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# Genetic variation of the *FADS1 FADS2* gene cluster and *n*-6 PUFA composition in erythrocyte membranes in the European Prospective Investigation into Cancer and Nutrition-Potsdam study

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Delta-5 (D5D) and delta-6 (D6D) desaturases are key enzymes in PUFA metabolism. Several factors (e.g. hyperglycaemia, hypertension, blood lipids, statins and fatty acids in diet and biological tissues) may influence desaturase activity. The goals were to evaluate the associations between variation in genes encoding these desaturases (*FADS1* and *FADS2*) and blood concentrations of *n*-6 PUFA and estimated D5D and D6D activities (evaluated as product/precursor ratio), and to investigate whether other factors influencing the activity of desaturases modify these associations. A random sample of 2066 participants from the European Prospective Investigation into Cancer and Nutrition-Potsdam study (*n* 27 548) was utilised in the analyses. Crude and adjusted associations between rs174546 genotypes (reflecting genetic variation in the *FADS1 FADS2* gene cluster), *n*-6 PUFA in erythrocytes and estimated desaturase activities were evaluated using multiple linear regression. Potential effect modification was determined by performing stratified analyses and evaluating interaction terms. We found rs174546 genotypes to be related to linoleic ($r^2$ 0·060), γ-linolenic ($r^2$ 0·041), eicosadienoic ($r^2$ 0·034), arachidonic ($r^2$ 0·026), docosatetraenoic acids ($r^2$ 0·028), estimated D6D activity ($r^2$ 0·052) and particularly strongly to dihomo-γ-linolenic acid (DGLA, $r^2$ 0·182) and D5D activity ($r^2$ 0·231). We did not observe effect modifications with regard to the estimated D5D activity, DGLA and arachidonic acid (AA) for most of the factors evaluated; however, the genetic effect on D5D activity and DGLA may be modified by the dietary *n*-6:*n*-3-ratio (*P*-values for interaction: 0·008 and 0·002), and the genetic effect on DGLA and AA may be modified by lipid-lowering medication (*P*-values for interaction: 0·0004 and 0·006). In conclusion, genetic variation in the *FADS1 FADS2* gene cluster affects *n*-6 PUFA profiles in erythrocytes reflecting altered D5D activity.

**Arachidonic acid: Fatty acid desaturases: Delta-5 desaturase: Delta-6 desaturase: Effect modification: Fatty acids, unsaturated: γ-Linolenic acid: Linolenic acid: Linoleoyl-CoA desaturase**

Fatty acids determine the biophysical properties of membranes and hence alter cell function. PUFA are believed to be associated with a variety of chronic diseases, e.g. type 2 diabetes mellitus, CHD and Alzheimer's disease[1]. While pools of longer-chain PUFA in the human body may originate from dietary intake, individual longer-chain PUFA are also the result of elongation and desaturation of their dietary precursors, α-linolenic acid (ALA, 18 : 3*n*-3) and linoleic acid (LA, 18 : 2*n*-6). Key enzymes in these biosynthesis pathways are delta-5 (D5D) and delta-6 desaturases (D6D) (a scheme is presented in Lattka *et al.*[2]). With regard to *n*-6 PUFA, D6D converts LA to γ-linolenic acid (GLA, 18 : 3*n*-6) and D5D converts dihomo-GLA (DGLA, 20 : 3*n*-6) to arachidonic acid (AA, 20 : 4*n*-6). GLA is elongated to DGLA, LA is elongated to eicosadienoic acid (EDA, 20 : 2*n*-6) and AA is elongated to docosatetraenoic acid (DTA, 22 : 4*n*-6) by the enzyme elongase. Recently, it was shown that delta-8 desaturase converts EDA to DGLA[3]. With regard to *n*-3 PUFA, ALA is converted by D5D and D6D to EPA (20 : 5*n*-3). EPA is elongated to docosapentaenoic acid (22 : 5*n*-3) and converted

**Abbreviations:** AA, arachidonic acid; ALA, α-linolenic acid; CC, homozygote carrier of the major C-allele; CT, carrier of the minor T-allele; D5D, delta-5 desaturase; D6D, delta-6 desaturase; DGLA, dihomo-γ-linolenic acid; DTA, docosatetraenoic acid; EDA, eicosadienoic acid; GLA, γ-linolenic acid; LA, linoleic acid; LD, linkage disequilibrium.

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by D6D to DHA (22 : 6*n*-3). D5D and D6D are expressed in the majority of human tissues, with the highest levels being found in the liver, brain, heart and lung. Currently, studies on substrate specificity and enzyme kinetics have identified only DGLA and eicosatetraenoic acid (20 : 4*n*-3) as substrates for D5D. D6D seems to have broader substrate specificity with at least five substrates (LA, ALA, 24 : 4*n*-6, 24 : 5*n*-3 and 16 : 0)[4].

Although the activity of D5D and D6D is relatively low in human subjects when compared with other species like rats and mice[5], there is a common notion that endogenous desaturation of fatty acids is important in humans[6]. *In vivo* studies in human subjects using $^{2}$H-labelled LA and ALA indicated significant interconversion to AA, EPA and longer-chain PUFA. The available human data indicate that the amount of AA formed by desaturation of LA normally exceeds the dietary intake of AA[7].

D5D and D6D are encoded by the *FADS1* and *FADS2* genes, which are localised head to head as a cluster on chromosome 11, with exon 1 of both the genes being separated by an 11 kb region. The proximity of the promoters suggests the possibility that transcription of *FADS1* and *FADS2* may be coordinately controlled by common regulatory sequences within the 11 kb region[8]. Common genetic variants of *FADS1* and *FADS2* have been reported to be in strong linkage disequilibrium (LD) with one LD block spanning over *FADS1* and the intergenic region well into the promoter region of *FADS2*[9–11]. Although an influence on promoter activity and binding affinity of nuclear protein complexes was observed for rs968567, a SNP within the promoter region of *FADS2*, consequences of genetic variation within *FADS1* and *FADS2* on desaturase expression or activity and its biological importance are largely unknown[2]. However, associations between several SNP or haplotypes in the *FADS* gene cluster and PUFA concentrations in different human tissues have been observed[9,12–15]. Minor alleles of SNP in *FADS1* and/or *FADS2* were commonly associated with increased LA concentrations and decreased AA concentrations[9–12,14]. However, the effect of SNP on *n*-6 PUFA such as DGLA has been investigated by few studies so far[9,13,14].

