( n = 3 \) family, were significantly lower in the plasma phospholipids for the minor-allele carriers, whereas osbond acid (22:5n−6), its \( n = 6 \) counterpart, could not be quantified (because it was below the limit of sensitivity). Substantially analogous results were shown in human milk except for GLA and ALA, the proportions of which did not significantly vary according to genotype.

The results generally agreed with an additive model [an increase or decrease of fatty acid proportions with an increasing number of minor alleles (ie, proportionate differences between carriers homozygous for the major allele, heterozygous, and homozygous for the minor allele)]. The pattern of decreased or increased fatty acid proportions was similar for the 3 SNPs, indicating that none of the SNPs were uniquely related to precursors or conversion products of D5D or D6D.

Fish-oil intake and \( n = 3 \) LC-PUFA status

In the current study, 12 women (3.9%) consumed ≥2 portions of fatty fish/wk, whereas 117 women (37.9%) reported having no fatty fish intake at all. The remaining 180 women (58.2%) had intakes in between these amounts. Also, 12 women (3.9%) used a fish-oil supplement during pregnancy, and all of them had eaten <2 portions of fatty fish/wk during pregnancy. Regarding the FFEq calculated from dietary intake of fish and fish-oil supplements, 249 women (80.6%) consumed <2 FFEq (mean ± SD intake: 1.19 ± 0.89).

FFEq intake was strongly related with DHA proportions in plasma phospholipids (regression coefficient ± SE: 0.508 ± 0.042; \( P < 10^{-10} \)); ie, the DHA proportions increased 0.508 wt % with each portion per week of FFEq. Similarly, FFEq was strongly related with EPA+DPA proportions in plasma phospholipids (regression coefficient ± SE: 0.184 ± 0.023; \( P < 10^{-10} \)). FFEq intake was also strongly related with DHA proportions in human milk (regression coefficient ± SE: 0.102 ± 0.013; \( P < 10^{-10} \)) and with EPA+DPA proportions in human milk (regression coefficient ± SE: 0.053 ± 0.007; \( P < 10^{-10} \)).

In the week before breast-milk sampling, 107 women consumed fatty fish, 4 took fish-oil supplements, and 3 consumed fatty fish and took fish-oil supplements. When the FFEq intake was adapted by using this information, this did not essentially modify the prediction of the proportions of DHA and EPA+DPA in human milk (always \( P < 10^{-10} \)), and therefore, FFEq intake during pregnancy was used as estimate of intake of sources of

### Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>M/m alleles</th>
<th>Genotype</th>
<th>MAF (%)</th>
<th>HWE (%)</th>
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</thead>
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<td>166 (53.7)</td>
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<td></td>
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<td>18 (5.8)</td>
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<td>C/G</td>
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<td></td>
<td></td>
<td>GG</td>
<td>15 (4.9)</td>
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</tr>
<tr>
<td>rs3834458</td>
<td>Intergenic</td>
<td>T/del</td>
<td>TT</td>
<td>144 (46.6)</td>
<td>0.058</td>
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<td></td>
<td></td>
<td>Tdel</td>
<td>144 (46.6)</td>
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<td></td>
<td></td>
<td></td>
<td>deldel</td>
<td>21 (6.8)</td>
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</tbody>
</table>

1 M/m, major and minor alleles; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium; del, deletion.
2 \( P \) values of deviation from HWE among all subjects were tested by chi-square tests.
3 Number of subjects for each genotype; percentage in parentheses (all such values).
<table>
<thead>
<tr>
<th>SNP</th>
<th>Fatty acid</th>
<th>Plasma phospholipid proportions</th>
<th>Human milk proportions</th>
<th>Correlation r</th>
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<td>MM: 0.73 ± 0.20, Mm: 0.76 ± 0.22, mm: 0.70 ± 0.18</td>
<td>MM: 2.49 ± 0.80, Mm: 2.43 ± 0.75, mm: 2.37 ± 0.76</td>
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<td>20:5n-3</td>
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<tr>
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<td>cis 18:1n-7</td>
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<td>rs17455</td>
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<td>cis 20:1n-9</td>
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<tr>
<td>rs174561</td>
<td>22:6n-3 (DHA)</td>
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<td>20:5n-3</td>
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</tbody>
</table>

