α-Linolenic acid, Δ⁶-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction[1-3]

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ABSTRACT

Background: Δ^6-Desaturase (FADS2) is the rate-limiting step in the polyunsaturated fatty acid (PUFA) biosynthetic pathway.

Objective: The aim was to test whether the common deletion [*T*/-] in the promoter of *FADS2* affects the PUFA biosynthetic pathway and consequently modifies the effect of α-linolenic acid (ALA) on myocardial infarction (MI).

Design: Case subjects ($n = 1694$) with a first nonfatal acute MI were matched by age, sex, and area of residence to 1694 population-based control subjects in Costa Rica. PUFAs were quantified by gas-liquid chromatography from plasma and adipose tissue samples. Least-squares means from generalized linear models and odds ratios (ORs) and 95% CIs from multiple conditional logistic regression models were estimated.

Results: The prevalence of the variant *T*/- allele was 48%. Eicosa-pentaenoic acid, γ-linolenic acid, and arachidonic acid decreased in adipose tissue and plasma with increasing number of copies of the variant allele with a monotonic trend ($P < 0.05$ for all). Fasting plasma triacylglycerols by genotype were 2.08 mmol/L for *TT*, 2.16 mmol/L for *T*-, and 2.26 mmol/L for - - [ie, homozygous for the variant (deletion) allele] ($P = 0.03$). The *FADS2* deletion was not associated with MI and did not significantly modify the association between adipose tissue ALA and the risk of MI.

Conclusions: The *FADS2* deletion may prevent the conversion of ALA into very-long-chain PUFAs. However, this metabolic effect is not translated into an attenuated risk between ALA and MI among carriers of the variant. It is possible that, at current intakes of ALA, any potential defect in the transcription of the gene is masked by the availability of substrate. Further research in populations deficient in ALA intake is warranted. *Am J Clin Nutr* 2007;85:554–60.

KEY WORDS Myocardial infarction, genetics, diet, fatty acids, epidemiology

INTRODUCTION

Very-long-chain polyunsaturated fatty acid (PUFA) pools originate from the diet and endogenously from elongation and desaturation of their dietary precursors, α-linolenic acid (n−3) and linoleic acid (n−6). Both families of fatty acids, n−3 and n−6, share and compete for the same enzymes (Δ^6-desaturase, Δ^5-desaturase, and elongases) in their biosynthesis, and Δ^6-desaturase is the rate-limiting step (1–3) (**Figure 1**). The Δ^6-desaturase converts α-linolenic acid (18:3n–3) into stearidonic acid (18:4n–3) and linoleic acid (18:2n–6) into γ-linolenic acid (18:3n–6). Stearidonic acid and γ-linolenic acid are then

converted into eicosapentaenoic acid (EPA) and arachidonic acid (20:4n–6), respectively, through other desaturase (Δ^5-desaturase) and elongase steps. However, most α-linolenic acid and linoleic acid is either β-oxidized to produce energy or stored (4). Only a small part of these fatty acids follow the main biosynthetic pathway or are elongated into 20:3n–3 or 20:2n–6, respectively, in what it is considered a dead-end of the pathway, given that the enzymes for further metabolism are not present in mammals (5, 6).

The Δ^6-desaturase gene (*FADS2*) is located in chromosome 11 and expressed in almost all human tissues, with the highest expression in the liver, heart, and brain (7). Because *FADS2* was cloned recently (7), the metabolic effects of polymorphisms in this gene or their effects on the risk of ischemic heart disease (IHD) are unknown. It is known that a large proportion of regulatory single-nucleotide polymorphisms (SNPs) that affect gene expression are located in the promoter regions (8). One of them, the rs3834458 (*T*/-) polymorphism could play an important role in metabolism because of its location near potential regulatory regions, such as binding sites for sterol regulatory element–binding protein 1c (SREBP-1c) (9) and peroxisome proliferator-activated receptor α (PPAR-α) (10), which regulate the transcription of Δ^6-desaturase (3). The frequency of the variant *T*/- allele is high, which makes it an ideal candidate for gene-diet interaction studies and risk of IHD.