Besides genetic variants, several other factors have been found to have an influence on the activity of desaturases and thus on the PUFA concentrations[1,8,16,17]. These factors include ageing, hyperglycaemia, diabetes, hypertension, metabolic syndrome, blood lipids and alcohol consumption[1]. Treatment with statins and glitazones, physical activity and cigarette smoking are also hypothesised to relate to concentrations of DGLA and AA by affecting desaturase activities[1,17–19]. Furthermore, the activity of D5D and D6D is modulated by dietary fatty acid intake and the level of fatty acids in biological tissues. In general, desaturase activities are influenced by the amount of and relationship between the substrates LA and ALA, while the desaturation products AA and long-chain *n*-3 fatty acids inhibit the conversion of LA and ALA to their longer chain metabolites[7,18]. Additionally, saturated and *trans* fats are regarded as inhibitors of D5D and D6D[1]. Although previous studies on *FADS* polymorphisms and PUFA concentrations adjusted in their analyses for some characteristics like age, sex and BMI[9,11–13,20], other factors potentially affecting desaturase activities have rarely been considered. While it cannot be expected that the associations between genetic polymorphisms and concentrations of PUFA are confounded by these factors, the genetic effect on PUFA concentrations might differ depending on other factors that modify the activity of desaturases.

The goals of the research study described herein were to evaluate the associations between rs174546 in *FADS1* (which was considered to be a proxy for the *FADS1 FADS2* gene cluster) and concentrations of *n*-6 PUFA and estimated D5D and D6D activities (evaluated as product/precursor ratio), and to investigate whether the following factors modify these associations: sex, age, smoking, obesity, alcohol consumption, level of physical activity, presence of hypertension, dyslipidaemia or hyperglycaemia, use of lipid-lowering medication, and fatty acid proportions in diet and erythrocytes.

## Subjects and methods

### *Study population*

The European Prospective Investigation into Cancer and Nutrition-Potsdam study is part of the German arm of the multicentre prospective cohort study European Prospective Investigation into Cancer and Nutrition[21]. In Potsdam, Germany, 27 548 subjects (16 644 women and 10 904 men) were recruited from the general population between 1994 and 1998. The age range was mainly 35–65 years in women and 40–65 years in men. The baseline examination included the collection of blood samples, anthropometric measurements, a self-administered validated FFQ, and a personal interview including a questionnaire about socio-demographic and lifestyle characteristics. Information on educational attainment, smoking and physical activity was assessed with a self-administered questionnaire and a personal interview. Informed consent was obtained from all the participants, and approval was given by the Ethics Committee of the State of Brandenburg, Germany.

A random subcohort of 2500 individuals was selected from all the participants of the European Prospective Investigation into Cancer and Nutrition-Potsdam study population who provided blood samples (*n* 26 444). We excluded ninety participants for whom not all relevant biomarkers were available and twelve individuals with implausible values in biomarkers. Furthermore, we excluded 332 participants for whom the measurement of *n*-6 PUFA concentrations was considered to be unreliable. Altogether, 2066 participants were included for analyses.

### *Measurement of biochemical variables*

Peripheral venous blood samples taken from the baseline examination that were performed were centrifuged at 1000 ***g*** for 10 min at 4°C. Plasma, erythrocytes and buffy coat were aliquoted and stored at −80°C. Fatty acids in erythrocytes were utilised for the study of fatty acid metabolism in the present study (in accordance with the recommendations made by Lattka *et al.*[4], Malerba *et al.*[12]) and were measured between February and June 2008 at the Laboratory of Dutch National Institute for Public Health and the Environment. For each sample of 200 µl erythrocytes, 800 µl distilled water were added and centrifuged for 10 min at 3000 rpm. The resulting pellets, which contained the phospholipid

membranes, were washed with 800 μl distilled water and centrifuged again. After the addition of 400 μl distilled water and 3 ml chloroform–methanol (1:1, v/v), the mixture was shaken. The chloroform layer was transferred into another tube, and the solvent was removed by evaporation. The phospholipids were hydrolysed and methylated simultaneously with a mixture of 100 μl toluene and 0·5 ml $BF_3$–MeOH for 60 min at 100°C in a heating block. After cooling, 800 μl distilled water and 800 μl hexane were added. After shaking and settling, the hexane layer (upper layer) containing the fatty acid methyl ester was transferred to GC vials and stored at −20°C until analysis. The fatty acid methyl esters were separated on a 100 m × 0·25 mm internal diameter wall-coated open tubular fused silica capillary column and were coated with 0·25 μm of CP-Select CB, provided by Varian Ass., using a Varian Ass. GC-3900 gas chromatograph equipped with a CP 8400 auto injector. The Galaxie software was used for quantification and identification of peaks. Baseline separation of over fifty fatty acid methyl ester peaks was accomplished by means of mixed fatty acid methyl ester standards (Sigma, St Louis, MO, USA). The analytical conditions employed were as follows: volume injected 1 μl; carrier gas $N_2$ (1·1 ml/min); injector temperature 250°C; flame ionisation detection 275°C; split ratio 1:20 and oven temperature from 185 to 245°C with stepped temperature program: within total run time 57 min. The fatty acids have been expressed as % of the total fatty acids present in the chromatogram.

Plasma concentrations of glucose, HDL-cholesterol and TAG were measured with the automatic ADVIA 1650 analyser (Siemens Medical Solutions, Erlangen, Germany). Non-HDL-cholesterol was calculated as the difference between total cholesterol and HDL-cholesterol.

### *SNP selection and genotyping*

We genotyped rs174546 which is located on chromosome 11 (11q12–13·1) in *FADS1*. Rs174546 belongs to a highly preserved LD block involving other SNP in *FADS1*, several variants in the intergenic region between *FADS1* and *FADS2*, several variants within *FADS2*, as well as other variants near *FADS1* (a scheme is presented in Schaeffer *et al.*[9]). Reconstructed haplotypes within this LD block have been reported to represent mainly either carrying the major alleles at all loci or carrying only the minor alleles at all loci[9,13]. Pairwise tagging of SNP with minor allele frequencies > 0·05 and an $r^2$ threshold of 0·8 based on HapMap CEU data release 21 revealed that rs174546 tags fifteen other SNP within this LD block with many of them carrying synonymous information ($r^2$ 1 for rs174535, rs174536, rs174537, rs102275, rs174538, rs174545, rs174546, rs174547, rs174550, rs1535, rs174576, rs174577). Rs174546 is also strongly correlated with rs3834458, a minor deletion mutation in the promoter region of *FADS2*[9]. Several reports have linked rs174546 or strongly correlated SNP to PUFA concentrations in plasma or erythrocytes[9,10,13,15,20]. Furthermore, the strongest association with *n*-6 PUFA in whole-genome association studies was observed for rs174537 near *FADS1*[11] which is in complete LD with rs174546. Rs174546 has also been shown to reach whole-genome significance to *FADS1* gene expression in lymphoblastoid cells[22]. Although high LD between SNP most likely prohibits identification of the real functional variant in association studies, rs174546 was picked as a plausible proxy. It should be mentioned that other SNP in the *FADS* region not being in LD with rs174546 have been reported to be associated with PUFA concentrations; however, associations were considerably weaker[9]. Genotyping of rs174546 was performed by TaqMan technology (Applied Biosystems, Foster City, CA, USA) on 384-well plates. Genotyping error was < 0·5 %, and genotypes were in Hardy–Weinberg equilibrium ($P > 0·05$).

