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Maternal polymorphisms in the FADS1 and FADS2 genes modify the association between PUFA ingestion and plasma concentrations of omega-3 polyunsaturated fatty acids

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ABSTRACT

There is a lack of studies about polymorphisms in FADS genes in pregnant women. We aimed to verify the interaction between three FADS gene polymorphisms (rs174561; rs174575; rs3834458) and dietary α-linolenic acid (ALA) or linoleic/α-linolenic acid ratio (LA/ALA) and plasma concentrations of omega-3 ($n-3$) PUFAs in pregnant women. Of the 250 women evaluated, the homozygous for the rs174561 and rs3834458 minor allele had high plasma ALA concentrations at the highest ALA and LA/ALA ratio tertile ($p < 0.05$). Plasma concentrations of EPA and DHA were not influenced by diet. For the rs174575 SNP, pregnant women who carried the minor allele presented lower proportions of plasma EPA in the second LA/ALA ratio tertile ($p < 0.05$). Increased dietary intake of ALA and LA/ALA ratio promoted plasma ALA accumulation in homozygotes for the minor allele rs174561 and rs3834458. Moderate intake of LA/ALA ratio may reduce plasma concentration of EPA in pregnants carrying the rs174575 minor allele.

1. Introduction

Western diet changes in the last few decades have culminated in increased of omega-6 ($n-6$) and reduction of omega-3 ($n-3$) fatty acids intake, which are characterized by an essentially pro-inflammatory activity [1,2]. This dietary pattern is particularly detrimental in pregnancy when large amounts of docosahexaenoic acid (DHA, 22: 6, $n-3$), the major long-chain polyunsaturated fatty acid (LC-PUFA) $n-3$, is deposited in fetal retina and tissues, influencing cognitive development and visual acuity in the infant's early years [3,4].

The α-linolenic (ALA, 18: 3 $n-3$) and linoleic (LA, 18: 2 $n-6$) fatty acids are not synthesized by the body due to the lack of delta-15 and −12 desaturases, respectively, and must be acquired through ingestion [2,5]. The LCPUFAs - eicosapentaenoic acid (EPA; 20: 5; $n-3$), docosahexaenoic acid (DHA; 22: 6; $n-3$), and arachidonic acid (AA: 20: 4; $n-6$) - are the active forms of $n-3$ and $n-6$ fatty acids in the body and can be obtained by dietary intake or through endogenous synthesis from ALA or LA, respectively [6–8].

LA and ALA compete for the same enzymes in LC-PUFAs metabolism (Fig. 1). For conversion of dietary LA and ALA to AA and EPA, respectively, three enzymatic steps are required, including two

Abbreviations: AA, arachidonic acid; ALA, α-Linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturases; FFQ, food frequency questionnaire; HWE, hardy-weinberg equilibrium; LA, linoleic acid; LA/Ala, linoleic/α-linolenic acid ratio; LC-Pufa, long-chain polyunsaturated fatty acid; MAF, minor allele frequencies; Mm, heterozygote; MM, homozygote for the major allele; Mm, homozygote for the minor allele; NISAMI, maternal and child health research group; $n-3$, omega-3; $n-6$, omega-6; PUFAs, polyunsaturated fatty acids; SNP, single nucleotide polymorphisms; UFRB, Universidade Federal do Recôncavo da Bahia

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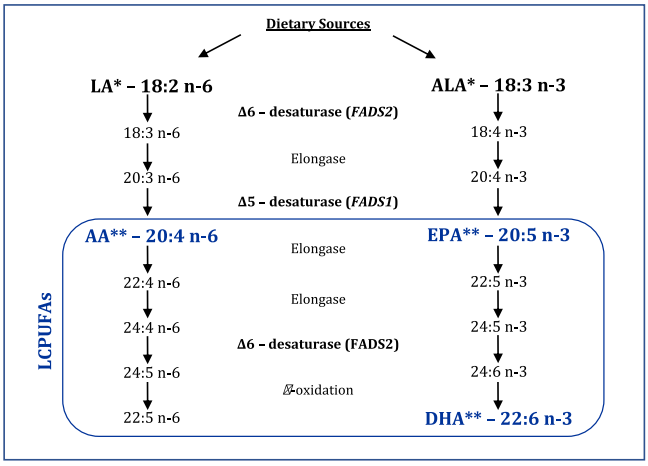

Fig. 1. Schematic representation of the metabolic pathway of omega-3 and omega-6 polyunsaturated fatty acids in humans.
* Dietary form. ** Active form.
PUFAs = Polyunsaturated fatty acids. LCPUFAs = Long-chain polyunsaturated fatty acids. LA = Linoleic Acid. ALA = α-Linolenic Acid. AA = Arachidonic acid. EPA = Eicosapentaenoic Acid. DHA = Docosahexaenoic acid.

desaturation steps regulated by FADS2 and FADS1, in that order. Conversion of EPA to DHA requires three additional enzymatic steps: one step of desaturation regulated by FADS2 and one B-oxidation step [1,9–11].

Due to the genetic regulation of enzyme steps, the existence of single nucleotide polymorphisms (SNPs) in FADS genes has been associated with changes in blood concentrations of polyunsaturated fatty acids (PUFAs). The high prevalence of minor alleles in the general population (11–40%) and their relevance in metabolism of essential fatty acids makes these genetic variants an important health problem [12]. Studies also show controversial results, because only some SNPs can reduce LCPUFA concentrations, but in certain studies the same loci may have no effects [13] or the results are contradictory [14].