1 The numbers of women who were homozygous for the major allele (MM), heterozygous (Mm), and homozygous for the minor allele (mm) were as follows: 166, 125, and 18, respectively, for single nucleotide polymorphism (SNP) rs174561; 168, 126, and 15, respectively, for SNP rs174575; and 144, 144, and 21, respectively, for SNP rs3834458. LA, linoleic acid; DGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Differences between plasma phospholipids and human milk fatty acid proportions between genotypes were assessed by using ANOVA assuming an additive model (FADS SNP genotypes coded 1, 2, and 3 for homozygous for the major allele, heterozygous, and homozygous for the minor allele, respectively).

2 Mean ± SD (all such values).
3 Significant at 0.01 (2-tailed).
4 Significant difference (P < 0.05).
n−3 LC-PUFAs during pregnancy and lactation in further analyses. The total fat content of breast milk was not associated with FFEq intake.

Gene-diet interaction for fish intake and FADS SNPs

As expected, there were no significant differences in FFEq intake between women with different genotypes. DHA and EPA+DPA proportions in plasma phospholipids increased with FFEq intake in all 3 genotypes for rs174575 (Figure 2, A and C). Although DHA proportions (Figure 2A) were lower in the women homozygous for the minor allele over the whole range of FFEq intake, the DHA proportions increased with higher FFEq to a similar extent for the 3 genotypes (the slopes of the regression lines were similar); this was also true for EPA+DPA proportions (Figure 2B). The differences of slopes between the minor genotypes compared with major genotypes were tested by assessing the interaction with FFEq intake in linear regression (with 10log transformation of DHA and EPA+DPA to derive a normal distribution); P values for this test of gene-diet interaction were >0.05, indicating no significant differences between the slopes. Results for the other 2 SNPs were essentially the same (data not shown), with gene-diet interaction P > 0.05.

In human milk, by contrast, significant relations between FFEq intake and DHA and EPA+DPA proportions were shown in the carriers of the major allele but not in the women homozygous for the minor allele; ie, proportions of these fatty acids in milk did not increase with higher FFEq intakes in the minor allele homozygotes (Figure 2, B and D; flat regression lines with slope near zero). In the test for the statistical interaction, P values for differences between slopes of different genotypes of the SNP rs174575 in human milk were \( P = 0.077 \) for DHA and \( P = 0.019 \) for EPA+DPA (regression coefficient ± SE for interaction = \(-0.081 ± 0.046 \) for DHA and \(-0.084 ± 0.019 \) for EPA+DPA). Results for the other 2 SNPs were essentially the same (P values for gene-diet interaction: 0.090 for DHA and 0.036 for EPA+DPA for SNP rs174561; 0.071 for DHA; and 0.021 for EPA+DPA for SNP rs3834458).

Haplotype association with DHA and gene-diet interaction

Analyzing the data at the allelic level, we showed 7 haplotypes for combinations of the SNPs rs174561, rs174575, and rs3834458. Ordered according to the number of alleles in each group and considering A, B, and C to represent the major alleles for the SNPs rs174561, rs174575 and rs3834458, respectively, we showed that...
and a, b, and c to represent the minor alleles for the SNPs rs174561, rs174575 and rs3834458, respectively, the haplotypes were: ABC (n = 428), abc (n = 128), aBc (n = 27), abC (n = 26), Abc (n = 4), ABo (n = 3) and aBo (n = 2). These totals add up to 618 (2 alleles for each SNP × 309 subjects). The alleles of the markers were in some linkage disequilibrium (D > 0.10, R² > 0.19), which may explain why the abc haplotype was far more common (128 of 618; 20.7% observed) than expected from random combination of the minor allele frequencies (2.0% expected; this is the product of minor allele frequencies shown in Table 1). To assess whether the results were different at the haplotype level than at the genotype level (as presented in Table 2), we combined the 2 most common haplotypes across the maternal and paternal chromosomes to create the following 3 haplotype groups: subjects homozygous for all major alleles on all 3 SNPs (ABC/ABC, n = 140 subjects), subjects homozygous for all minor alleles (abc/abc, n = 12), and subjects heterozygotes for all SNPs (ABC/abc and vice versa, n = 97), leaving out the group with other combinations (n = 60).