### *Dietary assessment*

All the participants were asked to complete a semi-quantitative FFQ. This FFQ assessed the average intake frequency and portion size of 148 foods consumed during the 12 months before examination. Intake frequency was measured using ten categories, ranging from 'never' to 'five times per d or more'. Portion sizes were estimated using photographs of standard portion sizes. Information on intake frequency and portion size was used to calculate the amount of each food item in g consumed on average per d. Nutrient intake was calculated from the food items according to the German Food Code and Nutrient Data Base[23] version II.3. Intakes of fatty acids were expressed as proportions of total fat intake. The validity and reproducibility of the FFQ have been described previously[24–26]. Briefly, energy-adjusted correlation coefficients (corrected for intra-individual variation in dietary recall data) between the FFQ (non-calibrated) and twelve 24 h recalls were 0·76 for SFA, 0·77 for MUFA and 0·70 for PUFA[26].

### *Statistical analysis*

D5D activity was estimated as the AA:DGLA ratio, and D6D activity was estimated as the GLA:LA ratio. We used multiple linear regression models with *n*-6 PUFA concentrations and D5D and D6D activities as dependent variables, and with rs174546 genotypes as the independent variable. Crude as well as adjusted associations were calculated to investigate possible confounding effects. Model 1 was adjusted for sex and age (< 45, 45–49, 50–54, 55–59 and ≥ 60 years), model 2 was further adjusted for smoking status (never, past, current < 20 cigarettes/d and current ≥ 20 cigarettes/d), alcohol consumption (0, > 0– < 15, 15– < 30, 30– < 40 and ≥ 40 g/d), sport activities (0, > 0–2, > 2–4, > 4–6 and > 6 h/week) and education (in training or no training or vocational training, technical school or technical college or university degree), and model 3 was further adjusted for hypertension (% with medication or systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg), as well as for sex-specific quintiles of BMI, waist circumference, and plasma concentrations of TAG, HDL-cholesterol, non-HDL-cholesterol and glucose.

For stratified analyses, continuous variables were dichotomised based on established disease or risk thresholds for which associations with desaturase activities have previously been reported[1] (waist circumference: men ≥ 94 cm and women ≥ 80 cm; fasting plasma glucose ≥ 5·6 mmol/l or non-fasting plasma glucose ≥ 11·1 mmol/l; TAG > 1·7 mmol/l; HDL-cholesterol: men ≥ 1·03 mmol/l and women ≥ 1·29 mmol/l; non-HDL-cholesterol ≥ 4·1 mmol/l; systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg;

**Table 1.** Characteristics of the study population according to rs174546 genotypes (EPIC-Potsdam study, *n* 2066)
(Mean values and 95 % confidence intervals or percentages)