Genetic variants in FADS genes may also be an important link between LCPUFAs ingestion and their blood profile [15,16]. However, there is a lack of studies about polymorphisms rs174561 (FADS1), rs174575, and rs3834458 (FADS2) in pregnant women.

Considering that gene-nutrient interactions influence the plasma concentrations of long-chain polyunsaturated fatty acids and their ALA and LA precursors [17], this study aimed to verify if FADS1 (rs174561) and FADS2 (rs174575 and rs3834458) polymorphisms affect the relationship between dietary α-linolenic acid (ALA) or LA/ALA ratio and plasma concentrations of omega-3 polyunsaturated fatty acid in pregnant women.

2. Materials and methods

2.1. Study design and subjects

A cross-sectional nested-case study examined a prospective cohort of pregnant women of the Maternal and Child Health Research Group (NISAMI - *Núcleo de Investigação em Saúde Materno Infantil)*. The NISAMI cohort was developed in the urban area of Santo Antônio de Jesus, Bahia, Brazil, between 2008 and 2016, and aimed to investigate determinants of maternal and infant health.

Pregnant women over 18 years old present in the cohort between August 2013 and December 2014 were invited to participate (Fig. 2). Those who agreed underwent additional assessments of dietary intake and blood collection for fatty acid analyzes and genotyping at 34 weeks of gestation or less. Exclusion criteria were multiple gestation; HIV infection; contagious, immunological and metabolic diseases, as well as vegan diet users.

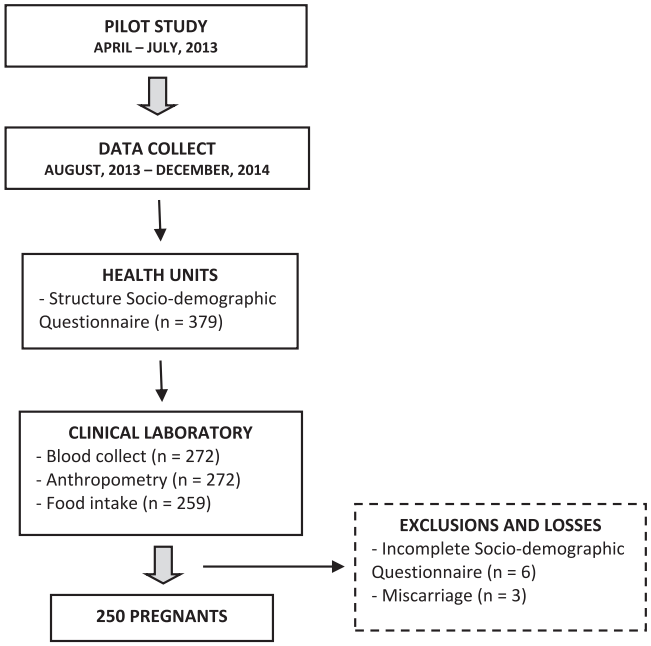

Fig. 2. Flowchart of study design and participant adhesion from adults pregnants participants from pregnant cohort of NISAMI, Santo Antônio de Jesus, Bahia, Brazil.

2.2. Data collect

The data collection was performed after a pilot study to calibrate the instruments. During first contact, which occurred in the health units where they received prenatal care, pregnant women signed a written informed consent form and answered the socioecomic questionnaire. Then, the expectant mothers were referred to a clinical laboratory in the city, where their food intake and anthropometry were evaluated, and blood was collected after overnight fasting.

2.3. Maternal characteristics and intake of polyunsaturated fatty acids

Socio-demographic and pre-natal data were obtained by a structured questionnaire. The anthropometric evaluation was performed in triplicate, according to protocols recommended by Jelliffe [18].

Fatty acid intake was estimated by means of a nutrient-specific semiquantitative Food Frequency Questionnaire (FFQ), constructed and validated for this purpose. The FFQ contained 89 items and estimated the consumption of PUFAS food sources during gestation. Participants were questioned about food consumption frequency and number of servings at a time. Amount of daily ingested food was used to calculate nutrients, using the Brazilian Food Composition Database [19] as reference or the USDA Food Composition Database [20], when the food or nutrient was not found in the Brazilian Database. In case of preparations, ingredients were estimated and calculated individually [21].

2.4. Plasma fatty acid composition

Plasma was separated from red cells and buffy coat by centrifugation at 2500 rpm for 15 min, and immediately frozen in liquid nitrogen until analysis. Lipids were extracted at the Biochemical Nutrition Laboratory of the Universidade Federal de Viçosa (UFV), Viçosa, MG, Brazil, from 600 mcL of plasma by the Folch method (Folch solution -chloroform/methanol 2:1) [22]. The derivatization step was performed by the Hartman and Lago method [23].

Separation of fatty acid methyl esters was performed on a SHIMADZU CG solution gas chromatography equipped with a flame ionization detector (FID). Fatty acids were identified by comparing sample esters

retention time with reference standards FAME mix (Sigma-Aldrich®, USA). Fatty acid relative content was calculated by the GC solution program and was based on the contribution of each fatty acid to the total area of the chromatogram, expressed as fatty acid percentage (%).

2.5. Genetic analyzes

Genomic DNA was extracted from the buffy coat within 72 h of collection, using the Qiagen FlexiGene® DNA Kit (Qiagen, CA, USA). Extraction was carried out at the UFRB Human Genetics Laboratory, where it was stored at −20 °C.

