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 Lactation1,2

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Abstract

The enzymes encoded by fatty acid desaturase (FADS) 1 and FADS2 are rate-limiting enzymes in the desaturation of linoleic acid [LA; 18:2(n-6)] to arachidonic acid [ARA; 20:4(n-6)], and α-linolenic acid [ALA; 18:3(n-3)] to eicosapentaenoic acid [EPA; 20:5(n-3)] and docosahexaenoic acid [DHA; 22:6(n-3)]. ARA, EPA, and DHA play central roles in infant growth, neural development, and immune function. The maternal ARA, EPA, and DHA status in gestation influences maternal-to-infant transfer and breast milk provides fatty acids for infants after birth. We determined if single nucleotide polymorphisms in FADS1 and FADS2 influence plasma phospholipid and erythrocyte ethanolamine phosphoglyceride (EPG) (n-6) and (n-3) fatty acids of women in pregnancy or their breast milk during lactation. We genotyped rs174553, rs99780, rs174575, and rs174583 in the FADS1 FADS2 gene cluster and analyzed plasma and erythrocyte fatty acids and dietary intake for 69 pregnant women and breast milk for a subset of 54 women exclusively breast-feeding at 1 mo postpartum. Minor allele homozygotes of rs174553(GG), rs99780(TT), and rs174583(TT) had lower ARA but higher LA in plasma phospholipids and erythrocyte EPG and decreased (n-6) and (n-3) fatty acid product:precursor ratios at 16 and 36 wk of gestation. Breast milk fatty acids were influenced by genotype, with significantly lower 14:0, ARA, and EPA but higher 20:2(n-6) in the minor allele homozygotes of rs174553(GG), rs99780(TT), and rs174583(TT) and lower ARA, EPA, 22:5(n-3), and DHA in the minor allele homozygotes G/G of rs174575. We showed that genetic variants of FADS1 and FADS2 influence blood lipid and breast milk essential fatty acids in pregnancy and lactation. J. Nutr. 138: 2222–2228, 2008.

Introduction

The enzymes Δ5 desaturase and Δ6 desaturase, encoded by fatty acid desaturase (FADS)1 and FADS2, respectively, are the rate-limiting enzymes in synthesis of the long-chain PUFA arachidonic acid [ARA; 20:4(n-6)] and eicosapentaenoic acid [EPA; 20:5(n-3)] and docosahexaenoic acid [DHA; 22:6(n-3)] from their dietary precursors linoleic acid [LA; 18:2(n-6)] and α-linolenic acid [ALA; 18:3(n-3)], respectively (Fig. 1) (1–6). ARA, EPA, and DHA are esterified in membrane phospholipids where they contribute to membrane-dependent enzyme, receptor, and ion channel activities and provide a reservoir for the regulated release of ARA and EPA, and DHA for synthesis of eicosanoids and docosanoids, respectively, and for regulation of gene expression (1,7–10). ARA fulfills the essential role of (n-6) fatty acids in growth and is a precursor for synthesis of multiple eicosanoids, including prostaglandin E2, which is important in normal development of many organs and cells, including the central nervous system (11–16). DHA is enriched in membrane lipids of the brain and retina and plays critical roles in neurogenesis, neurotransmitter metabolism, neuroprotection, and the kinetics of the visual cycle (1,7,9,11,17–19). During gestation and breast-feeding, ARA, EPA, and DHA and their precursor (n-6) and (n-3) fatty acids are transferred from the mother by placental transfer and in breast milk, respectively (20,21), and higher levels are positively associated with pre- and postnatal growth and better development of visual, neural, and immune function (21–30).

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Abbreviations used: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; LA, linoleic acid; SNP, single nucleotide polymorphism.

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Subjects and Methods

Subjects. The present study involved pregnant women, 24–40 y of age, with no known maternal or fetal complications, all of whom were participants in a larger study of maternal fatty acid status and infant development (29). Each subject was enrolled at 16 wk of gestation and each subject was seen again at 36 wk of pregnancy and 1 mo postpartum. Women consuming a vegan diet or with any known metabolic disease, including diabetes, immune disorders, or communicable diseases, were not enrolled. In addition, women taking supplemental sources of EPA or DHA were not included in the present study. The protocol and procedures were approved by the Committee for Ethical Review of Research Involving Human Subjects at the University of British Columbia and the British Columbia’s Children’s and Women’s Hospital. All subjects provided written informed consent prior to participation.