| | | | | rs174546 genotype | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total population (*n* 2066) | | | CC (*n* 946) | | | CT (*n* 875) | | | TT (*n* 245) | | | |
| Characteristic | % | Mean | 95 % CI | % | Mean | 95 % CI | % | Mean | 95 % CI | % | Mean | 95 % CI | *P** |
| General characteristics | | | | | | | | | | | | | |
| Sex (men) | 38·6 | | | 37·3 | | | 39·1 | | | 41·6 | | | 0·43 |
| Age (years) | | 49·9 | 49·5, 50·3 | | 49·9 | 49·3, 50·4 | | 49·9 | 49·3, 50·4 | | 50·2 | 49·1, 51·3 | 0·84 |
| Education (with at least technical school) | 62·4 | | | 62·8 | | | 62·1 | | | 62·0 | | | 0·94 |
| Smoking (current smokers) | 21·6 | | | 22·8 | | | 21·1 | | | 18·8 | | | 0·35 |
| Alcohol consumption (g/d)† | | 7·1 | 7·1, 7·1 | | 6·9 | 6·9, 7·0 | | 7·2 | 7·1, 7·2 | | 7·2 | 7·1, 7·3 | 0·59 |
| Sport activity (h/week)† | | 1·9 | 1·9, 1·9 | | 2·0 | 1·9, 2·0 | | 1·9 | 1·9, 1·9 | | 1·9 | 1·9, 2·0 | 0·58 |
| BMI (kg/m$^2$) | | 26·1 | 25·9, 26·3 | | 26·0 | 25·7, 26·3 | | 26·3 | 26·0, 26·6 | | 26·0 | 25·5, 26·5 | 0·33 |
| Waist circumference (cm) | | 85·9 | 85·3, 86·4 | | 85·6 | 84·7; 86·4 | | 86·3 | 85·4, 87·1 | | 85·7 | 84·2, 87·2 | 0·49 |
| TAG (mmol/l)† | | 1·20 | 1·20, 1·20 | | 1·16 | 1·16, 1·16 | | 1·24 | 1·24, 1·24 | | 1·19 | 1·19, 1·20 | 0·043 |
| HDL-cholesterol (mmol/l) | | 1·36 | 1·35, 1·38 | | 1·38 | 1·36, 1·40 | | 1·34 | 1·32, 1·36 | | 1·39 | 1·34, 1·44 | 0·027 |
| Non-HDL-cholesterol (mmol/l) | | 3·17 | 3·13, 3·21 | | 3·19 | 3·14, 3·25 | | 3·16 | 3·11, 3·22 | | 3·10 | 2·99, 3·22 | 0·25 |
| Hypertension (with medication or SBP ≥ 140 mmHg or DBP ≥ 90 mmHg) | 50·2 | | | 47·9 | | | 52·3 | | | 51·4 | | | 0·15 |
| Lipid-lowering medication (with statin, fibrate medication) | 5·1 | | | 5·4 | | | 5·3 | | | 3·3 | | | 0·74 |
| Glucose (mmol/l)† | | 5·37 | 5·37, 5·37 | | 5·42 | 5·42, 5·43 | | 5·32 | 5·32, 5·32 | | 5·37 | 5·36, 5·38 | 0·17 |
| Elevated glucose (with type 2 diabetes or fasting glucose ≥ 5·6 mmol/l, non-fasting glucose ≥ 11·1 mmol/l) | 12·7 | | | 12·6 | | | 12·3 | | | 14·3 | | | 0·72 |
| Metabolic syndrome‡ | 27·7 | | | 25·9 | | | 29·0 | | | 29·8 | | | 0·24 |
| Dietary fatty acids (% of total fat intake) | | | | | | | | | | | | | |
| SFA | | 40·9 | 40·7, 41·1 | | 40·9 | 40·6, 41·1 | | 41·0 | 40·7, 41·2 | | 40·7 | 40·2, 41·2 | 0·70 |
| LA | | 15·1 | 14·9, 15·3 | | 15·3 | 15·0, 15·5 | | 14·9 | 14·7, 15·2 | | 15·1 | 14·6, 15·5 | 0·19 |
| AA | | 0·21 | 0·21, 0·22 | | 0·21 | 0·21, 0·22 | | 0·21 | 0·21, 0·22 | | 0·21 | 0·21, 0·22 | 0·84 |
| ALA† | | 1·85 | 1·85, 1·85 | | 1·87 | 1·87, 1·87 | | 1·83 | 1·83, 1·83 | | 1·84 | 1·84, 1·85 | 0·039 |
| EPA† | | 0·12 | 0·12, 0·12 | | 0·11 | 0·11, 0·11 | | 0·11 | 0·11, 0·12 | | 0·13 | 0·12, 0·13 | 0·26 |
| DHA† | | 0·21 | 0·21, 0·21 | | 0·21 | 0·21, 0·21 | | 0·21 | 0·21, 0·21 | | 0·22 | 0·22, 0·22 | 0·31 |
| *n*-6:*n*-3 ratio | | 6·62 | 6·55, 6·69 | | 6·64 | 6·64, 6·64 | | 6·62 | 6·62, 6·62 | | 6·54 | 6·53, 6·55 | 0·65 |
| Fatty acids in erythrocytes (% of total FA) | | | | | | | | | | | | | |
| *n*-6 PUFA | | | | | | | | | | | | | |
| LA | | 10·67 | 10·61, 10·73 | | 10·39 | 10·32, 10·47 | | 10·77 | 10·69, 10·86 | | 11·40 | 11·22, 11·58 | <0·0001 |
| GLA† | | 0·0515 | 0·0515, 0·0515 | | 0·0558 | 0·0558, 0·0559 | | 0·0500 | 0·0500, 0·0500 | | 0·0421 | 0·0419, 0·0422 | <0·0001 |
| EDA | | 0·256 | 0·254, 0·258 | | 0·250 | 0·247, 0·252 | | 0·259 | 0·256, 0·262 | | 0·273 | 0·267, 0·279 | <0·0001 |
| DGLA | | 1·50 | 1·48, 0·151 | | 1·37 | 1·36, 1·39 | | 1·55 | 1·53, 1·57 | | 1·79 | 1·74, 1·85 | <0·0001 |
| AA | | 12·60 | 12·49, 12·70 | | 12·95 | 12·79, 13·10 | | 12·46 | 12·30, 12·62 | | 11·73 | 11·44, 12·02 | <0·0001 |
| DTA | | 2·62 | 2·59, 2·65 | | 2·72 | 2·68, 2·76 | | 2·58 | 2·53, 2·62 | | 2·37 | 2·30, 2·45 | <0·0001 |
| *n*-3 PUFA | | | | | | | | | | | | | |
| ALA | | 0·16 | 0·15, 0·16 | | 0·15 | 0·14, 0·15 | | 0·16 | 0·16, 0·17 | | 0·18 | 0·17, 0·18 | <0·0001 |
| EPA† | | 0·73 | 0·73, 0·73 | | 0·74 | 0·74, 0·75 | | 0·72 | 0·71, 0·72 | | 0·70 | 0·70, 0·70 | 0·069 |
| DPA | | 2·25 | 2·23, 2·28 | | 2·26 | 2·23, 2·30 | | 2·25 | 2·21, 2·28 | | 2·23 | 2·16, 2·30 | 0·54 |
| DHA | | 4·60 | 4·54, 4·66 | | 4·60 | 4·51, 4·68 | | 4·59 | 4·50, 4·68 | | 4·69 | 4·51, 4·87 | 0·60 |
| *Trans*-fatty acids† | | 0·71 | 0·71, 0·71 | | 0·71 | 0·71, 0·71 | | 0·71 | 0·71, 0·71 | | 0·71 | 0·71, 0·71 | 0·82 |
| SFA† | | 43·93 | 43·93, 43·94 | | 44·04 | 44·03, 44·05 | | 43·89 | 43·88, 43·89 | | 43·71 | 43·68, 43·73 | 0·035 |

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**Table 1.** *Continued*

| Characteristic | Total population (*n* 2066) | | | rs174546 genotype | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | CC (*n* 946) | | | CT (*n* 875) | | | TT (*n* 245) | | | |
| | % | Mean | 95 % CI | % | Mean | 95 % CI | % | Mean | 95 % CI | % | Mean | 95 % CI | *P** |
| Estimated desaturase activities | | | | | | | | | | | | | |
| Estimated delta-5 desaturase activity (20 : 4*n*-6/20 : 3*n*-6 ratio) | | 8·63 | 8·54, 8·71 | | 9·56 | 9·44, 9·67 | | 8·16 | 8·05, 8·27 | | 6·71 | 6·51, 6·91 | <0·0001 |
| Estimated delta-6 desaturase activity (18 : 3*n*-6/18 : 2*n*-6 ratio) | | 0·0055 | 0·0054, 0·0056 | | 0·0061 | 0·0059, 0·0063 | | 0·0052 | 0·0051, 0·0054 | | 0·0042 | 0·0039, 0·0045 | <0·0001 |

CC, homozygote carrier of the major C-allele; CT, heterozygote carrier of the minor T-allele; TT, homozygote carrier of the minor T-allele; SBP, systolic blood pressure, DBP, diastolic blood pressure; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-γ-linolenic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid.

* $\chi^2$ test is used for categorical variables, ANOVA is used for normally distributed variables, rank-sum test is used for continuous variables that are not normally distributed.

† Data represent geometric means and 95 % confidence limits because variable was not normally distributed.