Dietary α-linolenic acid, the n−3 fatty acid precursor abundant in vegetable oils, is beneficial for IHD (11–17). Using adipose tissue as a biomarker of α-linolenic acid intake, we have shown that α-linolenic acid is associated with a large and significant reduction in the risk of nonfatal acute myocardial infarction (MI) (17). The potentially protective effect of α-linolenic acid can be achieved directly on its own (18) or through conversion to very-long-chain n−3 fatty acids including EPA and docosahexaenoic acid (DHA). Linoleic acid, the n−6 precursor also
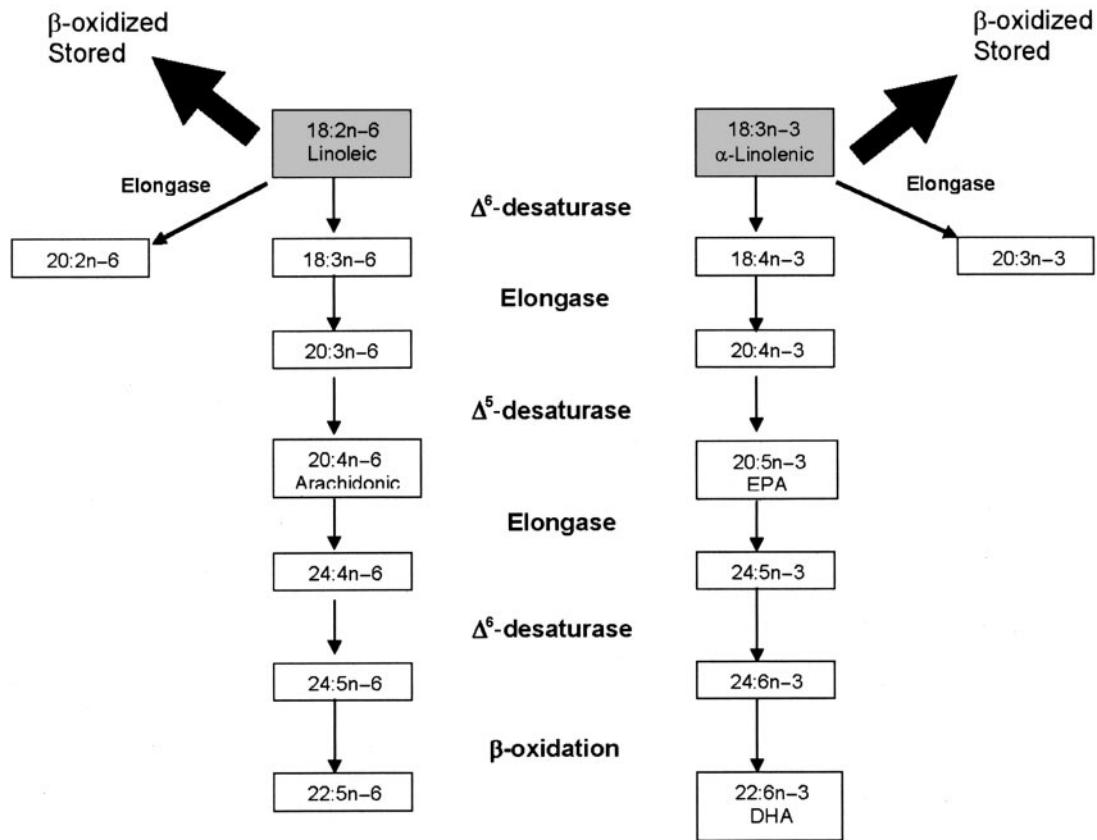
FIGURE 1. Metabolic pathway of polyunsaturated fatty acids. Both families, n−3 and n−6 fatty acids, share the same enzymes. The Δ⁶-desaturase converts linoleic acid (18:2n−6) into γ-linolenic acid (18:3n-6) and α-linolenic acid (18:3n–3) into stearidonic acid (18:4n–3). γ-Linolenic acid and stearidonic acid are converted into arachidonic acid and eicosapentaenoic acid (EPA), respectively, through other desaturase (Δ⁵-desaturase) and elongase steps. However, only a small part of linoleic and α-linolenic acids follows the biosynthetic pathway. Most is either β-oxidized to produce energy or stored, but part of it can also be elongated into 20:2n−6 and 20:3n–3, respectively, in what it is considered a dead-end of the pathway.

abundant in vegetable oils, has been associated with reduced IHD, although some biosynthetic products such as arachidonic acid may be proatherogenic (19, 20).