Results for the haplogenotypes were very similar compared with results for the single SNPs, with the homozygous minor alleles (abc/abc) having lower proportions of products of the desaturases than of substrates compared with the homozygous wild types (ABC/ABC), and again, the results for the heterozygotes were in between (see supplemental Table 1 under “Supplemental data” in the online issue).

The gene-diet interaction was also very similar for the haplogenotypes compared with the individual SNPs genotypes (see supplemental Figure 1 and Table 2 under “Supplemental data” in the online issue). Contrasting the homozygous haplotype groups ABC/ABC and abc/abc, their slopes of increasing DHA proportions in human milk with increasing FFEq diverged similarly as shown in Figure 2B, and the test for interaction gave a P value of 0.081. For the proportions of EPA+DPA in human milk, this divergence of slopes between haplogenotype groups ABC/ABC and abc/abc was similar to the divergence shown in Figure 2D, and was significant (P = 0.022). For DHA and EPA+DPA in plasma phospholipids, the slopes were parallel for the haplogenotype groups, similar to the results for the single SNPs (Figure 2, A and C), and not significantly different (P > 0.35).

DISCUSSION

In the present study we provided evidence from a group of 309 women that LC-PUFAs proportions in their plasma phospholipids at the 34th wk of pregnancy and in their milk at 1 mo postpartum depended on their genotypes for 3 selected SNPs on the FADS1 FADS2 gene cluster. In particular, we observed that DHA proportions in plasma phospholipids and milk were decreased in the women homozygous for the major allele compared with the proportions in carriers of the major allele, and, for the first time to our knowledge, showed that a higher fish (or fish-oil) intake compensated for the lower DHA proportions in the women’s plasma phospholipids but not in their milk.

Our results of the fatty acid composition of plasma phospholipids and human milk by FADS gene variants agree well with those of previous studies (14–16, 18, 19). Also in agreement with other studies (14–17), the haplotype analyses confirmed the results of the single markers. This agrees with the fact that the SNPs were highly linked with each other and shows that each SNP conveys most of the information contained in the other SNPs. In general, previous studies point to a lower activity or transcription of the desaturases to explain the observed increases in the proportions of the substrates of the mentioned enzymes and the subsequent decreases in the proportions of their products. Our results follow this same pattern, with increased proportions of substrates (LA and DGLA), decreased proportions of products (GLA, AA, EPA), and changes in other fatty acid proportions in the expected direction (decreased proportions of 22:4n-6, DPA, and DHA). By the time that our study was finished, an article (20) reported that the most significant SNP for AA was rs174537 on the FADS1 FADS2 gene cluster. However, this marker was not included in our list of candidate SNPs because none of the previously published works genotyped it.

One of the most important implications for the development of newborns is that the percentages of AA and DHA in plasma phospholipids and milk of the mothers homozygous for the minor allele were lower compared with the percentages for carriers of the major allele. However, we expected that higher fish and fish-oil intake could permit those women homozygous for the minor allele to compensate for their lower desaturase activity/expression (because of incorporation of the preformed fatty acids including DHA in fatty fish and fish-oil supplements) and to reach DHA proportions not far below the DHA proportions observed for the carriers of the major allele, both in their plasma and milk. Indeed, in plasma phospholipids, DHA proportions increased with fish and fish-oil intake for all of the 3 genotypes and to a similar extent (parallel slope in Figure 2A), and this was also apparent for EPA+DPA proportions (Figure 2C). By contrast, proportions of DHA and EPA+DPA in milk only increased with fish and fish-oil intake in the major-allele carriers, whereas no increase was seen in the mothers homozygous for the minor allele, leading to an augmentation of the genetic differences at higher FFEq intakes. The finding of a different response to the intake of n-3 LC-PUFA depending on the FADS1 FADS2 genotype can be described as a gene-diet interaction, and may indicate that mothers homozygous for the minor allele take no advantage of fish-oil intake or at least have less advantage than the major-allele carriers.