‡ Metabolic syndrome: men: waist circumference ≥ 94 cm and at least two of the following characters: TAG > 1·7 mmol/l; HDL-cholesterol < 1·03 mmol/l; SBP ≥ 130 mmHg or DBP ≥ 85 mmHg; or medication for hypertension; fasting glucose ≥ 5·6 mmol/l or non-fasting glucose ≥ 11·1 mmol/l; or self-reported diabetes and women: waist circumference ≥ 80 cm and at least two of the following characters: TAG > 1·7 mmol/l; HDL-cholesterol < 1·29 mmol/l; SBP ≥ 130 mmHg or DBP ≥ 85 mmHg; or medication for hypertension; fasting glucose ≥ 5·6 mmol/l or non-fasting glucose ≥ 11·1 mmol/l; or diabetes mellitus.

moderate alcohol consumption (≥ 12 g/d), regular sport activity (> 1 h/week)) or on the population median (fatty acids in erythrocytes (% of total fatty acids): EPA, > 0·72 %; *n*-3-PUFA, > 8·0 %; *trans*-fatty acid, > 0·71 %; SFA, > 43·9 %; *n*-6:*n*-3 ratio, ≥ 3·68 and dietary fatty acids (% of total fat intake): LA, > 14·9 %; AA, > 0·208 %; α-linolenic acid, > 1·82 %; EPA, > 0·122 %; DHA, > 0·212 %; SFA, > 40·6 %; *n*-6:*n*-3 ratio, 6·48). We present means for DGLA and AA concentrations and the estimated D5D activity stratified by the afore-mentioned factors. Effect modification was evaluated by including interaction terms in the multiple linear regression with DGLA, AA and the estimated D5D activity as dependent variables (model 3). Analysing the interaction terms within the crude model and model 1 did not change the conclusions gained using model 3; therefore, results of these additional analyses are not presented. Using Quanto (developed by Gauderman and Morrison, version 1.2), the power was 0·99 for all investigated gene–environment interaction terms.

All statistical analyses were performed using Statistical Analysis Systems statistical software package version 9.2 (SAS Institute, Cary, NC, USA). All *P*-values presented are two-sided; $P<0·05$ was considered statistically significant.

## Results

Of all the study participants, 46 % were homozygote carriers of the major C-allele (CC), 42 % were heterozygote, and 12 % were homozygote carriers of the minor T-allele (TT).

### *Genetic variation in the* FADS1 FADS2 *gene cluster and* n-6 *PUFA in erythrocyte membranes*

Table 1 shows general characteristics, dietary fatty acid intakes, proportions of fatty acids in erythrocyte membranes and estimated activities of D5D and D6D by rs174546 genotypes. There was no significant association between rs174546 genotypes and most general characteristics including sex, age, lifestyle variables, and certain biochemical and medical characteristics. Significant differences between the genotypes were observed for plasma TAG ($P=0·043$) and HDL-cholesterol ($P=0·027$); however, differences were modest in magnitude, and there was no clear trend across the genotypes. Similarly, dietary fatty acid intakes generally showed no significant relation to the genotype with the exception of ALA ($P=0·039$) whose intake was slightly higher in CC carriers. Strong associations with the genotype were observed for a number of fatty acids in erythrocyte membranes. Relations of *n*-6 PUFA in erythrocyte membranes to the genotype were all highly significant ($P<0·0001$). Here, we clearly observed higher proportions of LA, EDA and DGLA, and lower proportions of GLA, AA and DTA for the minor allele carriers. With regard to *n*-3 PUFA in erythrocyte membranes, ALA proportions were significantly higher ($P<0·0001$), while EPA levels were lower in the minor allele carriers, although the latter relation was only borderline significant ($P=0·069$). Docosapentaenoic acid and DHA did not differ significantly between the genotypes. Finally, lower concentrations of SFA in erythrocyte membranes were observed among carriers of the minor T-allele, while *trans*-fatty acid levels did not differ significantly between

the genotypes. Very strong and highly significant associations were observed between the genotypes of rs174546 and estimated activities of D5D and D6D ($P<0{\cdot}0001$). The estimated activities of both the desaturases strongly decreased with increasing number of the minor T-allele.

Table 2 presents the results of multivariate regression models with different levels of adjustment. We did not find any substantial difference between crude and adjusted effect estimates. Positive associations with LA, EDA and DGLA, negative associations with GLA, AA and DTA and the estimated D5D and D6D activities remained significant even after the adjustment for anthropometry, hypertension, blood lipid levels and glucose levels. The highest genetically explained variance was observed for the estimated D5D activity (crude $r^2$ 0·23). Compared with the CC genotype, the CT genotype was related to 1·396 units lower D5D activity, and the TT genotype was related to 2·845 units lower D5D activity. DGLA was also strongly associated with rs174546 genotype (CT, + 0·177 and homozygote carrier of the minor T-allele, + 0·423 % contribution to all fatty acids

**Table 2.** Association* between rs174546 genotypes and fatty acid composition in erythrocyte membranes, EPIC-Potsdam study (*n* 2066)
(Estimates and 95 % confidence intervals)

