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Docosahexaenoic acid (DHA) supplementation in pregnancy differentially modulates arachidonic acid and DHA status across FADS genotypes in pregnancy^{1–3}

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Abstract

Some FADS alleles are associated with lower DHA and ARA status assessed by the relative amount of arachidonic acid (ARA) and docosahexaenoic acid (DHA) in plasma and red blood cell (RBC) phospholipids (PL). We determined two FADS single nucleotide polymorphisms (SNPs) in a cohort of pregnant women and examined the relationship of FADS1rs174533 and FADS2rs174575 to DHA and ARA status before and after supplementation with 600 mg per day of DHA. The 205 pregnant women studied were randomly assigned to placebo (mixed soy and corn oil) (n= 96) or 600 mg algal DHA (n=109) in 3 capsules per day for the last two trimesters of pregnancy. Women homozygous for the minor allele of FADS1rs174533 (but not FADS2rs174575) had lower DHA and ARA status at baseline. At delivery, minor allele homozygotes of FADS1rs174533 in the placebo group had lower RBC-DHA compared to major-allele carriers ($P = 0.031$), while in the DHA-supplemented group, all genotypes had higher DHA status compared to baseline ($P = 0.001$) and status did not differ by genotype ($P = 0.941$). Surprisingly, DHA but not the placebo decreased ARA status of minor allele homozygotes of both FADS SNPs but not major allele homozygotes at delivery. Any physiological effects of changing the DHA to ARA ratio by increasing DHA intake appears to be greater in minor allele homozygotes of some FADS SNPs.

Keywords

arachidonic acid; docosahexaenoic acid; FADS SNPs; pregnancy

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Introduction

The long chain polyunsaturated fatty acids, docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) are important constituents of neural tissue that play an important role in cognitive and visual development [1]. While ARA and DHA can be provided directly by the diet from animal fats, they may also be synthesized endogenously from their essential dietary precursors, linoleic acid (18:2 n-6) and α -linolenic acid (18:3n-3), respectively. The conversion pathway consists of a succession of desaturations and elongations, and two key enzymes, Δ -5 and Δ -6 desaturase (encoded by FADS1 and FADS2, respectively) govern their rate of synthesis. FADS1 and 2 are located in a cluster on chromosome 11 (11q12-13.1) with head-to-head orientation. Both Δ -5 and Δ -6 desaturase are expressed in the majority of human tissues, but the highest expression is found in the liver, brain, heart, and lung [2, 3].

Several studies have demonstrated that specific single nucleotide polymorphisms (SNPs) in FADS1 and 2 influence plasma and RBC fatty acid composition in infants and adults with minor allele carriers having lower product to precursor ratios [4–12]. While some studies have also shown reduced proportions of ARA and DHA in plasma and RBC PL [5–7], others have not found an association between FADS SNPs and these long-chain metabolites, especially DHA [5, 13, 14]. To our knowledge, previous studies have not examined the interaction between DHA and ARA status and FADS alleles after DHA supplementation although Cormier et al. [15] reported a FADS genotype predicted the effect of fish oil supplementation on serum triacylglycerol concentration. The goals of our study were a) to determine DHA and ARA status using RBC-PL-DHA and ARA as biomarkers in a population of pregnant, low fish consumers and b) to determine the effect of DHA supplementation on RBC-PL-DHA and ARA across FADS1rs174553 and FADS2 rs174575 genotypes. FADS1rs174553 and FADS2rs174575 SNPs were selected among those studied previously due to their relatively common minor allele frequencies (33% and 24%, respectively) and previously observed association with blood lipid and breast milk DHA [5, 6, 8].