We hypothesized that a deletion in the promoter region of *FADS2* that potentially affect gene transcription modifies the conversion of α-linolenic and linoleic acid into very-long-chain PUFA and could attenuate the protective association between α-linolenic acid and MI. An attenuation of the α-linolenic acid effect on MI risk would suggest that this protection occurs via the conversion of α-linolenic acid to very-long-chain n−3 fatty acids.

SUBJECTS AND METHODS

Study population

The study design and population has been described previously (17, 21). Briefly, eligible case subjects (cases) were men and women who were diagnosed as survivors of a first acute MI by 2 independent cardiologists at any of the 6 recruiting hospitals in the Central Valley of Costa Rica for the period of 1994 to 2004 (21). All cases met the World Health Organization criteria for MI, which require typical symptoms plus either elevations in cardiac enzyme concentrations or diagnostic changes in the electrocardiogram (22). Enrollment was carried out while cases were in the hospital's step-down-unit. One free-living control subject (control) for each case, matched for age (±5 y), sex, and area of

residence (county), was randomly selected by using the information available at the National Census and Statistics Bureau of Costa Rica. Thus, controls are a representative sample of the Costa Rican population. Participation was 98% for cases and 88% for controls. Because of the comprehensive social services provided in Costa Rica, all persons living in the recruiting area had access to medical care without regard to income. Therefore, controls come from the same population that gave rise to the cases and were not likely to have cardiovascular disease that was not diagnosed because of poor access to medical care. Controls were ineligible if they had ever had an MI or if they were physically or mentally unable to answer the questionnaires.

The population in the recruiting area for the present study (the Costa Rican Central Valley) fulfills 2 major prerequisites necessary for the effective search of susceptibility genes for human diseases. It derives from a relatively small number of founders, and the expansion of the population has occurred by reproduction rather than by immigration. All participants gave informed consent on documents approved by the Human Subjects Committee of the Harvard School of Public Health and the University of Costa Rica.

Data collection

Trained personnel visited all study participants at their homes for data collection, biological specimen collection, and anthropometric measurements. Sociodemographic characteristics,

medical history, and lifestyle habits were collected by using a general questionnaire. Dietary intake was collected by using a food-frequency questionnaire that has been developed and validated specifically to assess fatty acid intake among the Costa Rican population (23, 24). The instrument also inquired about type of fat most frequently used for home cooking or frying; it was confirmed by visual identification at the subject's home. Biological samples were always collected in the morning after an overnight fast. Subcutaneous adipose biopsies were collected from the upper buttock with a 16 gauge needle by using a modified version of the method described by Beynen et al (25). Blood samples (20 mL) were drawn during the same visit into 0.1% EDTA-coated tubes after a 12–14 h fast. Blood sample tubes were immediately stored at 4 °C and protected from light. Within 36 h, they were centrifuged at $1430 \times g$ for 20 min at 4 °C to isolate and aliquot plasma and white blood cells. Blood samples were sealed and stored under nitrogen at −80 °C until analysis in our laboratory.

Fatty acid analysis

Fatty acids from adipose tissue and plasma were quantified by gas-liquid chromatography (24, 26). Peak retention times and area percentages of total fatty acids were identified by using known standards (NuCheck Prep, Elysium, MN) and analyzed with the Agilent Technologies ChemStation A.08.03 software (Agilent Technologies, Santa Clara, CA). Twelve duplicate samples, indistinguishable from others, were analyzed throughout the study for quality control purposes. Fatty acids in plasma were quantified in a subsample of the study population ($n = 196$). Interassay CVs in adipose tissue and plasma were 3.87% and 3.15% for α-linolenic acid, 2.63% and 0.85% for linoleic acid, 5.87% and 1.79% for arachidonic acid, and 14.2% and 7.95% for EPA.

SNP selection and genotyping

Of the 2 SNPs reported in the public databases in the core promoter region, the rs3834458 (*T*/-) polymorphism was selected because of its high frequency and its proximity to potential regulatory regions, such as the binding sites for SREBP-1c (9) and PPAR-α (10), which regulate the transcription of Δ^6-desaturase (3). An approximate 1000 bp fragment containing regulatory regions in the promoter of the *FADS2* gene was resequenced in 96 participants, but there were no novel SNPs at this site among the Costa Ricans.