| | | CT | | TT | |
|---|---|---|---|---|---|
| | Adjusted $r^2$ | Estimate | 95 % CI | Estimate | 95 % CI |
| LA | | | | | |
| Crude | 0·060 | 0·377 | 0·261, 0·494 | 1·003 | 0·825, 1·182 |
| Model 1† | 0·074 | 0·383 | 0·267, 0·499 | 1·018 | 0·841, 1·195 |
| Model 2‡ | 0·118 | 0·384 | 0·271, 0·498 | 1·053 | 0·880, 1·227 |
| Model 3§ | 0·146 | 0·388 | 0·275, 0·500 | 1·045 | 0·873, 1·217 |
| GLA | | | | | |
| Crude | 0·041 | −0·007 | −0·009, −0·004 | −0·016 | −0·019, −0·012 |
| Model 1† | 0·075 | −0·007 | −0·009, −0·005 | −0·016 | −0·019, −0·013 |
| Model 2‡ | 0·087 | −0·007 | −0·009, −0·005 | −0·016 | −0·020, −0·013 |
| Model 3§ | 0·163 | −0·007 | −0·009, −0·005 | −0·016 | −0·019, −0·013 |
| EDA | | | | | |
| Crude | 0·034 | 0·009 | 0·006, 0·013 | 0·023 | 0·018, 0·029 |
| Model 1† | 0·060 | 0·009 | 0·006, 0·013 | 0·024 | 0·018, 0·029 |
| Model 2‡ | 0·066 | 0·009 | 0·006, 0·013 | 0·024 | 0·018, 0·029 |
| Model 3§ | 0·076 | 0·008 | 0·005, 0·012 | 0·023 | 0·017, 0·028 |
| DGLA | | | | | |
| Crude | 0·182 | 0·177 | 0·150, 0·203 | 0·423 | 0·382, 0·464 |
| Model 1† | 0·186 | 0·177 | 0·151, 0·204 | 0·425 | 0·384, 0·466 |
| Model 2‡ | 0·186 | 0·178 | 0·151, 0·204 | 0·428 | 0·387, 0·469 |
| Model 3§ | 0·221 | 0·168 | 0·142, 0·195 | 0·429 | 0·389, 0·470 |
| AA | | | | | |
| Crude | 0·026 | −0·490 | −0·711, −0·268 | −1·220 | −1·559, −0·882 |
| Model 1† | 0·042 | −0·483 | −0·702, −0·263 | −1·194 | −1·529, −0·858 |
| Model 2‡ | 0·039 | −0·487 | −0·708, −0·267 | −1·191 | −1·529, −0·854 |
| Model 3§ | 0·048 | −0·499 | −0·721, −0·277 | −1·215 | −1·553, −0·876 |
| DTA | | | | | |
| Crude | 0·028 | −0·143 | −0·204, −0·082 | −0·346 | −0·439, −0·253 |
| Model 1† | 0·073 | −0·140 | −0·199, −0·080 | −0·333 | −0·424, −0·242 |
| Model 2‡ | 0·074 | −0·142 | −0·202, −0·083 | −0·333 | −0·425, −0·242 |
| Model 3§ | 0·080 | −0·152 | −0·212, −0·092 | −0·339 | −0·430, −0·247 |
| D5D | | | | | |
| Crude | 0·231 | −1·396 | −1·558, −1·234 | −2·845 | −3·092, −2·598 |
| Model 1† | 0·240 | −1·392 | −1·553, −1·231 | −2·830 | −3·076, −2·585 |
| Model 2‡ | 0·242 | −1·399 | −1·560, −1·238 | −2·851 | −3·097, −2·604 |
| Model 3§ | 0·284 | −1·351 | −1·508, −1·193 | −2·875 | −3·116, −2·634 |
| D6D | | | | | |
| Crude | 0·052 | −0·0008 | −0·001, −0·0006 | −0·0019 | −0·0023, −0·0015 |
| Model 1† | 0·095 | −0·0009 | −0·001, −0·0006 | −0·0019 | −0·0023, −0·0016 |
| Model 2‡ | 0·124 | −0·0009 | −0·001, −0·0006 | −0·0020 | −0·0023, −0·0016 |
| Model 3§ | 0·194 | −0·0009 | −0·001, −0·0007 | −0·0020 | −0·0023, −0·0016 |

CT, heterozygote carrier of the minor T-allele; TT, homozygote carrier of the minor T-allele; LA, linoleic acid (18 : 2*n*-6); GLA, γ-linolenic acid (18 : 3*n*-6); EDA, eicosadienoic acid (20 : 2*n*-6); DGLA, dihomo-γ-linolenic acid (20 : 3*n*-6); AA, arachidonic acid (20 : 4*n*-6); DTA, docosatetraenoic acid (22 : 4*n*-6); D5D, estimated delta-5 desaturase activity (20 : 4*n*-6/20 : 3*n*-6); D6D, estimated delta 6 desaturase activity (18 : 3*n*-6/18 : 2*n*-6)

* Linear regression models with homozygote carriers of the major C-allele as reference.

† Model 1 was adjusted for sex and age (< 45, 45–49, 50–54, 55–59 and ≥ 60 years).

‡ Model 2 was further adjusted for smoking status (never, past, current < 20 cigarettes/d and current ≥ 20 cigarettes/d), alcohol consumption (0, > 0– < 15, 15– < 30, 30– < 40 and ≥ 40 g/d), sport activities (0, > 0–2, > 2–4, > 4–6 and > 6 h/week) and education (in training or no training or vocational training, technical school or technical college or university degree).

§ Model 3 was further adjusted for BMI (sex-specific quintiles), waist circumference (sex-specific quintiles), TAG (sex-specific quintiles), HDL-cholesterol (sex-specific quintiles), non-HDL-cholesterol (sex-specific quintiles), glucose (sex-specific quintiles) and hypertension (medication or systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg).

in the chromatogram compared with CC), which explained 18 % of its variance. The proportion of explained variance was between 3 and 6 % for most other *n*-6 PUFA and the D6D activity. Because the association between rs174546 and D6D was much less pronounced than the association between rs174546 and D5D (D6D, $r^2$ 0·05 and D5D, $r^2$ 0·23), the decision was made to focus on D5D in subsequent analyses to minimise the problem of multiple testing.

### *Stratified analyses for dihomo-γ-linolenic acid, arachidonic acid and estimated delta-5 desaturase activity*

Additional analyses were performed to assess whether the observed associations between rs174546 and D5D activity as well as DGLA and AA as determining precursor and product were modified by the presence of factors that are discussed to inhibit D5D activity. With regard to general characteristics, we found that participants with male sex, high age, large waist circumference, hypertension, high concentrations of TAG, non-HDL-cholesterol and glucose and low concentrations of HDL-cholesterol had significantly lower estimated D5D activity, while smoking, alcohol consumption, sport activity and lipid-lowering medication were not related to estimated D5D activity (Table 3). There was no significant effect modification between estimated D5D activity and almost all of these variables, except alcohol consumption and non-HDL-cholesterol concentrations (*P*-values for interaction=0·02). While lipid-lowering medication did not modify the genetic effect on D5D activity overall, participants with the TT genotype who took lipid-lowering medication (eight persons) had unexpectedly low concentrations of DGLA and very low concentrations of AA (Table 3, *P*-values for interaction: DGLA, 0·0004 and AA, 0·0065). Further significant effect modification was only observed for hypertension, TAG and non-HDL-cholesterol concentrations with DGLA (*P*-value for interaction $<$0·02). Because of the unexpected results regarding lipid-lowering medication, other *n*-6 PUFA were investigated to identify potential interactions with lipid-lowering medication. Significant effect modifications were observed for DTA (*P*-values for interaction: 0·005), but were not observed for LA, GLA and EDA (*P*-values $>$0·25; data not shown).

With respect to fatty acids in erythrocyte membranes, we observed significantly lower D5D activity among persons with lower concentrations of EPA, total *n*-3 PUFA and high *n*-6:*n*-3 ratio in erythrocyte membranes (Table 3). This finding was triggered by high AA concentrations in the group with high *n*-3 PUFA concentrations. A significantly lower D5D activity was found for persons with higher SFA concentrations in erythrocyte membranes, which is explained by very low AA concentrations in this group. We investigated whether these fatty acids in erythrocyte membranes modify the genotype–D5D association (Table 3), and we found no clear effect modification. Dietary intakes of LA, ALA, EPA, DHA, SFA and the dietary *n*-6:*n*-3 ratio were not significantly related to the estimated D5D activity when comparing high intakes with low intakes. However, the subjects with low AA intake had lower estimated D5D activity in our study population. There was no significant interaction of these dietary fatty acids with rs174546 genotypes, except for the dietary *n*-6:*n*-3 ratio. The association between rs174546 genotypes and estimated D5D activity appeared to be stronger in the subjects with a high dietary *n*-6:*n*-3 ratio compared with the subjects with a low *n*-6:*n*-3 ratio (*P* for interaction: 0·008). Similarly, the association between rs174546 genotypes and DGLA concentrations was slightly stronger for persons with a high dietary *n*-6:*n*-3 ratio (*P*-value for interaction: 0·002).