SNP selection was due to their relation with plasma concentrations of long chain polyunsaturated fatty acids in children and pregnant women [12,24–26]. Samples were genotyped at Center of Human and Molecular Genetics of the Universidade Federal do Espírito Santo (UFES), Vitória, ES, Brazil, using pre-designed (rs174575 and rs174561) and customized (rs3834458) TaqMan® assays, using conditions suggested by the manufacturer. The following primers and probes were used for the SNP rs3834458: Primer (5′−3′) foward AGAGCAG AGGTTCCGCAATT and reverse GCCTTGGATTAGAGGGCTTTGAA and Probes (5′−3′) T allele VIC/TCAGACAATCTTAGAAAAG-NFQ and allele Del FAM/TCAGACAATCTTGAAAAG-NFQ.

2.6. Statistical analysis

Data were analyzed by the Stata software version 14. Categorical variables were presented by absolute and relative frequencies. Discrete and continuous variables were described as mean (Standard Deviation). Assymetric variables were evaluated by the Kolmogorov-Smirnov test or by means of scatter plots and histograms.

During the study period, 349 pregnant women were monitored. However, genetic information was only available for 250 of them. Considering that a sample was not estimated to detect a mean difference between polyunsaturated fatty acid intake according to the highest frequency heterozygous allele for rs174575, rs174561 and rs3834458 SNPs, a posteriori power was calculated, with a confidence level of 95%. Under these circumstances, the power calculated to detect mean difference between according to the highest frequency heterozygous allele for the rs174575 was 97%, rs174561 was 88% and rs3834458 was 98% with a 95% confidence level.

Genotype frequencies and Minor Allele Frequencies (MAF) were calculated. SNPs with allele frequencies between 1% and 5% were considered rare and excluded to avoid weak statistical analyzes [27,28]. Genotypes distribution was tested by Hardy-Weinberg equilibrium using the chi-square test ($p > 0.05$) in the Arlequin version 3.5.2.2 software. Pregnant women were coded as: homozygous for the major allele {1}, heterozygous {2}, and homozygotes for the minor allele {3}.

Pregnant women were classified according to the tertile of α-Linolenic acid consumption and the relation Linoleic acid:α-Linolenic acid (LA:ALA). ANOVA one way and Tukey's test were used to compare the mean PUFAS plasma concentrations according to genotypes; and the mean plasma ratios of $n-3$ PUFAS according to genotypes in each tertile of ALA and LA:ALA consumption. The level of significance used in the analyzes was 5%.

2.7. Ethical statement

The study was approved by the Research Ethics Committee involving Human Beings, of the Universidade Federal do Recôncavo da Bahia (UFRB), number: 241.225 dated 04/09/2013. All study proceedings has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The informed consent was obtained for experimentation with human subjects and the privacy rights of human subjects was observed.

Table 1
Characteristics of pregnant from the sample ($n = 250$), Santo Antônio de Jesus - Bahia, Brazil, 2016.

Variables	Value
Maternal Characteristics	
Age (years)	27.24 ± 6.01
Race	
- Black	105 (42%)
- Brown	105 (42%)
- White	25 (10%)
- Others	15 (6%)
Schooling (years)	4.62 ± 1.53
-High School	208 (83.2%)
-Elementary School	42 (16.8%)
Socioeconomic class ($n = 244$)	
-A	2 (0.8%)
-B	39 (15.6%)
-C	155 (62%)
-D	46 (18.4%)
-E	2 (0.8%)
Smoker, yes/no	18 (7.2%): 232 (92.8%)
Consume alcohol, yes/no	21 (8.4%): 229 (91.6%)
Weight (Kg)	67.17 ± 12.82
Height (m)	1.61 ± 0.06
BMI (kg/m^2)	26.07 ± 5.01
BMI before pregnancy (kg/m^2)	24.17 ± 4.90
First Pregnancy ($n = 249$)	106 (42.4%)
Number of pregnancies	0.96 ± 1.14
Gestational weeks ($n = 243$)	19.79 ± 7.18
$n-3$ PUFA intake	
- ALA (g/day)	1.9 ± 0.9
- EPA (mg/day)	65.6 ± 73.1
- DHA (mg/day)	209.9 ± 211.7
$n-6$ PUFA intake	
- LA (g/day)	18.2 ± 8.8
- AA (mg/day)	186.2 ± 283.1

BMI = Body Mass Index. $n-3$ PUFA = omega-3 Polyunsaturated fatty acids. ALA = α-linolenic acid (18:3; $n-3$). EPA = Eicosapentaenoic Acid (20:5; $n-3$). DHA = Docosahexaenoic acid (22:6, $n-3$). $n-6$ PUFA = $n-6$ polyunsaturated fatty acids. LA = Linoleic acid (18:2; $n-6$). AA = arachidonic acid (20:4 $n-6$).

3. RESULTS

3.1. Sample characteristics

The final sample of the study consisted of 250 women, with a mean age of 27.24 years (SD = 6,01), low income (88,4%; $n = 203$), and 83,2% (208) had the high school (Table 1; Supplemental Table 1). Pregestacional BMI presented mean was 24.17 kg/m^2 (SD = 4.90). Fatty acid intake did not differ between genotypes (Supplemental Table 1).

3.2. Allelic and genotypic frequencies

FADS1 (rs174561) and FADS2 (rs174575 and rs3834458) polymorphisms were genotyped with a success rate of 100%. The minor alleles ranged from 22.0% to 28.8% and the genetic variants were in Hardy–Weinberg equilibrium ($p > 0.05$; Table 2).