Genotyping was carried out by using a variation of the allele-specific assay (ASA). The SNP genotyping procedure consisted of 3 steps. In step one, DNA fragments were obtained by using PCR primers designed according to each SNP's vicinity sequence. The reverse primers contained an artificially introduced sequence (derived from the bacteriophage M13) at the 5′ end, which was identical across all SNPs. In step 2, the SNP was genotyped by ASA with the use of allele-specific forward primers and a reverse primer whose sequence is universal for all SNPs. Universal primers were labeled at the 5′ end with one of 3 fluorescent dyes (fluorescein, hexachlorofluorescein, or tetra-chlorofluorescein). In step 3, ASA products were separated by capillary electrophoresis with the ABI Prism 310 genetic analyzer (Applied Biosystems, Perkin-Elmer, Wellesley, MA) and analyzed by using the Genotyper software (Applied Biosystems, Foster City, CA). Eight control samples were genotyped for each

plate throughout the study to assess the genotyping reproducibility. Reproducibility was 99.9%, and <1% of control and unknown samples had missing values. All samples, cases and controls, were blinded.

Statistical analysis

All data were analyzed with the Statistical Analysis Systems software (SAS Institute Inc, Cary, NC). The number of controls with available fatty acid information was 1820 for adipose tissue and 196 for plasma. The subsample with available plasma is a random sample of the whole study population and general characteristics of those participants with plasma information did not differ significantly from those without it. Least-squares means and 95% CIs from generalized linear models adjusted for potential confounders were used to report the relation between adipose tissue and plasma fatty acids and *FADS2* genotype. For those variables that did not have a normal distribution, log transformations were carried out and geometric means are reported instead. The significance of differences in health characteristics and potential confounders were assessed by McNemar's test and paired *t* tests if data were normally distributed or by Wilcoxon's signed-rank test if data were not normally distributed. After deleting missing values and unmatched participants, a total of 1694 case-control pairs with complete data on adipose fatty acids, *FADS2* genotypes, and potential confounders were used in the final analysis to evaluate the effect of *FADS2* genotypes on the risk of MI. More controls are available for the fatty acids analysis than for the MI analysis because the design is matched and, when deleting one subject due to missing values (either case or control), their pair has to be deleted too. Odds ratios (ORs) and 95% CIs were estimated from multiple conditional logistic regression models. Medians were used to classify people in low and high adipose tissue α-linolenic acid groups. Likelihood ratio tests were used to test for interactions.

RESULTS

The general characteristics of the population by case-control status are shown in **Table 1**. The risk factors by case-control status are distributed as expected in an adult population from a country in nutrition transition. The prevalence of the variant allele (deletion) in the Costa Rican population was 48%. The observed allele frequencies were in Hardy-Weinberg equilibrium.

We evaluated the effect of the *FADS2* common deletion on adipose tissue PUFA concentrations among the controls ($n = 1820$) in an attempt to assess the effect of this polymorphism on the enzyme activity. To isolate the effect of the genotype on the metabolic levels of very-long-chain PUFA, we adjusted for dietary concentrations of these fatty acids and other dietary n−3 or n−6 fatty acids. Dietary variables are expressed as percentage of total energy intake. Adjustment for total energy intake and further adjustments for other potential confounders did not significantly change the results. As shown in **Figure 2** for adipose tissue, EPA, γ-linolenic acid, and arachidonic acid decreased with the number of copies of the variant allele (one or 2 deletions) in a monotonic trend. In contrast, the "dead-end" products 20:3n−3 and 20:2n−6 increased with the number of deletions (*P* value < 0.05 for all comparisons). These results were consistent when the analysis was conducted in a subgroup of 196 randomly selected plasma samples from the same population (**Table 2**).

TABLE 1
General characteristics of the study population[1]