Dietary assessments. Sociodemographic data, including age, parity, and highest level of education, family income, and ethnic background, were collected from each subject by questionnaire. Dietary assessments were conducted at 16 and 36 wk of gestation using an interview-administered FFQ designed to collect information on fat and fatty acid intakes and their food sources (34). Information from the dietary record was entered into a nutrient database (FOOD PROCESOR 11; ESHA Research) containing the Canadian Nutrient File, updated with the fatty acid composition of foods analyzed in our laboratory (34).

Sample collection and fatty acid analyses. Venous blood was collected from fasting subjects in the outpatient laboratory of the British Columbia’s Children’s Hospital. The erythrocytes were separated from plasma by centrifugation (2000 × g; 15 min at 4°C), washed 2 times in normal saline, then the plasma and erythrocytes were frozen at −70°C until later analyses (24,28,29). Samples of breast milk, collected from a subset of 34 women who were exclusively breast-feeding their infants at 1 mo postpartum, were stored at −70°C until analysis (28,35). Total lipids were extracted from the RBC and plasma, then the plasma phospholipids and RBC ethanolamine phosphoglyceride (EPG) were separated by HPLC, recovered, and their fatty acids separated and quantified by GLC (24,28,29). The milk samples were thawed in ice-cold water, directly transmethylated to avoid potential loss of medium-chain fatty acids, and the FAME separated and quantified by GLC (28,35).

Genotyping. We selected 6 SNP covering a 34-kb-long genomic region of the FADS1 FADS2 gene cluster in which the promoter region was included for SNP analysis. Four of the SNP analyzed by us, rs174553, rs174561, rs174583, and rs99780, have been reported to be associated with differences in the composition of serum lipid (n-6) and (n-3) fatty acids (31,32). We included analyses of 2 additional SNP, rs498793 and rs174575. SNP information was derived from NCBI dbSNP Build 128 (36). Genomic DNA was extracted from whole blood using the QIAamp DNA blood Mini kit (QIAGEN) and genotyping was performed with TaqMan SNP Genotyping assays (Applied Biosystems) using real-time PCR.

Statistical analysis. Results are expressed as means ± SD unless otherwise specified and were analyzed with SPSS 15.0 software. The mean levels of fatty acids were compared among the major and minor allele homozygotes and heterozygotes using the Kruskal-Wallis test. We used Mann-Whitney U tests to determine differences in the Δ6 plus Δ5 desaturase fatty acid product-precursor ratios between major allele carriers and minor allele homozygotes. P-values ≤ 0.05 were considered significant. The Haplovfreq program (version 4.0) was used to test the Hardy-Weinberg equilibrium for each genotype and linkage disequilibrium (LD) was calculated for paired SNP as described by others (32). The frequency of all haplotypes occurring among the women with a frequency >1% was calculated using the Haplovfreq program (version 4.0).

Results

The women in the present study were 33.5 ± 3.6 y of age and 74% were Caucasian. All the subjects delivered 1 healthy, full-term infant (37–41 wk of gestation), as specified by our study inclusion criteria. The SNP in the FADS1 FADS2 gene cluster were genotyped with a success rate of 100% and, consistent with public databases, the 6 SNP we analyzed were polymorphic (Table 1). SNP rs174561 and rs498793 were not consistent with Hardy-Weinberg equilibrium and further analyses relating to these SNP to maternal or breast milk fatty acid status were not conducted. The other 4 SNP, rs174553, rs99780, rs174575, and
The women's intakes of LA, ALA, ARA, EPA and DHA were 13.1 ± 6.76 g/d, 1.86 ± 1.24 g/d, 83.3 ± 39.6 mg/d, 76.1 ± 65.5 mg/d, and 118 ± 122 mg/d, respectively (n = 69). The intakes of (n-6) and (n-3) fatty acids did not differ among the women grouped by the rs174553 allele (Table 2). Genotype influenced the plasma phospholipid levels of LA, ARA, 22:5(n-6), and 22:6(n-6) (P < 0.005) and tended to affect those of ALA (P = 0.056) and EPA (P = 0.079). For rs174575, the plasma phospholipid levels of LA did not differ among C/C (n = 33), C/G (n = 28), and G/G (n = 8) carriers and were 21.0 ± 2.94, 20.5 ± 3.29, and 22.6 ± 3.45 g/100 g fatty acids (P > 0.05), whereas concentrations of ARA were influenced by genotype with levels of 10.7 ± 1.70, 9.9 ± 1.58, and 8.5 ± 1.49 g/100 g fatty acids in the 3 groups, respectively (P = 0.003). Plasma phospholipid levels of the other (n-6) and (n-3) fatty acids did not differ among the pregnant women when grouped by rs174575 genotype (data not shown).