3.3. Plasma fatty acids according to FADS gene polymorphisms

Plasma concentrations of PUFAs according to genotypes are shown in Table 3. Plasma concentrations of $n-6$ PUFAs did not differ between genotypes. For $n-3$ PUFAs, plasma concentrations of ALA were high in pregnant women homozygous for the minor alleles for polymorphism rs174561 and rs3834458, compared to homozygotes for the major allele ($p < 0.05$; Table 3). No significant differences were found in plasma concentrations of EPA, DHA and EPA/ALA ratio among the genotypes.

Table 2
Characteristics of the three polymorphisms of the desaturase gene (FADS) from the sample of pregnant women (n = 250).

Gene/SNP	Genótipo (%)			MAF	HWE
	MM	Mm	mm	%	p[a]
FADS2					
rs174575	CC (49.6)	CG (43.2)	GG (7.2)	28.8	0.44490
FADS1					
rs174561	TT (61.6)	TC (32.8)	CC (5.6)	22.0	0.46273
Intergênico (*FADS2*)					
rs3834458	TT (58.8)	TDel (34.8)	DelDel (6.4)	23.8	0.72693

SNP = Single nucleotide polymorphism. MM = Homozygote for the major allele. Mm = Heterozygote. mm = Homozygote for the minor allele. MAF = Minor Allele Frequency. HWE = Hardy-Weinberg equilibrium. Del = Deletion.
[1a] Derived from the Chi-square test of the Hardy-Weinberg equilibrium.

3.4. Plasma fatty acids according to tertile of ALA ingestion and FADS gene polymorphisms

Regardless of the level of ALA ingestion, plasma concentrations of $n-3$ PUFAS did not differ among genotypes, when rs174575 polymorphism was considered. For polymorphisms rs174561 and rs3834458, in women with high ALA intake, plasma ALA concentrations were high in homozygotes for the minor allele ($p < 0.05$), compared to those who carried the major allele (MM and Mm). No significant differences were found in ALA plasma concentrations for pregnant women classified in the first two ALA ingestion tertiles. Plasma EPA and DHA concentrations were not influenced by ALA intake (Supplemental Table 2; Fig. 3).

3.5. Plasma fatty acids according to La/ALA tertiles ratio and FADS gene polymorphisms

For rs174575 polymorphisms, only EPA plasma concentrations were influenced by the LA/ALA ratio, and no significant differences were observed in the plasma concentrations of ALA and DHA. In the second tertile of LA/ALA ratio ingestion, women who carried the minor allele (Mm and mm) had low plasma EPA levels when compared to women homozygous for the major allele ($p < 0.05$; Supplemental Table 3, Fig. 4).

For polymorphisms rs174561 and rs3834458, in the third tertile of LA/ALA ingestion ratio, ALA plasma concentrations were high in women homozygous for the minor allele compared to the those who carried the major allele ($p < 0.05$) (Supplemental Table 2, Fig. 4). No significant differences were observed in the plasma concentrations of desaturase gene products (EPA and DHA) among genotypes (Supplemental Table 3; Fig. 4).

4. Discussion

Our results suggest a possible gene-nutrient interaction in plasma concentrations of $n-3$ PUFAS. The high intake of ALA and the LA/ALA ratio promoted increased ALA plasma concentrations in pregnant women homozygous for the minor alleles for polymorphisms rs174561 and rs3834458, but did not change the concentrations of desaturase, EPA, and DHA gene products. Considering the rs174575 polymorphism, only EPA plasma concentrations were altered, being reduced in the second tertile of LA/ALA ratio intake in pregnant women who carried the minor allele, compared to those homozygous for the major allele.

The limited enzymatic conversion of LCPUFAs may be assumed in pregnant women homozygous for the rs174561 and rs3834458 minor alleles, as they had high plasma ALA concentrations, regardless of dietary fatty acid intake. The highest ALA concentration profile has been associated with reduced activity of $\Delta-5$ and $\Delta-6$ desaturase enzymes [12–14]. Genetic variants of FADS1 and FADS2 genes may reduce gene transcription and enzymatic conversion rate of desaturases, which would result in enhanced amounts of $n-3$ long chain PUFAs precursor (ALA) and reduced amounts of products (EPA and DHA) [12,29–32]. SNP rs174575 showed a distinct behavior, as the plasma concentrations of all $n-3$ and $n-6$ PUFAs were similar when food consumption was considered, regardless of the genotype.

Some studies have found no association between FADS gene polymorphisms and DHA blood concentration, probably because the source of this is mainly dietary [16,31]. It has been found that individuals with at least one minor allele (Mm or mm) can compensate for the low conversion of omega-3 LCPUFAs by increasing α-linolenic acid (ALA) consumption [16,17,33], which was not observed in the present study. ALA consumption could have an additional benefit in relation to the consumption of $n-3$ LCPUFAs, because ALA can prevent the conversion pathway of $n-6$ LCPUFAs [17], as it competes with LA in the first steps of PUFAS metabolism [2,9,17]. Furthermore, epigenetic mechanisms could link LCPUFA concentrations to the transcriptional machinery involved in the metabolism of polyunsaturated fatty acids [1].

In normal conditions, the endogenous conversion of ALA to DHA is low, with rates varying from 1 to 10% depending on genetic factors [8]. Therefore, in individuals genetically predisposed to limited DHA biosynthesis, the main strategy to improve plasma concentrations would be through direct ingestion of foods rich in this nutrient, such as fish, or through EPA and DHA supplementation [16,31,33,34]. Thus, it is possible that pregnant women homozygous for minor alleles for polymorphisms rs174561 and rs3834458 benefit less from ALA intake, and they may have more health benefits from DHA consumption.