	Control subjects ($n = 1694$)	Case subjects ($n = 1694$)
Women (%)	26.5	26.5
Rural area (%)	25.5	25.5
Age (y)	58.2 ± 11.2[2]	58.5 ± 11.0
Visceral adiposity[3]	0.95 ± 0.07	0.97 ± 0.07[4]
Physical activity (MET)	1.54 ± 0.63	1.50 ± 0.67[4]
Household income (US$)[5]	579 ± 422	504 ± 384[4]
History of diabetes (%)	14.8	24.9[4]
History of hypertension (%)	30.2	38.7[4]
History of angina (%)[6]	4.1	12.5[4]
Current smoker (%)	20.8	39.4[4]
Total energy (MJ/d)	10.21 ± 3.15	11.27 ± 3.98[4]
Saturated fat (% of energy)	10.4 ± 2.7	11.1 ± 2.9[4]
Monounsaturated fat (% of energy)	11.8 ± 3.8	11.9 ± 3.5[4]
Polyunsaturated fat (% of energy)	6.3 ± 2.1	6.0 ± 2.0[4]
trans Fat (% of energy)	1.16 ± 0.59	1.19 ± 0.57
Carbohydrates (% of energy)	55.3 ± 7.3	54.2 ± 7.6[4]
Fiber (g/d)[7]	25.0 ± 6.0	23.9 ± 6.3[4]
Folate (g/d)[7]	447 ± 114	435 ± 126[4]
Use of vitamin E supplements (%)[8]	3.9	3.4
Alcohol drinker (%)	40.7	37.3[4]
α-Linolenic acid (% of energy)	0.61 ± 0.23	0.58 ± 0.23[4]
EPA (% of energy)	0.04 ± 0.04	0.04 ± 0.03
DHA (% of energy)	0.07 ± 0.06	0.07 ± 0.06
Linoleic acid (% of energy)	6.32 ± 2.20	6.03 ± 2.18[4]
γ-Linolenic acid (% of energy)	0.01 ± 0.02	0.01 ± 0.02
Arachidonic acid (% of energy)	0.09 ± 0.04	0.10 ± 0.05[4]
Genotype frequency (%)		
TT	26.7	26.4
T-	49.9	50.8
- -[9]	23.4	22.8
Allele frequency, deletion (%)	48.3	48.2

[1] MET, metabolic equivalents; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[2] $\bar{x}$ ± SD (all such values).

[3] Measured as the waist-to-hip ratio.

[4] Significantly different from control subjects, $P < 0.05$.

[5] n = 234 with missing values.

[6] $n = 7$ with missing values.

[7] Adjusted for total energy intake by using the residuals method.

[8] $n = 2$ with missing values.

[9] Homozygous for the variant (deletion) allele.

α-Linolenic and linoleic acid did not differ significantly across genotypes in adipose tissue, plasma, and diet.

We then evaluated if this polymorphism affected plasma lipids. Fasting plasma triacylglycerols (geometric means and 95% CIs) by genotype were 2.08 mmol/L (2.00, 2.17), 2.16 mmol/L (2.09, 2.22), and 2.26 mmol/L (2.16, 2.36) ($P = 0.03$) for the homozygous for the wild type, heterozygous, and homozygous for the variant groups, respectively, after adjustment for intake of α-linolenic acid, EPA, DHA, and other potential confounders. Total, LDL, or HDL cholesterol were not associated with the genotype.

The effect of α-linolenic acid and *FADS2* genotype on the risk of MI is shown in **Table 3**. High α-linolenic acid was associated with a decreased risk of MI regardless of *FADS2* genotype. *FADS2* was not significantly associated with MI risk. ORs and 95% CIs in a comparison with homozygous of the wild type

group were 0.97 (0.82, 1.14) and 1.01 (0.83, 1.23) for the heterozygous and homozygous of the variant groups, respectively. Adjustment for potential confounders did not significantly change the results.

DISCUSSION

We found that a common deletion in the *FADS2* promoter was associated with low EPA and arachidonic acid in tissues. The consistency of the observed pattern in both n−3 and n−6 fatty acids in 2 different tissues (adipose and plasma) together with the monotonic trend by genotype gives substantial plausibility to the hypothesis of decreased Δ⁶-desaturase activity with an increased number of copies of the common deletion (*T*/-). The increase in fasting plasma triacylglycerols associated with the variant is consistent with the known effect of very-long-chain n−3 fatty acids on triacylglycerol concentrations. This polymorphism is not associated with the risk of MI and does not modify the association of α-linolenic acid and MI in the whole population.