Subjects and Methods

Subjects

The study population was a subset of 205 women (placebo, n= 96; DHA, n=109) who enrolled in an NICHD-funded Phase-III clinical trial (NCT00266825) to determine the effects of consuming 600 mg/day of DHA throughout gestation on maternal and infant/toddler outcomes. Women (n=208) whose children remained in follow-up after birth were asked to consent for genotype testing. Of those, one woman refused consent and 2 did not have a postpartum blood sample leaving a final sample of 205. The demographic characteristics of the women who enrolled for the larger study have been published [16]. Women were eligible for the primary study if they were English-speaking, between 16 to 35.99 years of age, and between the 8th and 20th week of gestation. Subjects were excluded if they were expecting multiple infants or had any serious health condition likely to affect the growth and development of their fetus or the postnatal growth and development of their

newborn infants. These included, but were not limited to cancer, lupus, hepatitis, HIV/AIDS, and pre-pregnancy or gestational diabetes mellitus.

Women were also excluded if they had a baseline BMI ≥ 40 or systolic blood pressure ≥ 140 mm Hg as morbid obesity and elevated blood pressure present a high risk for obstetric complications. The research protocol and informed consent forms adhered to the Declaration of Helsinki (including the October 1996 amendment) and were approved by the Institutional Review Board/ethics committee at the participating institution, the University of Kansas Medical Center (HSC #10186).

The demographic characteristics of the subset studied are shown by group in Table 1. Women enrolled in the study answered questions about their intake of foods that contained DHA and about their supplement intake at baseline. In general, they were low consumers of food sources of DHA and were not consuming DHA supplements at baseline. Low consumption is consistent with the low DHA status of women when they began the study: baseline RBC-PL-DHA was 4.3 ± 1.2 (mean \pm SD) (Table 1). Compared to the subset of women who did not provide a DNA sample, those included in the present analysis were older, had achieved a higher level of education at enrollment, and were more likely to be Caucasian (all $P < 0.001$). They also consumed, on average, a greater number of capsules per week ($P < 0.001$) (data not shown). A description of women enrolled in the primary study has been reported [16].

Supplementation

Women were randomly assigned to capsules of a marine algal oil source of DHA or capsules containing half soybean and half corn oil (DSM, formerly Martek Biosciences, Columbia, MD) at a mean of 14.5 weeks gestation [16]. The fatty acid composition and other details about the capsules are shown in Table 2. All subjects were asked to consume three of their assigned capsules daily from enrollment until they delivered their baby. DSM Nutritional Products donated the capsules for the study but had no role in the study design, analysis, interpretation or dissemination. The University of Kansas Medical Center Investigational Pharmacy mailed placebo or DHA capsules to women in accordance with a randomization schedule provided by a biostatistician. The Pharmacy maintained a record of returned capsules. The weekly and overall capsule intake of each subject was calculated at the end of the treatment phase [16].

Analysis of red blood cell phospholipid DHA and ARA

Women provided a blood sample at baseline and before breakfast on the morning following birth. Blood samples were collected by venipuncture into 4 mL K₂EDTA tubes (BD Vacutainer, Franklin Lakes, NJ). Plasma and RBC were separated by centrifugation ($3000 \times g$, 10 minutes; $4^{\circ}C$), frozen, and stored under nitrogen at $-80^{\circ}C$ until analysis. Lipids were isolated according to a modification of Folch et al. [17], and RBC lipids were fractionated [18] by thin-layer chromatography. RBC phospholipids were transmethylated with boron trifluoride-methanol [19], and the resulting fatty acid methyl esters (FAME) were separated using a Varian 3900 gas chromatograph with an SP-2560 capillary column (100 m; Sigma Aldrich) and a Star 6.41 Chromatography Workstation for peak integration

and analysis as previously reported [20]. Injector and detector temperatures were programmed at 260°C. The temperature program for the 41-minute column run was: 140°C, 5 minutes; 4°C increase/minute to 240°C; 240°C, 11 minutes. Individual peaks were identified by comparison with a qualitative standard (PUFA No. 1 Marine Source 100 mg; PUFA No. 2 Animal Source 100 mg; Sigma Aldrich) and a weighed standard mixture (Supelco 37 Component FAME mix, Sigma Aldrich) was employed to adjust area percent to weight percent.