We have shown that higher LA/ALA ingestion increased plasma ALA

Table 3
Plasma concentrations of polyunsaturated fatty acids (%) according to desaturase gene polymorphisms (FADS) in the sample of pregnant women.

	rs174575				rs174561				rs3834458			
	MM	Mm	mm	*P* value[a]	MM	Mm	mm	*P* value[a]	MM	Mm	mm	*P* value[a]
PUFAS $n-6$												
LA (n = 193)	12.60	13.53	15.57	0.101	13.10	13.17	16.07	0.217	13.04	13.25	15.30	0.320
AA (n = 226)	1.53	1.88	1.52	0.126	1.65	1.61	2.56	0.051	1.63	1.66	2.28	0.184
AA/LA (n = 183)	0.11	0.15	0.15	0.125	0.13	0.13	0.17	0.655	0.13	0.13	0.15	0.895
PUFAS $n-3$												
ALA (n = 235)	1.75	1.76	1.46	0.752	1.71[a]	1.55[a]	3.14[b]	0.004	1.66[a]	1.66[a]	2.76[b]	0.028
EPA (n = 227)	2.95	2.24	2.59	0.053	2.58	2.75	2.22	0.689	2.57	2.77	2.15	0.554
DHA (n = 101)	2.29	2.83	2.96	0.426	2.30	3.06	2.19	0.201	2.37	2.93	2.16	0.367
EPA/ALA (n = 226)	2.21	1.61	1.89	0.088	1.94	2.00	1.23	0.454	1.98	1.95	1.32	0.464

MM = Homozygote for the major allele. Mm = Heterozygote. mm = Homozygote for the minor allele. PUFAS $n-6$ = Polyunsaturated fatty acids of the $n-6$ series. LA: Linoleic acid (18: 2; $n-6$). AA = Arachidonic acid (20: 4; $n-6$). AA/LA = Arachidonic acid/linoleic acid ratio. PUFAs $n-3$ = omega-3 polyunsaturated fatty acids. ALA = α-linolenic acid (18: 3; $n-3$). EPA = Eicosapentaenoic Acid (20: 5; $n-3$); DHA = Docosahexaenoic acid - (22: 6, $n-3$). EPA/ALA = Eicosapentaenoic acid/α-linolenic acid ratio.
[a] One Way ANOVA. Means followed by the same letter did not differ statistically by the Tukey test ($p < 0.05$).