Our findings on the effect of the *FADS2* promoter *T*/-deletion on fatty acids are consistent with findings from a recent study that also showed a metabolic effect of polymorphisms and haplotypes of *FADS2* and *FADS1* (Δ⁵-desaturase) on serum phospholipid fatty acids (27). The mechanism for the observed effects of the *T*/- deletion on the PUFA biosynthetic pathway probably involves a decrease in Δ⁶-desaturase transcription that affects the conversion of α-linolenic acid and linoleic acid into the very-long-chain PUFAs arachidonic acid and EPA. It is known that the fatty acid 20:2n−6 represents a dead-end product in the biosynthetic PUFA pathway (Figure 1), given that the enzyme necessary for its conversion is not present in mammals (5, 6). It is therefore not surprising that carriers of the deletion have higher concentrations of 20:6n−6 than do noncarriers. Consistently, EPA concentrations decreased with the number of *T*/- deletions, and 20:3n−3 increased in both adipose tissue and plasma. Our data also suggest that our findings cannot be attributed to substrate availability, because α-linolenic acid and linoleic acid (in adipose tissue, plasma, and diet) did not differ significantly by genotype.

Our finding of an association between the *T*/- deletion and higher triacylglycerol concentrations, although modest, is not surprising. The hypotriacylglycerolemic effect of dietary very-long-chain n−3 fatty acids is well described in the literature (28). The effect of endogenous pools of very-long-chain n−3 fatty acids could affect plasma triacylglycerol concentrations through similar mechanisms. Very-long-chain n−3 fatty acids increase lipoprotein lipase expression in adipose tissue, which may result in an increase in lipoprotein lipase-mediated triacylglycerol clearance (28). The *T*/- deletion has strong effects on very-long-chain PUFAs. For example, the interquartile range for arachidonic acid (0.37% to 0.56% of total fatty acids) is comparable to the difference in adipose tissue arachidonic acid between the homozygous for the *FADS2* variant (- -) and the homozygous for the wild type (*TT*) (0.41% and 0.52% of total fatty acids, respectively). Thus, on one hand, the *T*/- deletion may prevent the synthesis of very-long-chain n−3 fatty acids, which are protective (29, 30), and increase fasting triacylglycerols, which are an independent risk factor for IHD (28). On the other hand, the *T*/-deletion could prevent the accumulation of arachidonic acid in tissues. Adipose tissue arachidonic acid has been associated with an increased risk of MI (19, 20), suggesting that endogenous
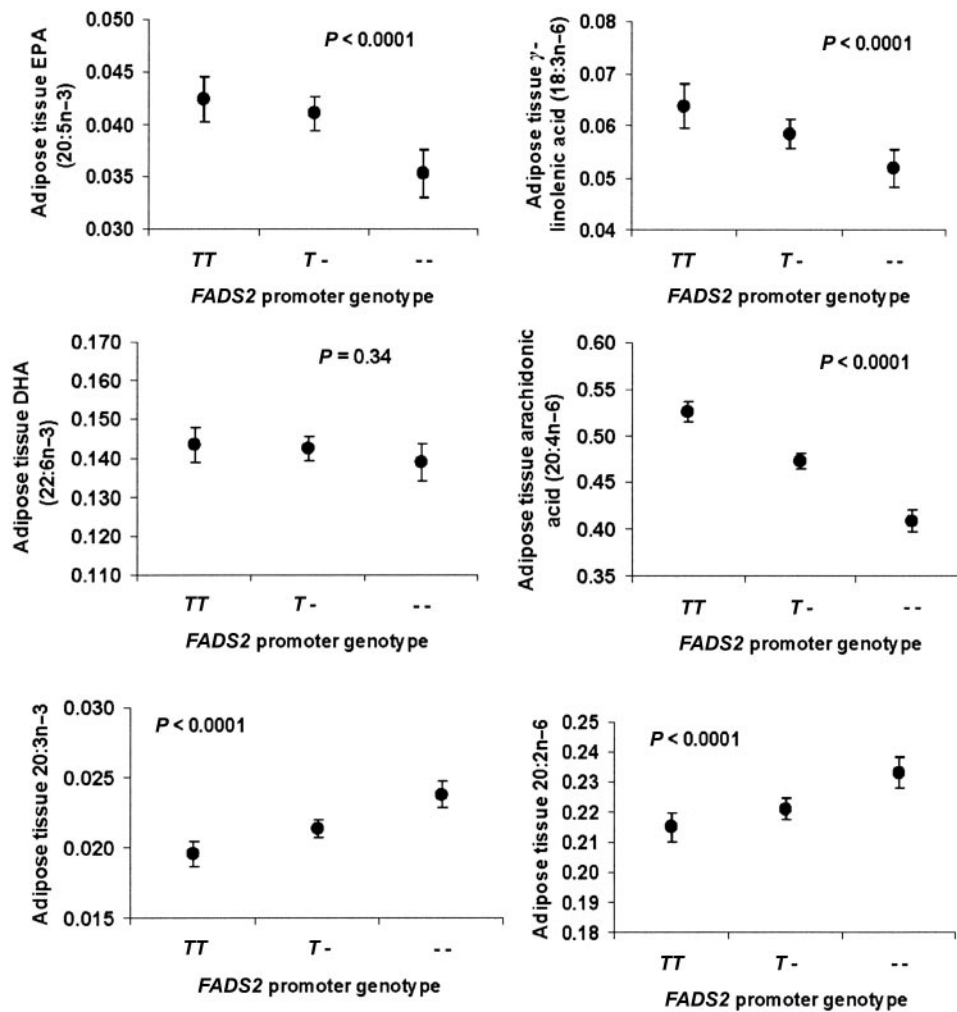
FIGURE 2. Mean (95% CI) adipose tissue fatty acids by *FADS2* promoter genotype among control subjects. - -, Homozygous for the variant (deletion) allele. ●, least-squares means adjusted for age, sex, area of residence, BMI, and fatty acids from diet [linoleic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)]; bars are 95% CIs. The model for adipose tissue γ-linolenic acid is also adjusted for γ-linolenic acid obtained in the diet, and geometric means are reported instead. $n = 1820$ except for 18:3n–6 ($n = 1743$), 20:4n–6 ($n = 1819$), 20:2n–6 ($n = 1812$), and 20:5n–3 ($n = 1475$). Absolute genotype frequencies were the following: $TT = 486$, $T- = 908$, and - - $= 426$.