Genotyping

Genomic DNA was extracted from buccal collection brushes using the Genra Puregene Buccal Cell Kit (QIAGEN, Hilden, Germany), and genotyping was performed with made-to-order TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA) using real-time polymerase chain reaction. Five-microliter total reactions were prepared according to manufacturer instructions, and individual genotypes were determined with StepOne Software (Version 2.0; Applied Biosystems). Hardy –Weinberg equilibrium was assessed using chi-square analysis. Both FADS SNPs evaluated were in Hardy-Weinberg Equilibrium ($P = 0.62$ and $P=0.73$ for FADS1rs17553 and FADS2rs174575, respectively). Call rate was 100%. The observed genotypic and minor allele frequencies for each SNP are shown in Table 3.

Statistical analysis

One way ANOVA was used to compare RBC-DHA and ARA across maternal FADS genotypes in samples collected at baseline and delivery. When indicated, Fisher's Least Significant Difference (LSD) was used to conduct pairwise comparisons. All data were analyzed with SPSS Statistics 17.0 software (SPSS, Chicago, IL), and P -values ≤ 0.05 were considered significant.

Although the frequency of FADS minor alleles differs between individuals of European and African descent [21, 22], race was not included as a covariate in the present analyses. Neither did we include other covariates as these would have introduced multicollinearity into the model and dramatically reduced our power to observe differences in RBC-DHA and ARA across maternal genotypes. Preliminary analyses were performed to evaluate the homogeneity-of-regression (slopes) assumption. The normality and homogeneity of variance assumptions were satisfied, and the preliminary analysis evaluating the homogeneity-of-regression (slope) assumption indicated that the relationship between average weekly capsule intake and postpartum RBC-DHA and ARA did not differ significantly as a function of genotype ($P = 0.421$ and 0.519 for FADS1 DHA and ARA, respectively; $P = 0.449$ and 0.827 for FADS2 DHA and ARA, respectively) so no adjustment was made for capsule compliance.

Results

Enrollment

Carlson et al (2013) reported significant effects of prenatal supplementation on DHA with this sample; this observation held true for the subset of the sample for whom SNP analyses

were conducted; the two groups did not vary statistically at enrollment (placebo: 4.30%, SD = 1.25; supplemented: 4.43%, SD = 1.12), but did vary ($P < 0.001$) at delivery (placebo: 4.71%, SD = 1.19; supplemented: 7.54%, SD = 2.10). Means for all subsequent analyses of FADS genotypes as a function of group assignment are presented in Table 4.

FADS1—At enrollment, the FADS1 genotype was significantly related to DHA ($P = 0.034$); mothers with two minor alleles showed more than 20% less DHA than mothers with either one ($P = 0.028$) or two ($P = 0.009$) major alleles. The FADS1 genotype was similarly related to ARA ($P = 0.002$), as mothers with two minor alleles showed significantly less ARA than mothers with either one ($P = 0.014$) or two ($P = 0.002$) major alleles.

FADS2—The FADS2 genotype was not significantly associated with differences in either DHA ($P = 0.167$) or ARA ($P = 0.237$) at enrollment.

At baseline, FADS1rs174553 genotype was significantly related to both RBC-DHA ($P = 0.035$) and ARA ($P = 0.002$) (Table 4). Specifically, minor allele homozygotes had a lower proportion of RBC-DHA than major allele homozygotes and heterozygotes ($P = 0.010$ and 0.027 , respectively), and minor-allele carriers had a lower proportion of RBC-ARA than major allele homozygotes ($P = 0.009$ and 0.003 for A/G and G/G, respectively). FADS2 rs174575 genotype was unrelated to RBC-DHA ($P = 0.164$) or ARA ($P = 0.300$) at enrollment (Table 4).

Delivery

FADS1—As expected, mothers in the placebo group with the FADS1 genotype showed the pattern of DHA and ARA at the time they delivered their infants seen at enrollment. DHA was again significantly lower ($P = 0.018$) in individuals with two minor alleles than individuals with either one ($P = 0.030$) or two ($P = 0.005$) major alleles; thus, in mothers not receiving supplementation, genetic determination of DHA levels was sustained. ARA levels in mothers in the placebo group with the FADS1 genotype were also unaffected ($P = 0.252$).