Using the valid, but conservative, Bonferroni method for adjusting for multiple comparisons (twenty-four interaction models)[27], there was no significant effect modification for all investigated variables for D5D activity and AA. For DGLA, the interaction terms with lipid-lowering medication and with the dietary *n*-6:*n*-3 ratio remained significant.

## Discussion

We observed higher LA, EDA and DGLA concentrations in erythrocyte membranes among carriers of the rs174546 minor T-allele, while lower concentrations were observed for AA, GLA and DTA. Estimated D5D and D6D activities were significantly lower for carriers of the minor allele. The highest genetically explained variances were found for the estimated D5D activity and DGLA concentrations. The associations were largely independent of other characteristics. Medication for blood lipids modified the genetic effect on DGLA and AA concentrations, but not on their ratio. Furthermore, the dietary *n*-6:*n*-3 ratio modified the relation of rs174546 genotype to DGLA concentrations and estimated D5D activity.

The observed associations between rs174546 genotypes and *n*-6 PUFA proportions are in accordance with previous studies investigating SNP within the *FADS* gene cluster[9–12,15]. The associations seemed to be largely independent from the biological source used to analyse PUFA concentrations. One study used PUFA concentrations in serum phospholipids[9], one used concentrations in erythrocyte membranes[10], three compared the results gained from analyses in serum/plasma phospholipids and erythrocytes membranes[12–14], one used concentrations in plasma and erythrocytes[11], and one used PUFA concentrations in plasma and adipose tissue samples[20]. All these studies investigated at least one SNP in the *FADS1* and/or *FADS2* region. Higher LA concentrations and lower AA concentrations among carriers of minor alleles of SNP within the same LD block have been reported by all previous studies. Furthermore, similar to our observations, higher EDA concentrations were observed among carriers of minor alleles for almost all the SNP in the *FADS1* and *FADS2* regions that were investigated[9,12], while GLA and DTA concentrations were found to be lower in a previous study[9]. Higher DGLA concentrations were reported for minor allele carriers of all investigated SNP in *FADS1* and most of the SNP in *FADS2*[9,13]. Lattka *et al.*[15] emphasised the high genetically explained variance for AA in serum phospholipids. However, as in the study from Rzehak *et al.*[13], we observed the strongest association between genetic variants within the *FADS1 FADS2* gene cluster and DGLA in erythrocyte samples. We also observed significantly higher levels of ALA among the participants with the TT genotype, while EPA levels were lower, although they were only with borderline significance. Taken together, the findings of enhanced contents of desaturase substrates and decreased

**Table 3.** Means for dihomo-γ-linolenic acid (DGLA, 20 : 3*n*-6), arachidonic acid (AA, 20 : 4*n*-6) and the AA:DGLA ratio (estimated delta-5 desaturase activity, D5D) according to rs174546 genotypes, stratified for factors discussed to influence the D5D activity (EPIC-Potsdam study, *n* 2066)