We found it surprising that this response differed between plasma phospholipids and human milk and that the response was similar for DHA and EPA+DPA proportions. We speculate that, in the minor allele homozygous mothers, there is an alteration in the incorporation of n-3 LC-PUFAs into milk, and that the FADS gene cluster markers could be linked somehow to fatty acid transportation (for instance, related to common genetic regulation). In a recent genome-wide association study, Tanaka et al (20) showed EPA and DHA in plasma to be associated with the genotype for SNP rs2277324 in the gene SLC26A10 (GeneID 65012) on chromosome 12, a gene encoding for a solute carrier family member. Other possible genetic candidates are those that code for fatty acid binding proteins (FABP) and fatty acid transport proteins (FATP), but all known variants are located on different chromosomes than the FADS gene cluster (FABP5, expressed in the mammary gland, for instance, is located on chromosome 8). Interestingly, an FABP5 homolog, FABP5 like 7, is located at chromosome 11q12.1, close to FADS (11q12.2), but this is considered a nonfunctional pseudogene. Finally, a polymorphism in FABP2 was shown to be related to D6D...
activity in obese children (33), but FABP2 is not known to be expressed in the mammary gland.

If incorporation of preformed n-3 LC-PUFAs into milk is truly limited in mothers with certain FADS variants, this could imply that it is not helpful for these mothers to increase their fish or fish-oil intake to raise the content of n-3 LC-PUFAs (in particular DHA) in their milk, at least up to the studied upper range of 3 portions of fatty fish/wk. In any case, these results should be replicated in other studies, preferably by using stable isotopes to compute conversion rates and to calculate fluxes. In addition, our results are based on a limited number of subjects with the minor allele genotype and with a maximum intake of 3 equivalents of fatty fish/wk. Future supplementation studies could evaluate whether higher amounts of fatty fish intake or n-3 LC-PUFA supplementation than currently recommended can overcome the limited incorporation into the milk of mothers homozygous for the minor allele.

The majority of the population of the Netherlands and other Western European countries do not reach the recommended fatty fish intake of 2 times/wk. For those with low fish intake (0 or 1 time/wk), milk DHA proportions do not greatly differ according to genotype (Figure 2B). However, at the amount of 2 portions of fatty fish/wk, DHA proportions already differed in 0.2 wt% between subjects homozygous for the minor allele and subjects with the other 2 genotypes (Figure 2B; mean ±0.3 wt% for minor-allele homozygous subjects and ±0.5 wt% for major-allele carriers). This means that children at risk of an insufficient DHA supply are those whose mothers have low fish and fish-oil intake and the children from mothers homozygous for the minor allele, regardless of fish intake or supplementation at the recommended amounts. In addition, the child’s own genotype may be an important factor determining his or her LC-PUFA status. Caspi et al (30) suggested an association between children’s genotypes for the SNP rs174575 on FADS2 and their AA and DHA status. This observation and the findings of the present study raise new questions: eg, What would the DHA status of a breastfed child be if mother and child shared the minor allele homozygous genotype?

In conclusion, we confirmed the previously reported associations between FADS SNPs and fatty acid profiles in human milk within a different population and by using a larger dataset. To our knowledge, the major novelty in this study is the gene-diet interaction, which was shown to differ between plasma phospholipids and human milk for DHA and EPA+DPA proportions. If confirmed by supplementation studies, a challenge is to disentangle the possible mechanisms of incorporation of n-3 LC-PUFAs in human milk and its genetic regulation. Also, further investigation is warranted on whether these findings may have any functional consequences for the child. If so, a mother’s diet and the combination of FADS genotypes of the mother and child would be crucial for the child’s development and health.

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REFERENCES

16. Xie L, Innis SM. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J Nutr 2008;138:2222–8.