In DHA supplemented mothers, genotype was not significantly associated with DHA levels at delivery ($P = 0.954$). However, genotype significantly affected ARA ($P < 0.001$) in mothers at delivery in the supplemented group. Mothers with two minor alleles had significantly lower ARA than mothers with either one ($P = 0.004$) or two ($P < 0.001$) major alleles; in addition, heterozygous mothers had significantly ($P = 0.038$) lower ARA than mothers homozygous for major alleles. Thus, the lower DHA levels seen at enrollment in mothers who were homozygous for minor FADS1 alleles were successfully remediated by supplementation, but prenatal supplementation was associated with a significant drop in blood ARA levels at delivery.

FADS2—The FADS2 genotype was not associated with differences in DHA levels in mothers in the placebo ($P = 0.404$) or supplemented ($P = 0.573$) groups at delivery. In addition, there were no significant differences in ARA levels in mothers as a function of FADS2 genotype at delivery ($P = 0.852$). However, FADS2 was significantly associated with ARA levels at delivery in the supplemented group ($P = 0.049$); as with the FADS1

group, mothers homozygous for FADS2 minor alleles had lower ARA than mothers with either one ($P = 0.033$) or two ($P = 0.015$) major alleles.

Discussion

To our knowledge, this study is the first to examine the interaction among FADS1rs174553 and FADS2rs1747575 genotypes for DHA and ARA status in persons randomly assigned to DHA supplementation. As expected women supplemented with 600 mg DHA per day for the last two trimesters of pregnancy (approximately 26 weeks) had significantly higher DHA status (RBC-PL-DHA) at birth compared to placebo and compared to baseline. The lower DHA status of FADS1rs174553 minor allele homozygotes observed at baseline and in the placebo group at birth was not observed after DHA supplementation.

Previous studies regarding the influence of FADS alleles of various SNPs on ARA and DHA status have produced conflicting results. While some find lower proportions of ARA and DHA in plasma and RBC phospholipids [5–7], others do not find an association between FADS SNPs and ARA and DHA, especially DHA [7, 13]. For example, Xie and Innis [8] observed a significantly lower proportion of RBC-PL-ARA, but not DHA in minor allele homozygotes for the same FADS1 SNP studied here (rs174553). Interestingly, after DHA supplementation we also find no difference in DHA status but a significant difference in ARA status in women carrying the minor allele. Because Xie and Innis were studying women consuming their usual diet, a possible explanation for our differences is that our population has a lower usual DHA status than theirs. Indeed this is suggested by comparing RBC-PL-DHA of our cohorts at baseline (4.3% by weight of total fatty acids compared to approximately 7.9% in their subjects). The conflicting results in the literature may be due to differences in DHA intake among populations. Our data suggest that in populations consuming little DHA, minor allele carriers of some FADS SNPs could exhibit lower DHA and perhaps lower ARA, while those consuming more DHA may exhibit only lower ARA status. Indeed, the mean RBC-PL-DHA (weight %) in the population examined by Xie and Innis (7.9%) is similar to that observed in our cohort after DHA supplementation (7.5%) (Table 1).

It is generally accepted that ARA status is reduced by DHA supplementation, for example see [23–26]. Our results suggest this effect may be explained by a reduction in ARA status in individuals carrying some minor alleles of FADS1 and FADS2. Major-allele homozygotes of the two FADS SNPs studied here had similar ARA status regardless of whether they were assigned to the placebo or DHA group. Gillingham *et al.* [13] found lower plasma ARA in FADS1 (rs174561 and rs174537) and FADS2 (rs174583 and rs174545) minor-allele carriers consuming a diet enriched in the precursor of DHA, α -linolenic acid. The mechanism by which DHA reduces ARA status is likely different. α -Linolenic acid may compete with linoleic acid for the enzymes of elongation and desaturation, whereas the reduction in ARA with DHA supplementation may be due to a negative feedback of the product of elongation and desaturation. Studies in both rats and preterm infants show diminished FADS expression [27] and endogenous LCPUFA synthesis [28, 29] when diets containing LCPUFA are provided, Individuals susceptible to impaired enzymatic function and/or transcription (FADS minor-allele carriers) may be prone to

further reductions in endogenous LCPUFA production with either increased α -linolenic acid or DHA intake.