We calculated the ratio of the Δ6 and Δ5 desaturase products to their LA or ALA precursors in plasma phospholipids as indices of potential differences in desaturase activities among the women grouped as major allele carriers or minor homozygotes. However, we note that the ratios did not consider the possible effects of differences in the rate of utilization of the products. In the major allele carriers and minor allele homozygotes of rs174553 (rs99780 and rs174583), the (n-6) fatty acid product:LA ratios were 11.5 ± 1.4 (n = 49) and 8.8 ± 1.2 (n = 20) (P < 0.001) and for the (n-3) fatty acids, the desaturation product:ALA ratios were 20.4 ± 10.9 (n = 49) and 13.5 ± 5.8 (n = 20) (P = 0.007),

### Table 1: Characteristics of 6 SNP in the FADS1 FADS2 gene cluster

<table>
<thead>
<tr>
<th>dbSNP</th>
<th>Position (bp)</th>
<th>Gene</th>
<th>Allele</th>
<th>Genotype</th>
<th>Allele</th>
<th>H-W^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs174553</td>
<td>61331734</td>
<td>FADS1 intergenic</td>
<td>A/G</td>
<td>M/M</td>
<td>M</td>
<td>0.185</td>
</tr>
<tr>
<td>Rs174561</td>
<td>6139284</td>
<td>FADS1 intron</td>
<td>T/C</td>
<td>M/m</td>
<td>m</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rs99780</td>
<td>61353209</td>
<td>FADS2 intron</td>
<td>C/T</td>
<td>M/m</td>
<td>m/m</td>
<td>0.019</td>
</tr>
<tr>
<td>Rs174575</td>
<td>61358579</td>
<td>FADS2 intron</td>
<td>C/G</td>
<td>M</td>
<td>m</td>
<td>0.97</td>
</tr>
<tr>
<td>Rs174583</td>
<td>61366326</td>
<td>FADS2 intron</td>
<td>C/T</td>
<td>M/m</td>
<td>m/m</td>
<td>0.185</td>
</tr>
<tr>
<td>Rs986793</td>
<td>6138281</td>
<td>FADS2 intron</td>
<td>C/T</td>
<td>M/m</td>
<td>m/m</td>
<td>0.005</td>
</tr>
</tbody>
</table>

1 The position in base pairs was derived from NCBI dbSNP Build 128.

2 The number of women carrying M or m, the major and minor alleles, and carrying M/M, M/m, or m/m genotypes, respectively.

3 H-W, Hardy-Weinberg equilibrium.

### Table 2: Major (n-6) and (n-3) fatty acids in plasma phospholipids of pregnant women classified by rs174553 genotype

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>A/A, n = 20</th>
<th>A/G, n = 29</th>
<th>G/G, n = 20</th>
<th>P^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>19.7 ± 2.19</td>
<td>20.2 ± 3.26</td>
<td>23.3 ± 2.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20:2(n-6)</td>
<td>0.52 ± 0.10</td>
<td>0.97 ± 0.12</td>
<td>0.83 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>3.93 ± 0.66</td>
<td>4.08 ± 0.75</td>
<td>4.41 ± 0.93</td>
<td>ns^3</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>11.6 ± 1.19</td>
<td>10.4 ± 1.28</td>
<td>8.30 ± 1.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>22:6(n-6)</td>
<td>0.64 ± 0.29</td>
<td>0.65 ± 0.48</td>
<td>0.48 ± 0.15</td>
<td>ns</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.72 ± 0.16</td>
<td>0.61 ± 0.22</td>
<td>0.51 ± 0.16</td>
<td>0.003</td>
</tr>
<tr>
<td>18:3(n-3)</td>
<td>0.35 ± 0.15</td>
<td>0.35 ± 0.11</td>
<td>0.43 ± 0.13</td>
<td>ns</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.92 ± 0.51</td>
<td>1.34 ± 1.85</td>
<td>0.63 ± 0.34</td>
<td>ns</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>1.04 ± 0.24</td>
<td>0.87 ± 0.25</td>
<td>0.80 ± 0.17</td>
<td>0.005</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>5.65 ± 1.17</td>
<td>5.27 ± 1.50</td>
<td>4.65 ± 1.54</td>
<td>ns</td>
</tr>
</tbody>
</table>