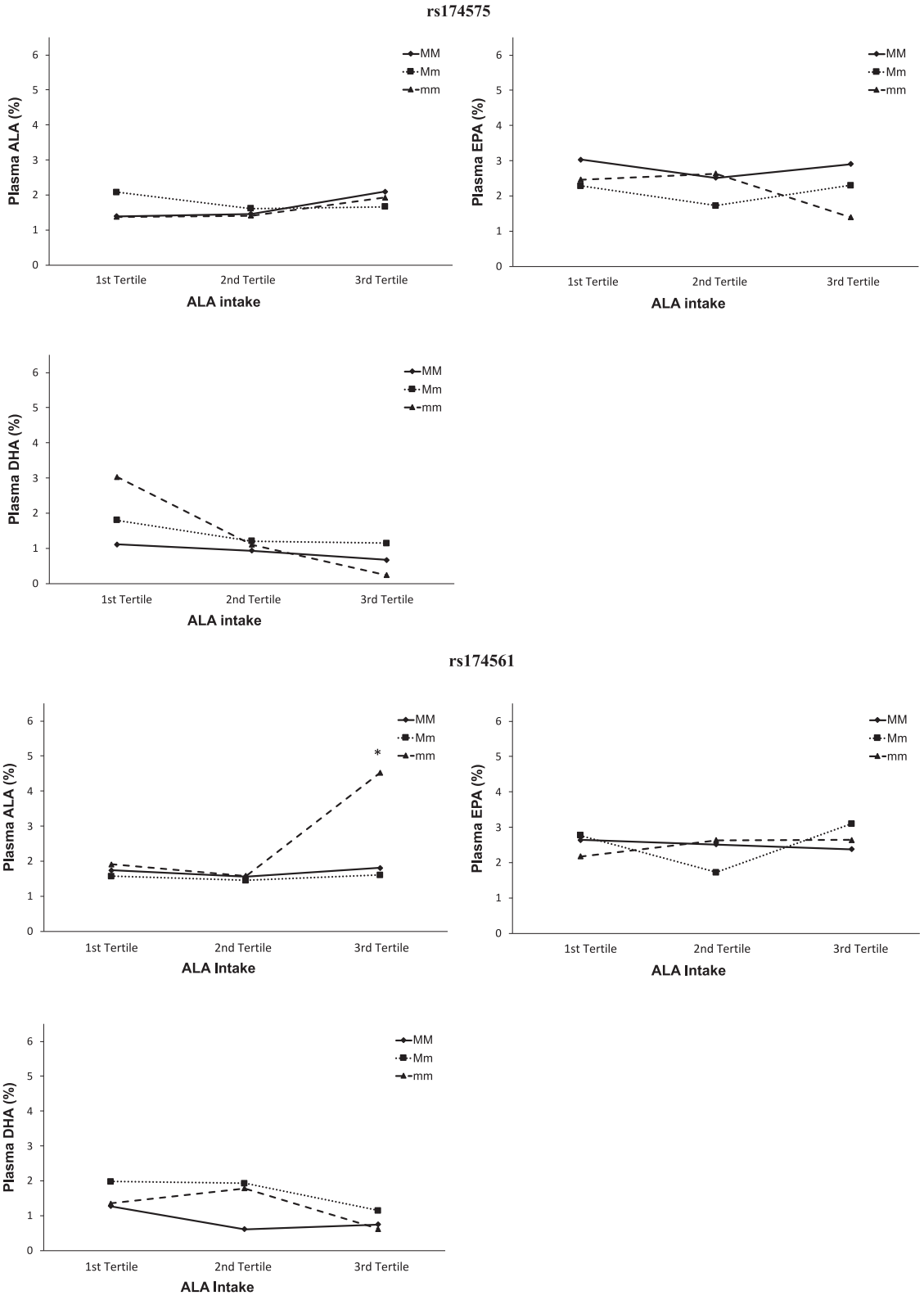

Fig. 3. Plasmatic concentrations of omega-3 fatty acids (%) according to tertile of ingestion of α-linolenic acid (ALA) and FADS1 (rs174561) and FADS2 (174575 and rs3834458) polymorphisms.
ALA = α-linolenic acid (18: 3; $n-3$). EPA = Eicosapentaenoic Acid (20: 5; $n-3$); DHA = Docosahexaenoic acid - (22: 6, $n-3$). rs174575 (MM = CC; Mm = CG; mm = GG). rs174561 (MM = TT; Mm = TC; mm = CC). rs3834458 (MM = TT; Mm = TDel; mm = DelDel).
* ANOVA One Way (Tukey's test, $p < 0.05$).

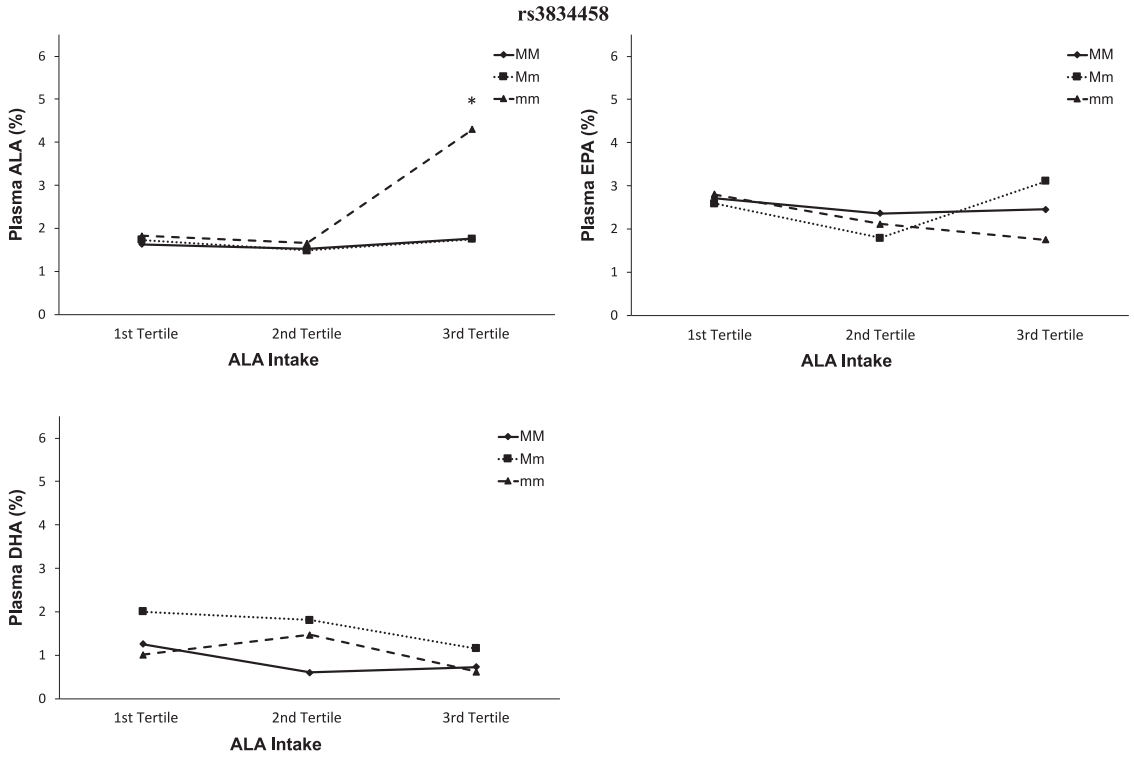

Fig. 3. (*continued*)

in women homozygous for minor alleles for polymorphisms rs174561 and rs3834458, as well as reduced plasma EPA concentrations in those with the minor allele. This result may be due to enzymatic competition between LA and ALA in the initial LCPUFAs metabolism stage [1,2,17]. EPA is considered an independent protective factor [16], as it competes for the cyclooxygenase and lipoxygenase enzymes with arachidonic acid, facilitating the synthesis of the odd series eicosanoids, which are less inflammatory [9]. In contrast with the present study, Gillingham et al. [16]. found that in the hyperlipidemic individuals homozygous for minor FADS1 SNPs (rs174561, rs174545, and rs174537) and FADS2 (rs174583), a diet rich in α-linolenic acid increased plasma concentrations of EPA but did not increase DHA. The authors suggested that consuming an ALA-rich diet (approximately 10% of the total caloric value) would compensate for the apparent low desaturase enzymatic activity in minor allele homozygotes, thereby raising plasma EPA concentrations.