A limitation of this study is that we used a sample of convenience from a trial powered to examine the influence of prenatal DHA supplementation on birth outcomes. Relatively few women were homozygous for the minor allele of the genes examined so small sample size is a potential concern. However, we did find statistically significant effects of DHA supplementation on ARA status of homozygote minor alleles for both SNPs studied. We also found a significant reduction in ARA status in the larger group of FADS1 allele heterozygotes (n=42). FADS 2 heterozygotes (n=44) trended lower. We do not consider the potential conversion of α -linolenic acid to DHA in the placebo group to be a limitation of the study. Consumption of 3 placebo capsules provided approximately 60 mg of α -linolenic acid, which is a precursor for DHA that could theoretically result in approximately 10 mg of DHA per day [30], an amount far below that provided by the treatment capsules (600 mg DHA/day).

In summary, we studied a group of pregnant women who had low DHA status and found that their DHA and ARA status at baseline were significantly lower if they were homozygous for the minor allele of FADS1rs174533 compared to women with at least one copy of the major allele. After DHA supplementation, DHA status increased and the genotype difference was lost. At the same time, ARA status was decreased by DHA supplementation in women homozygous for the minor allele of both FADS1rs174533 and FADS2rs1747575. Our data suggest that variations in DHA intake in populations could be related to the variability of DHA and ARA status in minor allele carriers observed among studies. In addition, DHA supplementation appears to have a larger impact on the DHA to ARA ratio in persons homozygous for the minor alleles of both FADS SNPs studied compared to persons carrying one or more major allele. The physiological significance of this effect on the DHA to ARA ratio, if any, is unknown.

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List of abbreviations

ARA	arachidonic acid
DHA	docosahexaenoic acid
M/m	major/minor alleles
MAF	minor allele frequency
RBCs	red blood cells
SNP	single nucleotide polymorphism

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Highlights

- RBC DHA and ARA are lower in women homozygous for the minor allele of FADS1rs174533
- DHA and ARA status were not significantly influenced by FADS2rs174575 genotype
- DHA supplementation during pregnancy resulted in higher RBC DHA in all genotypes
- DHA supplementation reduced ARA status in minor allele homozygotes of both genotypes
- DHA supplementation had no effect on ARA status of major allele homozygotes

Table 1Characteristics of pregnant women by group¹

	Placebo n = 96	DHA n = 109	P-value
Age (year \pm SD)	26.3 \pm 4.6	26.4 \pm 4.3	NS
Education (years \pm SD)	14.3 \pm 2.8	14.3 \pm 2.6	NS
Baseline BMI	26.8 \pm 4.9	27.8 \pm 5.3	NS
Baseline RBC PL DHA (%)	4.3 \pm 1.2	4.4 \pm 1.1	NS
Baseline RBC PL ARA (%)	15.0 \pm 2.2	14.9 \pm 1.9	NS
Postpartum RBC PL DHA (%)	4.7 \pm 1.2	7.5 \pm 2.1	P=0.001
Postpartum RBC PL ARA (%)	13.2 \pm 1.6	12.3 \pm 2.1	NS
Cord blood RBC PL DHA (%)	5.9 \pm 1.5	7.5 \pm 1.7	P=0.001
Cord blood RBC PL ARA (%)	16.8 \pm 2.2	16.2 \pm 2.7	NS
Total study capsule intake (#)	17.3 \pm 4.1	17.5 \pm 4.0	NS
Fish intake (servings/wk)	1.5 \pm 1.8	1.4 \pm 1.4	NS