1 Values are means ± SD determined at 16 wk gestation.

2 Data were analyzed using the Kruskal Wallis Test; a post hoc test for differences among groups was not conducted. Statistical differences in fatty acids for the women grouped by rs99780 or rs174583 allele did not differ.

3 ns, P > 0.05.
respectively, at 16 wk of gestation. For the major allele carriers
\((n = 61)\) and minor allele homozygotes \((n = 8)\) of rs174575, the
\((n-6)\) fatty acid desaturation product:precursor ratios were \(11.0 \pm 1.7\) and \(9.0 \pm 1.5\), respectively \((P = 0.005)\), and for the \((n-3)\) fatty acids, the desaturation product:ALA ratios were \(19.0 \pm 10.5\) and
\(13.2 \pm 5.1\) \((P = 0.119)\), respectively. The results thus indicate a
robust association between the minor alleles of the 4 SNP we
analyzed and lower levels of ARA and other long chain \((n-6)\) fatty
acids relative to the precursor, LA. Similar results were found
for the \((n-3)\) fatty acids, which were significant for rs174553,
rs99780, and rs174583 but not for rs174575 \((P = 0.119)\). Similar
results were also found for the relationship between SNP and
plasma phospholipid fatty acids at 36 wk and those at 16 wk of
gestation, but based on our sample size, analyses for interactions
between genotype and stage of gestation in influencing maternal
fatty acid status were inappropriate.

The levels of the major \((n-6)\) and \((n-3)\) fatty acids in the
erthrocyte EPG differed among the women classified by the
rs174553 allele \((n = 56)\) and rs99780 \((n = 99)\) at 16 wk of
gestation (Table 3). We found a significant effect of
genotype on the erythrocyte EPG levels of LA, 20:3\((n-6)\), ARA,
and 22:4\((n-6)\), with LA being higher and its desaturation
products being lower in minor allele carriers. The \((n-3)\) fatty
acids did not differ in the erythrocyte EPG of the women grouped
by rs174553 allele. Calculation of the \(\Delta 6\) and \(\Delta 5\) desaturase
products to their LA precursor ratio showed that the ratio was
lower, suggesting less desaturation, for the erythrocyte EPA fatty
acids of the minor allele homozygotes than major allele carriers
of rs174553 \((n = 56)\) (or rs99780 or rs174583) at both 16 wk \((P < 0.001)\)
and 36 wk \((P = 0.001)\) of gestation, with a similar pattern for the
\((n-3)\) series of fatty acids at wk 16 \((P = 0.086)\) and 36 \((P = 0.046)\)
(Table 4). For rs174575, the levels of individual \((n-6)\) and \((n-3)\)
fatty acids did not differ in the erythrocyte EPA \((\text{data not shown})\)
or in the ratio of 20 and 22 carbon chain \((n-6)\) or \((n-3)\) fatty
acids to their LA or ALA precursor, respectively, between the major
allele carriers and minor allele homozygotes at either 16 or 36 wk
of gestation (Table 4).

The analysis of breast milk fatty acid provides new data to
show that genetic variation in the FADS1 FADS2 gene cluster is
important to the composition of fatty acids provided to breast-fed
infants in mothers’ milk (Tables 5 and 6). The medium chain fatty
acid 14:0, which is the end product of the de novo fatty acid

\[\text{TABLE 3} \quad \text{Major (n-6) and (n-3) fatty acids in RBC EPG of pregnant women classified by rs174553 genotype}^{1}\]