Thus, to facilitate conversion of $n-3$ LCPUFAs, diet should increase ALA ingestion concomitant with low LA intake [1,2,17]. Hellstrand et al. [17] suggested that FADS1 gene variants act as modifiers of PUFAS intake and cardiovascular disease, probably because they alter blood levels of polyunsaturated fatty acids. In this study, gene-nutrient interaction was observed, as the greater consumption of ALA and the ALA/LA ratio was inversely related to cardiovascular disease risk in rs174546 SNP minor allele homozygotes (TT).

A well-controlled prospective cohort study, standardized data collection and the application of a food frequency questionnaire to evaluate the habitual consumption of fatty acids by pregnant women were the positive aspects of the study. Limitation was mainly sample loss, because the analysis is very sensitive. On the other hand, determining DHA is complex, because its concentration is usually very low in the blood components and can be confused with noises in the chromatograms, which makes their detection difficult. However, these aspects did not compromise the validity of the present study, considering that the data from this investigation came from a prospective cohort that adopted strategies to avoid data collection bias risk.

Otherwise, the present study evaluated whether the gene-nutrient interaction in plasma concentrations of PUFAS could be perceived in doses usually consumed by the general population without the use of DHA or fish oil supplements (DHA + EPA) by pregnant women. This provides information about the real contribution of food consumption in the metabolism of these fatty acids, considering that other studies that evaluated the gene-nutrient interaction used intervention through fatty acid supplementation. However, these studies use high doses of the nutrient, usually in amounts above that established by nutritional guidelines.

Thus, observational studies, such as the present approach, of gene-nutrient interaction, should be conducted, because they facilitate the understanding of the greater vulnerability of certain groups. In this sense, knowing the genotypes at risk facilitates the elaboration of individualized nutritional recommendations and food planning, which consider the genetic variants involved in metabolic alterations implied in phenotypes unfavorable to health [35]. We recommend the development of prospective studies in pregnant women to test the relationship between fatty acids and outcomes related to maternal and infant health. The sample size should be adequate for comparison between groups to identify results that are clinically and epidemiologically relevant.

It was possible to observe that rs174561 (FADS1) and rs3834458 (FADS2) polymorphisms act as modifiers that affect in the relationship between ALA and LA/ALA ratio and the plasma concentrations of omega-3 polyunsaturated fatty acids in pregnant women. In homozygotes with the minor allele of rs174561 and rs3834458, increased ALA consumption does not favor the conversion of long-chain polyunsaturated fatty acids, EPA and DHA; however, low intake of the LA/ALA ratio may prevent plasma accumulation of ALA. It is possible that, in the pregnant women homozygous for minor alleles for polymorphisms rs174561 and rs3834458, the consumption of food sources with DHA is recommended, considering the probable occurrence of less gene transcription and enzymatic conversion. Regarding rs174575 SNP, plasma EPA concentrations were reduced in response to increased LA/ALA ratio consumption in pregnant women carrying the minor allele. Probably, the low intake of LA in relation to ALA intake is important to