TABLE 2
Plasma fatty acids by genotype in a subsample of control subjects[1]

	FADS2 promoter genotype			
	TT ($n = 57$)	*T-* ($n = 93$)	- - ($n = 46$)	*P*
18:3n–3	0.48 (0.43, 0.53)	0.49 (0.46, 0.53)	0.52 (0.46, 0.58)	0.56
20:5n–3[2]	0.41 (0.32, 0.51)	0.34 (0.30, 0.39)	0.27 (0.23, 0.33)	0.03
22:6n–3	1.56 (1.47, 1.65)	1.52 (1.45, 1.59)	1.32 (1.22, 1.43)	0.002
20:3n–3	0.011 (0.008, 0.014)	0.015 (0.012, 0.017)	0.021 (0.017, 0.024)	0.0002
18:2n–6	27.82 (26.83, 28.81)	27.70 (26.92, 28.47)	29.13 (28.03, 30.23)	0.0958
18:3n–6[3]	0.49 (0.46, 0.53)	0.39 (0.36, 0.42)	0.26 (0.22, 0.30)	< 0.0001
20:4n–6	7.33 (7.02, 7.64)	5.99 (5.74, 6.23)	4.46 (4.11, 4.81)	< 0.0001
20:2n–6[2]	0.26 (0.24, 0.27)	0.29 (0.28, 0.30)	0.31 (0.29, 0.33)	< 0.0001

[1] Least-squares means; 95% CIs in parentheses (unless otherwise specified). - -, Homozygous for the variant (deletion) allele. Adjusted for age, sex, area of residence, BMI, and fatty acids from diet (linoleic acid, α-linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid). $n = 196$ except for 18:3n–3 ($n = 162$), 20:2n–6 ($n = 195$), and 20:5n–3 ($n = 62$).

[2] Values are geometric $\bar{x}$; 95% CI in parentheses.

[3] Also adjusted for γ-linolenic acid in diet.

TABLE 3
Modifying effect of the *FADS2* deletion polymorphism on the association between adipose tissue α-linolenic acid and myocardial infarction[1]

	Low α-linolenic (0.49%)	High α-linolenic (0.76%)	*P* for interaction
All subjects (*n* = 3388)	1.00	0.67 (0.55, 0.82)[2]	
Stratified by genotype (*n* = 3388)			
TT	1.00	0.60 (0.44, 0.83)	0.41
T–	1.00	0.74 (0.58, 0.95)	
– –[3]	1.00	0.61 (0.44, 0.85)	