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>A/A, n = 20</th>
<th>A/G, n = 29</th>
<th>G/G, n = 20</th>
<th>(\text{g/100 g fatty acids}^{2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2((n-6))</td>
<td>5.42 ± 0.56</td>
<td>5.60 ± 0.99</td>
<td>6.52 ± 1.39</td>
<td>0.007</td>
</tr>
<tr>
<td>20:2((n-6))</td>
<td>0.29 ± 0.09</td>
<td>0.37 ± 0.11</td>
<td>0.35 ± 0.09</td>
<td>(\text{ns}^{3})</td>
</tr>
<tr>
<td>20:3((n-6))</td>
<td>1.16 ± 0.14</td>
<td>1.43 ± 0.29</td>
<td>1.85 ± 0.60</td>
<td>(\text{ns}^{3})</td>
</tr>
<tr>
<td>20:4((n-6))</td>
<td>23.4 ± 1.75</td>
<td>22.4 ± 2.19</td>
<td>21.2 ± 1.72</td>
<td>0.003</td>
</tr>
<tr>
<td>22:4((n-6))</td>
<td>7.68 ± 1.38</td>
<td>7.18 ± 1.29</td>
<td>6.65 ± 1.51</td>
<td>0.038</td>
</tr>
<tr>
<td>22:5((n-6))</td>
<td>0.88 ± 0.29</td>
<td>0.75 ± 0.35</td>
<td>0.83 ± 0.20</td>
<td>(\text{ns})</td>
</tr>
<tr>
<td>18:3((n-3))</td>
<td>0.33 ± 0.07</td>
<td>0.33 ± 0.09</td>
<td>0.38 ± 0.15</td>
<td>(\text{ns})</td>
</tr>
<tr>
<td>20:3((n-3))</td>
<td>0.93 ± 0.29</td>
<td>1.07 ± 0.72</td>
<td>0.93 ± 0.39</td>
<td>(\text{ns})</td>
</tr>
<tr>
<td>22:5((n-3))</td>
<td>4.21 ± 0.75</td>
<td>4.06 ± 0.89</td>
<td>3.78 ± 0.51</td>
<td>(\text{ns})</td>
</tr>
<tr>
<td>22:6((n-3))</td>
<td>7.79 ± 1.67</td>
<td>8.18 ± 2.12</td>
<td>7.61 ± 2.45</td>
<td>(\text{ns})</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SD determined at 16 wk gestation.
\(^2\) Data were analyzed using the Krukal Wallis Test; a post hoc test for differences among groups was not conducted. Statistical differences in fatty acids for the women grouped by rs99780 or rs174583 allele did not differ.
\(^3\) ns, \(P > 0.05\).
TABLE 6 Major fatty acids in breast milk from women classified by rs174575 genotype

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>C/C, n = 25</th>
<th>C/G, n = 23</th>
<th>G/G, n = 6</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/100 g fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>0.87 ± 0.35</td>
<td>0.82 ± 0.42</td>
<td>0.85 ± 0.31</td>
<td>ns³</td>
</tr>
<tr>
<td>12:0</td>
<td>5.11 ± 2.35</td>
<td>4.24 ± 1.60</td>
<td>4.01 ± 2.10</td>
<td>ns</td>
</tr>
<tr>
<td>14:0</td>
<td>6.29 ± 2.47</td>
<td>5.30 ± 2.02</td>
<td>5.89 ± 0.94</td>
<td>ns</td>
</tr>
<tr>
<td>16:0</td>
<td>19.59 ± 2.60</td>
<td>19.71 ± 1.96</td>
<td>18.63 ± 2.33</td>
<td>ns</td>
</tr>
<tr>
<td>18:0</td>
<td>6.34 ± 1.21</td>
<td>6.00 ± 0.81</td>
<td>5.94 ± 0.95</td>
<td>ns</td>
</tr>
<tr>
<td>18:1(n-7)</td>
<td>2.18 ± 0.54</td>
<td>2.54 ± 0.56</td>
<td>2.58 ± 0.72</td>
<td>ns</td>
</tr>
<tr>
<td>18:2(n-6)</td>
<td>1.90 ± 0.33</td>
<td>2.07 ± 0.29</td>
<td>2.19 ± 0.29</td>
<td>0.035</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>13.7 ± 2.89</td>
<td>13.0 ± 2.60</td>
<td>14.8 ± 2.60</td>
<td>ns</td>
</tr>
<tr>
<td>18:4(n-3)</td>
<td>0.36 ± 0.07</td>
<td>0.34 ± 0.07</td>
<td>0.41 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>18:3(n-6)</td>
<td>0.10 ± 0.05</td>
<td>0.11 ± 0.04</td>
<td>0.10 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>20:3(n-6)</td>
<td>0.37 ± 0.10</td>
<td>0.37 ± 0.11</td>
<td>0.42 ± 0.11</td>
<td>ns</td>
</tr>
<tr>
<td>20:4(n-6)</td>
<td>0.43 ± 0.09</td>
<td>0.42 ± 0.08</td>
<td>0.33 ± 0.04</td>
<td>0.015</td>
</tr>
<tr>
<td>22:4(n-6)</td>
<td>0.07 ± 0.03</td>
<td>0.08 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>ns</td>
</tr>
<tr>
<td>22:5(n-6)</td>
<td>0.02 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.011</td>
</tr>
<tr>
<td>18:3(n-9)</td>
<td>1.65 ± 0.62</td>
<td>1.59 ± 0.54</td>
<td>1.54 ± 0.60</td>
<td>ns</td>
</tr>
<tr>
<td>20:5(n-3)</td>
<td>0.10 ± 0.08</td>
<td>0.07 ± 0.03</td>
<td>0.04 ± 0.02</td>
<td>0.011</td>
</tr>
<tr>
<td>22:5(n-3)</td>
<td>0.16 ± 0.07</td>
<td>0.14 ± 0.04</td>
<td>0.09 ± 0.02</td>
<td>0.003</td>
</tr>
<tr>
<td>22:6(n-3)</td>
<td>0.32 ± 0.25</td>
<td>0.24 ± 0.11</td>
<td>0.16 ± 0.07</td>
<td>0.044</td>
</tr>
</tbody>
</table>