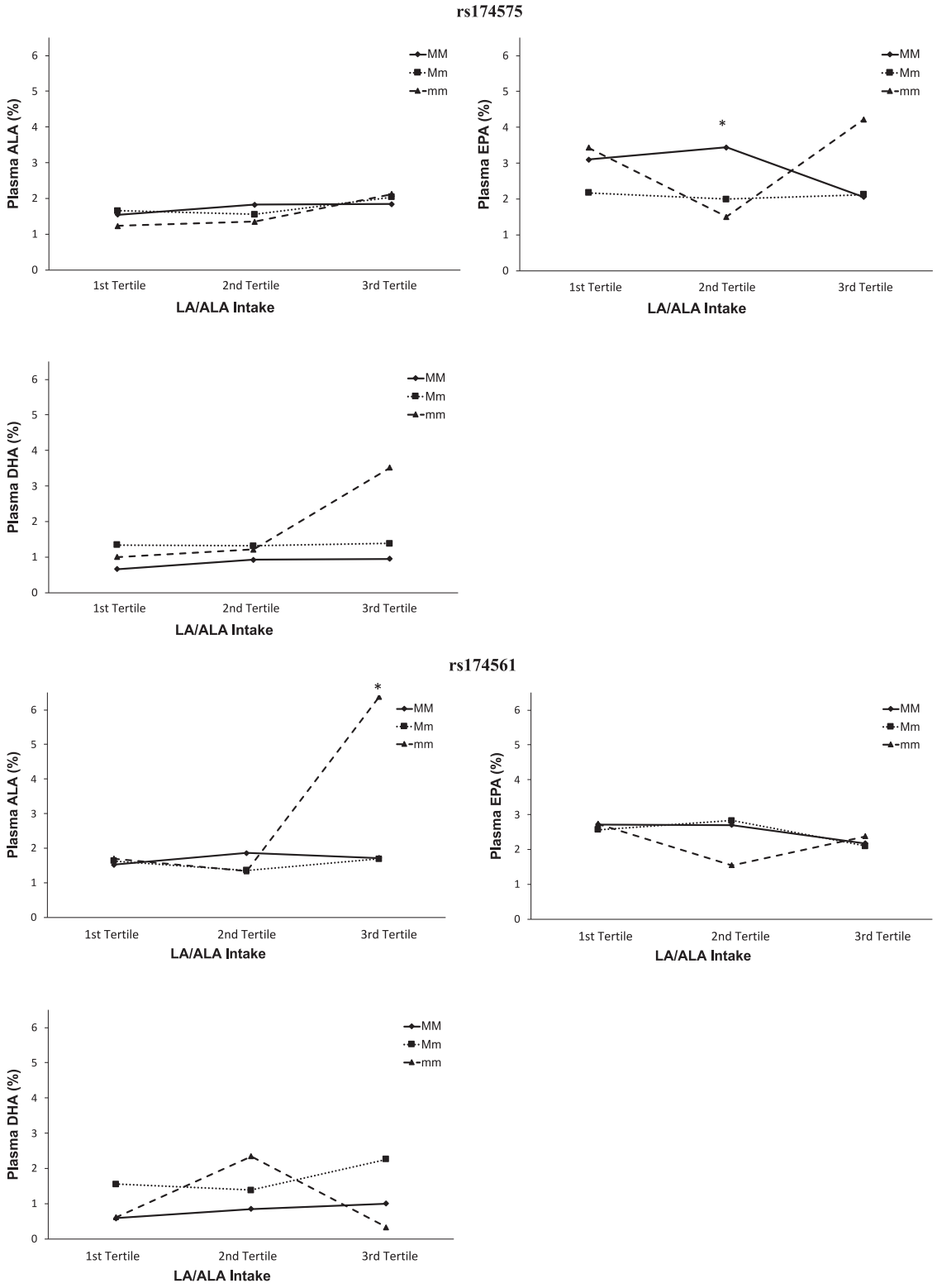

Fig. 4. Plasmatic concentrations of omega-3 fatty acids (%) according to tertile of ingestion of the linoleic acid/α-linolenic acid (LA/ALA) ratio and FADS1 (rs174561) and FADS2 (174575 and rs3834458) polymorphisms.

ALA = α-linolenic acid (18: 3; $n-3$). EPA = Eicosapentaenoic Acid (20: 5; $n-3$); DHA = Docosahexaenoic acid - (22: 6, $n-3$). rs174575 (MM = CC; Mm = CG; mm = GG). rs174561 (MM = TT; Mm = TC; mm = CC). rs3834458 (MM = TT; Mm = TDel; mm = DelDel).

* ANOVA One Way (Tukey's test, $p < 0.05$).

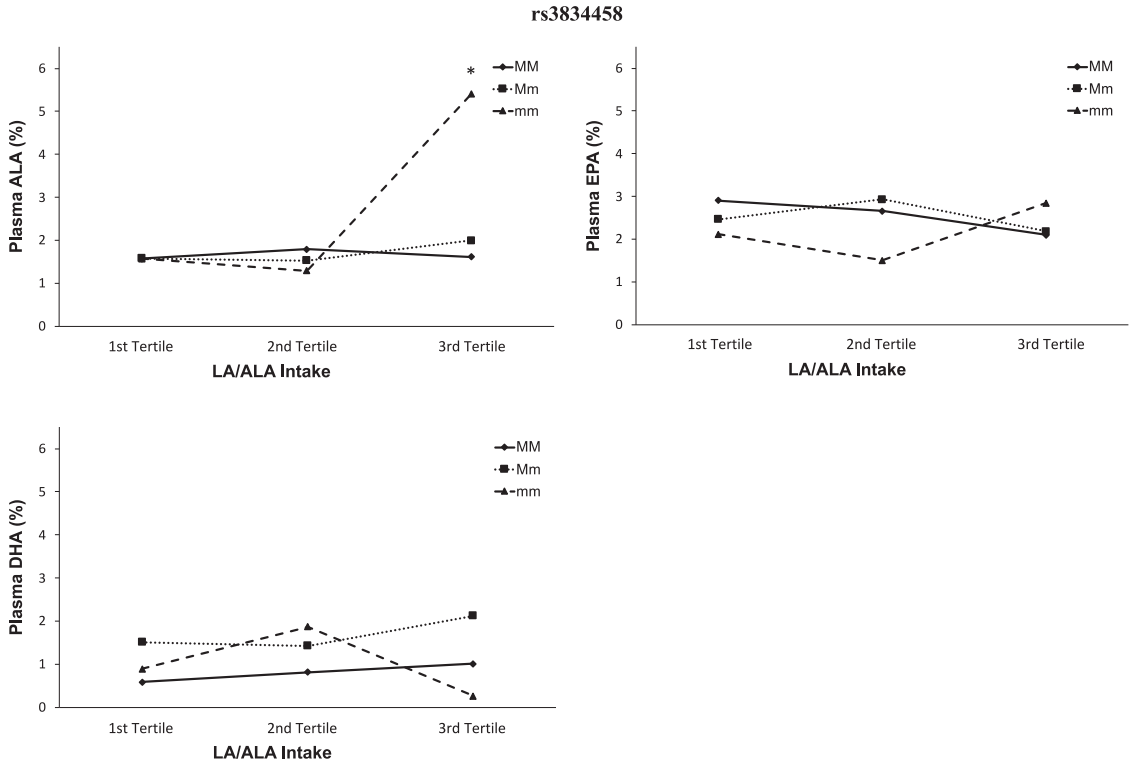

Fig. 4. (*continued*)

promote the adequate plasma profile of $n-3$ LCPUFAs in pregnant women.

Authorship

GQC formulated the conception and design of the study, the acquisition of data, the analysis and interpretation of data; drafted the article or revised it critically for important intellectual content. MPS participated to the acquisition of data, the analysis and interpretation of data. DBS and IDL participated to the design of the study, the analysis and interpretation of data. All authors read and approved the final version to be submitted.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.plefa.2019.09.004.

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