¹Values were analyzed using Student's *t*-test; NS, not significant

Table 2Capsule fatty acid composition¹

Fatty acid profile (weight percent)	Placebo	DHA
Caprylic acid (8:0) ²	1.5	None
Capric acid (10:0)	0.9	2.1
Lauric acid (12:0)	<0.1	5.4
Myristic acid (14:0)	<0.1	15.5
Palmitic acid (16:0)	10.6	14.6
Palmitoleic acid (16:1)	<0.1	1.6
Stearic acid (18:0)	4.6	0.6
Oleic acid (18:1n-9)	21.4	14.9
Vaccenic acid (18:1n-7c)	1.5	<0.1
Linoleic acid (18:2n-6)	51.2	0.8
Gamma-linolenic acid (18:3n-6)	0.4	<0.1
Alpha-linolenic acid (18:3n-3)	6.3	1.4
Arachidic acid (20:0)	0.4	5.0
Dihomo-gamma-linolenic acid (20:3n-6)	None	<0.1
Arachidonic acid (20:4n-6)	None	<0.1
Eicosapentaenoic acid (20:5n-3)	None	<0.1
Eicosenoic acid (20:1n-9)	0.3	None
Behenic acid (22:0)	0.5	None
Docosapentaenoic acid (22:5n-3)	None	0.2
Docosahexaenoic acid (22:6n-3)	None	41.5
Lignoceric acid (24:0)	0.2	None

¹ 500 mg capsules, size 10 oval, orange flavored; analysis provided by DSM Nutritional Products and rounded to the nearest 0.1%;

² fatty acid designation is #carbons:#double bonds:#carbons from the methyl carbon.

Table 3

Maternal FADS1 rs174553 and FADS2 rs174575 genotypic frequencies by group^{1,2}

Gene	SNP	M/m Alleles	Genotype	Subjects Per Group		MAF
				Placebo (n)	DHA (n)	
FADS1	rs174553	A/G	A/A	49	60	0.28
			A/G	37	42	
			G/G	10	7	
FADS2	rs174575	C/G	C/C	51	59	0.27
			C/G	35	44	
			G/G	10	6	

¹ SNP, single nucleotide polymorphism; M/m, major/minor alleles; MAF, minor allele frequency;

² both FADS SNPs evaluated were in Hardy-Weinberg Equilibrium ($P = 0.62$ and $P = 0.73$ for FADS1rs17553 and FADS2rs174575, respectively).

Table 4

Mean (\pm SE) RBC-PL-DHA and ARA (weight percent of total fatty acids) across FADS genotypes at enrollment and delivery¹

SNP	Genotype	Enrollment			Delivery		
		Entire Sample		Placebo		Supplemented	
		DHA	ARA	DHA	ARA	DHA	ARA
FADS1	A/A	4.47 \pm 0.11 ^a	15.36 \pm 0.19 ^a	4.93 \pm 0.16 ^a	13.40 \pm 0.23	7.53 \pm 0.28	12.97 \pm 0.26 ^a
	A/G	4.36 \pm 0.13 ^a	14.63 \pm 0.22 ^a	4.68 \pm 0.19 ^a	13.15 \pm 0.26	7.61 \pm 0.33	11.78 \pm 0.31 ^a
	G/G	3.67 \pm 0.28 ^b	13.73 \pm 0.48 ^b	3.78 \pm 0.36 ^b	12.50 \pm 0.50	7.35 \pm 0.80	10.09 \pm 0.74 ^b
FADS2	A/A	4.41 \pm 0.11	15.11 \pm 0.19	4.76 \pm 0.17	13.14 \pm 0.23	7.72 \pm 0.28	12.55 \pm 0.27 ^a
	A/G	4.41 \pm 0.13	14.86 \pm 0.23	4.78 \pm 0.20	13.23 \pm 0.27	7.39 \pm 0.33	12.30 \pm 0.32 ^a
	G/G	3.83 \pm 0.29	14.21 \pm 0.51	4.23 \pm 0.38	13.45 \pm 0.51	6.93 \pm 0.86	10.34 \pm 0.85 ^b

¹The table presents means \pm SEM. Definitions: ARA, arachidonic acid; DHA, docosahexaenoic acid; PL, phospholipid; RBC, red blood cell

* Rows within cells bearing different letters are significantly different ($P < 0.05$; ANOVA, Fisher Least Significant Difference post-hoc test).