¹ Values are means ± SD in women exclusively breast-feeding their term gestation infants at 1 mo postpartum.
² Data were analyzed using the Kruskal Wallis Test; a post hoc test for differences among groups was not conducted. Statistical differences in fatty acids for the women grouped by rs99780 or rs174583 allele did not differ from those shown.
³ ns, P > 0.05.

Discussion

In the present study, among healthy women of a predominantly Caucasian background, common genetic variations in the genes encoding for the Δ6 and Δ5 desaturases are associated with differences in (n-6) and (n-3) fatty acids in plasma and erythrocyte membrane lipids in gestation and in breast milk during lactation. We provide results to show that ARA was the lowest, and its precursors LA was highest, in minor allele homozygotes of rs174553 (GG), rs99780 (TT), and rs174583 (TT) in both plasma phospholipids and erythrocyte EPG (Fig. 1). A similar pattern was found for the (n-3) fatty acids, with higher LA and lower levels of its products in plasma phospholipids of women homzygous for the minor allele of these 3 SNP. We also show that genetic variations in the FADS1 FADS2 gene cluster are associated with differences in the saturated, monounsaturated, and (n-6) and (n-3) fatty acid composition of human milk, with the differences in (n-6) and (n-3) fatty acids similar to those in the maternal plasma phospholipids. As in the mothers’ plasma phospholipids, the minor alleles of rs174553, rs99780, and rs174583 were associated with lower milk levels of the 20- and 22-carbon chain (n-6) and (n-3) fatty acid desaturation products. Furthermore, the levels of ARA, as well as the levels of all of the long-chain (n-3) fatty acids, EPA, 22:5(n-3), and DHA, were lower in breast milk of women homozygous for the minor allele of rs174575, also reflecting differences in the (n-6) and (n-3) FADS product:precursor ratios in the maternal plasma phospholipids, although not in the erythrocyte EPG.

Recently, Malerba et al. (32) reported SNP association analysis with serum phospholipid and erythrocyte total lipid fatty aids in Northern Italians and found significant associations between lower circulating ARA and minor allele variants in the region of rs174545 up to rs174570 and higher circulating ALA in the minor allele carriers of rs174545, rs3834458, and rs174583.