[1] Models are adjusted for matching factors, income, history of diabetes, history of hypertension, physical activity, smoking status, adipose tissue *trans* 18:2 fatty acids, and adipose tissue linoleic acid. Further adjustment for other dietary variables, hormone replacement therapy use, and other potential confounders did not significantly change the results. α-Linolenic is divided into high or low, according to the median of the distribution of α-linolenic acid in adipose tissue.
[2] Odds ratio; 95% CI in parentheses (all such values).
[3] Homozygous for the variant (deletion) allele.

pools of arachidonic acid may have an effect on MI. These effects may be related to the role of arachidonic acid as a precursor of proinflammatory eicosanoids (31) or mediated by insulin sensitivity, because altered distribution of arachidonic acid in tissues has been correlated with alterations in lipogenesis and insulin action (32). Given that this polymorphism has both negative and favorable effects on metabolism, it is not surprising that the net effect of the *T*/-deletion on the risk of MI is null in this population.

Several studies have not found a protective association between α-linolenic acid and MI (33–36). Although part of the discrepancies may be explained by different ranges of intake and different compositions of the diet, it is possible that genetic background modifies the effect of dietary PUFA on the development of IHD, particularly if genetic mutations in the desaturases and elongases, which are directly responsible for PUFA biosynthesis, are present. Furthermore, from isotope-labeled α-linolenic feeding studies, the range of conversion of α-linolenic to EPA has been estimated between 0.2% and 21% (37). This considerable variation may be due, in part, to dietary influences (feedback regulation and n−6 competition for the shared enzymes) but also to genetic background. We did not find a modifying effect of the *FADS2* deletion on the protective effect of α-linolenic acid. However, note that the relation between adipose tissue α-linolenic acid and MI is not linear, suggesting that α-linolenic acid does not confer additional protection at higher adipose tissue levels (> 0.70% total fatty acids; A Baylin and H Campos, 2006, unpublished observations). Furthermore, the pathway is feedback regulated, and a high intake of α-linolenic and linoleic acid down-regulates the Δ⁶-desaturase. It is possible that at current intakes of α-linolenic acid any potential defect in the transcription of the gene is masked by the availability of substrate. Therefore, as a suggestion for further studies, we explored whether the *FADS2* deletion modified the effect of α-linolenic acid among those with low α-linolenic acid intake. In the Costa Rican population, the lowest intake is found among those that use palm oil for cooking, because palm oil does not contain α-linolenic acid. Interestingly, we found a significant interaction (*P* value for interaction = 0.047) between *FADS2* genotype, α-linolenic acid in adipose tissue, and the risk of MI among palm oil users, suggesting that at low intakes of α-linolenic acid, the protective effect of α-linolenic acid is attenuated among carriers of the variant allele. Further research in populations with low intake of α-linolenic acid and very-long-chain n−3 fatty acids is warranted.

Although there are no available epidemiologic studies on the *FADS2* gene, there is one case report in a patient with an inborn error of lipid metabolism (38). In this case, severe clinical symptoms described Δ⁶-desaturase deficiency (for example, 80% decrease in gene transcription) that was attributed to an insertion of a thymidine in the transcriptional regulatory region of the gene (38). Because this is the same allele that we found in 52% of the participants in our population (who do not present any clinical symptoms as described), it is possible that the allele identified by Nwankwo et al (38) was not responsible for the severe clinical phenotype described. Most likely, the high frequency of the *T* allele resulted in a spurious link to the phenotype, given that only 3 controls were evaluated.

The strengths of our study include the high participation rate in cases and population-based controls, the availability of fatty acid composition from both adipose tissue and plasma, and the use of biomarkers to assess dietary intake. As in all observational studies, we could not establish a causal relation; however, the consistency of our findings in 2 different tissues makes our hypothesis plausible, and results were not likely to be confounded by diet, age, sex, area of residence, or BMI. However, it is possible that the *T*/- deletion is in linkage disequilibrium with other functional SNPs in the *FADS2* gene. Because several fatty acid concentrations were assessed, multiple comparisons may be another limitation of the study. However, we limited the fatty acid comparisons to our a priori hypothesis, and the consistency of the association in both tissues makes chance an unlikely explanation for the findings. Further studies on this polymorphism and others in the *FADS2* gene are warranted to reassess the effect of n−3 and n−6 PUFA on IHD. These future studies will be of special importance in those populations in which the intake of α-linolenic acid and very-long-chain n−3 fatty acids from fish (EPA and DHA) is very low.   ❧

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