Another study of European adults reported higher levels of LA and lower ARA, as well as higher ALA and lower EPA and 22:5(n-3), among carriers of minor variants of SNP in the FADS1 FADS2 gene cluster (31). Our results are similar, with lower plasma phospholipid and erythrocyte EPG ARA but higher LA in minor allele carriers of rs174553, rs99780, and rs174583 among pregnant women in Canada. Our work also extends the latter findings to show lower ratios of carbon chain 20 and 22 (n-6) and (n-3) fatty acids to LA and ALA, respectively, among the minor allele carriers of these SNP, as well as rs174575, suggesting less desaturation of dietary LA and ALA among the minor allele variants. Previous studies have shown a positive correlation between maternal and fetal (newborn) cord plasma phospholipid (n-6) and (n-3) fatty acids (24), and increasing the maternal intake and blood levels of DHA in gestation is known to increase maternal to fetal DHA transfer, which has also been associated with increased maturation of visual and neural systems (20,25,26,29). Higher ARA, on the other hand, is positively associated with fetal growth (22–24). Whether differences in maternal blood levels of (n-6) and (n-3) fatty acids due to genetic variations in fatty acids metabolism or diet-gene interactions contribute to differences in placental fatty acid transfer and have relevance to fetal growth and development may be worth consideration.

Human milk provides a complex array of fatty acids that contribute energy and the essential (n-6) and (n-3) fatty acids to support the growth and development of the breast-fed infant (21). To our knowledge, the effect of genetic variations in FADS on the quality of human milk fatty acids has not been reported previously, although both Δ6 and Δ5 desaturase are present in the lactating mammary gland (37). The role of medium-chain fatty acids in human milk and a biological explanation as to why the mammatory fatty acid synthase complex truncates fatty acid synthesis at 14:0, rather than 16:0, as in the liver is not well understood (21). Early studies, however, suggested an important role for ketones derived from medium-chain fatty acid oxidation in sparing glucose and as a lipid precursor (38). In the latter context, we suggest that medium-chain fatty acid oxidation provides a source of malonyl CoA, which is important because lipogenesis is suppressed when a high-fat milk diet is fed yet is needed for cholesterol synthesis and fatty acid chain elongation. Our results provide novel findings for the rs174553, rs99780, and rs174583 loci to show that lower breast milk concentrations of 14:0 occur concomitantly with higher Δ9 desaturase products and LA and 20:2(n-6), suggesting that minor allele variants of these SNP have decreased endogenous fatty acid synthesis and lower Δ6 and Δ5 desaturation activities, with increased diversion of LA to 20:2(n-6) (Fig. 1). Whether this reflects differences in lipogenesis and desaturation activities in the mammary gland or...
differences in substrate flux from other organs, such as the liver, and any implications for milk total fat or metabolism in the breast-fed infant cannot be inferred from the present study, but seems worthy of more detailed study.

ARA is esterified to the sn-2 position of membrane phospholipids and is released to provide unesterified ARA for further metabolism to eicosanoids, which are important in many aspects of growth and development, including synaptic transmission and plasticity, development of the digestive tract epithelium, and immune and inflammatory responses (9,10,12–16,30). Our studies, consistent with previous studies in adults (31,32), confirm that minor allele variants of common SNP in FADS1 and FADS2 are associated with lower circulating lipid ARA, which in our study extended to similar effects in pregnant women and to the secretion of ARA in milk during lactation. Whether differences in maternal-to-infant transfer of ARA in breast milk due to genetic variation in maternal FADS are relevant to the young infant is not known. However, considerable attention has been given to the variability in DHA in human milk and the possibility that low milk levels of DHA may compromise early infant visual and neural system development (1,21,27,28,39–41). Observation and intervention studies suggest better visual and neural system development among infants receiving breast milk with >0.32 compared with 0.2 g DHA/100 g fatty acids or less (27,28,41). The present study provides new data to suggest that in addition to dietary DHA intake, genetically determined variations in FADS2 and FADS1 may influence the secretion of DHA in breast milk over a range that appears to have functional importance to the developing infant. In this regard, a recent study of large birth cohorts reported that children carrying the G/G allele in rs174575 had lower IQ scores than those carrying the C allele (33).

In summary, we have provided new evidence that genetic variation in FADS1 and FADS2 influence maternal plasma and erythrocyte phospholipid levels of (n-6) and (n-3) fatty acids during pregnancy and levels of saturated, monounsaturated, and (n-6) and (n-3) fatty acids in breast milk during lactation. Our results suggest that genetic variation among women may influence maternal-to-infant transfer of fatty acids during pregnancy and in lactation will, via breast milk, influence fatty acid nutrition of the breast-fed infant. The extent to which maternal SNP in FADS1 and FADS2 interact with the maternal dietary fatty acid composition to contribute to maternal health and early infant growth and development need to be considered in addressing those fatty acid requirements that best support human growth and development.

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Literature Cited