| | | 20 : 3*n*-6 (DGLA) | | | | | 20 : 4*n*-6 (AA) | | | | | Ratio AA:DGLA (D5D-activity) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | rs174546 genotype | | | | | rs174546 genotype | | | | | rs174546 genotype | | | | |
| Characteristic† | *n* | CC | CT | TT | Total population | *P*-value interaction | CC | CT | TT | Total population | *P*-value interaction | CC | CT | TT | Total population‡ | *P*-value interaction |
| General characteristics | | | | | | | | | | | | | | | | |
| Sex | | | | | | 0·58 | | | | | 0·79 | | | | | 0·86 |
| Men | 797 | 1·34 | 1·52 | 1·74 | 1·47 | | 12·6 | 12·2 | 11·5 | 12·3 | | 9·46 | 8·07 | 6·74 | 8·51* | |
| Women | 1269 | 1·39 | 1·57 | 1·84 | 1·51 | | 13·2 | 12·6 | 11·9 | 12·8 | | 9·62 | 8·22 | 6·69 | 8·70 | |
| Age (years) | | | | | | 0·22 | | | | | 0·79 | | | | | 0·33 |
| Low | 1053 | 1·36 | 1·55 | 1·82 | 1·50 | | 13·1 | 12·6 | 12·0 | 12·8 | | 9·74 | 8·22 | 6·78 | 8·75** | |
| High | 1013 | 1·38 | 1·54 | 1·77 | 1·50 | | 13·3 | 12·3 | 11·5 | 12·4 | | 9·37 | 8·09 | 6·65 | 8·50 | |
| Smoking | | | | | | 0·34 | | | | | 0·73 | | | | | 0·83 |
| Non-smoker | 1619 | 1·37 | 1·56 | 1·80 | 1·50 | | 12·9 | 12·4 | 11·8 | 12·6 | | 9·54 | 8·11 | 6·72 | 8·59 | |
| Current smoker | 447 | 1·38 | 1·52 | 1·78 | 1·48 | | 13·1 | 12·6 | 11·6 | 12·7 | | 9·61 | 8·34 | 6·67 | 8·78 | |
| Waist circumference (cm) | | | | | | 0·54 | | | | | 0·45 | | | | | 0·81 |
| Low | 1071 | 1·33 | 1·51 | 1·75 | 1·46 | | 12·9 | 12·6 | 11·9 | 12·6 | | 9·86 | 8·40 | 6·99 | 8·90*** | |
| High | 995 | 1·42 | 1·58 | 1·85 | 1·54 | | 13·0 | 12·4 | 11·6 | 12·5 | | 9·22 | 7·91 | 6·39 | 8·33 | |
| Alcohol | | | | | | 0·43 | | | | | 0·80 | | | | | 0·021 |
| Low | 1298 | 1·39 | 1·56 | 1·81 | 1·51 | | 13·0 | 12·5 | 11·8 | 12·6 | | 9·44 | 8·17 | 6·73 | 8·60 | |
| High | 768 | 1·34 | 1·53 | 1·77 | 1·47 | | 12·9 | 12·4 | 11·6 | 12·5 | | 9·76 | 8·15 | 6·67 | 8·68 | |
| Sport activity (h/week) | | | | | | 0·38 | | | | | 0·36 | | | | | 0·59 |
| Low | 1507 | 1·38 | 1·55 | 1·81 | 1·50 | | 13·0 | 12·4 | 11·8 | 12·6 | | 9·54 | 8·10 | 6·73 | 8·58 | |
| High | 559 | 1·36 | 1·54 | 1·75 | 1·48 | | 12·9 | 12·6 | 11·4 | 12·6 | | 9·59 | 8·35 | 6·67 | 8·75 | |
| Hypertension (mmHg) | | | | | | 0·010 | | | | | 0·47 | | | | | 0·25 |
| No | 1029 | 1·34 | 1·52 | 1·82 | 1·47 | | 13·0 | 12·5 | 12·0 | 12·7 | | 9·81 | 8·36 | 6·80 | 8·87*** | |
| Yes | 1037 | 1·40 | 1·57 | 1·77 | 1·52 | | 12·9 | 12·4 | 11·5 | 12·5 | | 9·28 | 7·98 | 6·63 | 8·38 | |
| TAG | | | | | | 0·005 | | | | | 0·09 | | | | | 0·70 |
| Low | 1541 | 1·35 | 1·55 | 1·81 | 1·49 | | 13·0 | 12·7 | 12·0 | 12·8 | | 9·73 | 8·32 | 6·83 | 8·81*** | |
| High | 525 | 1·43 | 1·54 | 1·76 | 1·52 | | 12·7 | 11·9 | 11·1 | 12·1 | | 8·99 | 7·73 | 6·38 | 8·10 | |
| HDL-cholesterol | | | | | | 0·31 | | | | | 0·50 | | | | | 0·52 |
| Low | 651 | 1·41 | 1·57 | 1·84 | 1·53 | | 13·0 | 12·3 | 11·5 | 12·5 | | 9·26 | 7·96 | 6·36 | 8·36*** | |
| High | 1415 | 1·35 | 1·54 | 1·78 | 1·48 | | 12·9 | 12·5 | 11·8 | 12·6 | | 9·68 | 8·26 | 6·85 | 8·75 | |
| Non-HDL-cholesterol | | | | | | 0·016 | | | | | 0·37 | | | | | 0·016 |
| Low | 1773 | 1·36 | 1·56 | 1·80 | 1·50 | | 13·0 | 12·5 | 11·9 | 12·6 | | 9·63 | 8·15 | 6·80 | 8·68** | |
| High | 293 | 1·42 | 1·49 | 1·79 | 1·51 | | 12·7 | 12·2 | 11·0 | 12·2 | | 9·05 | 8·23 | 6·28 | 8·30 | |
| Lipid-lowering medication | | | | | | 0·0004 | | | | | 0·0065 | | | | | 0·69 |
| No | 1961 | 1·37 | 1·55 | 1·81 | 1·50 | | 12·9 | 12·5 | 11·8 | 12·6 | | 9·57 | 8·18 | 6·74 | 8·64 | |
| yes | 105 | 1·41 | 1·60 | 1·43 | 1·49 | | 13·1 | 12·5 | 9·1 | 12·5 | | 9·38 | 7·82 | 5·97 | 8·44 | |
| Glucose/DM | | | | | | 0·41 | | | | | 0·89 | | | | | 0·74 |
| Low/no | 1804 | 1·37 | 1·55 | 1·80 | 1·50 | | 13·0 | 12·5 | 11·8 | 12·6 | | 9·59 | 8·19 | 6·71 | 8·66* | |
| High/yes | 262 | 1·39 | 1·55 | 1·75 | 1·50 | | 12·8 | 12·2 | 11·5 | 12·3 | | 9·31 | 7·92 | 6·74 | 8·39 | |
| Fatty acids in erythrocytes | | | | | | | | | | | | | | | | |
| EPA | | | | | | 0·029 | | | | | 0·06 | | | | | 0·82 |
| Low | 908 | 1·35 | 1·52 | 1·83 | 1·49 | | 12·1 | 11·7 | 11·4 | 11·8 | | 9·04 | 7·70 | 6·36 | 8·10*** | |
| High | 1158 | 1·39 | 1·57 | 1·76 | 1·50 | | 13·5 | 13·1 | 12·0 | 13·2 | | 9·92 | 8·53 | 7·07 | 9·04 | |
| *n*-3 PUFA | | | | | | 0·44 | | | | | 0·50 | | | | | 0·81 |
| Low | 1011 | 1·33 | 1·52 | 1·79 | 1·47 | | 12·0 | 11·6 | 11·0 | 11·7 | | 9·07 | 7·67 | 6·22 | 8·12*** | |
| High | 1055 | 1·41 | 1·58 | 1·80 | 1·52 | | 13·8 | 13·3 | 12·5 | 13·4 | | 10·0 | 8·64 | 7·22 | 9·12 | |

**Table 3.** *Continued*

| | | 20 : 3*n*-6 (DGLA) | | | | | 20 : 4*n*-6 (AA) | | | | | Ratio AA:DGLA (D5D-activity) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | rs174546 genotype | | | | | rs174546 genotype | | | | | rs174546 genotype | | | | |
| Characteristic† | *n* | CC | CT | TT | Total population | *P*-value interaction | CC | CT | TT | Total population | *P*-value interaction | CC | CT | TT | Total population‡ | *P*-value interaction |
| TFA | | | | | | 0·87 | | | | | 0·38 | | | | | 0·66 |
| Low | 1028 | 1·40 | 1·58 | 1·80 | 1·53 | | 13·2 | 12·8 | 11·8 | 12·9 | | 9·59 | 8·24 | 6·73 | 8·66 | |
| High | 1038 | 1·34 | 1·52 | 1·79 | 1·47 | | 12·7 | 12·1 | 11·6 | 12·3 | | 9·52 | 8·08 | 6·69 | 8·60 | |
| SFA | | | | | | 0·003 | | | | | 0·83 | | | | | 0·049 |
| Low | 1313 | 1·43 | 1·61 | 1·89 | 1·56 | | 14·0 | 13·5 | 12·7 | 13·6 | | 10·1 | 8·60 | 6·99 | 9·05*** | |
| High | 753 | 1·28 | 1·44 | 1·62 | 1·38 | | 11·2 | 10·6 | 9·9 | 10·8 | | 8·74 | 7·36 | 6·16 | 7·89 | |
| *n*-6:*n*-3 ratio | | | | | | 0·33 | | | | | 0·19 | | | | | 0·33 |
| Low | 1122 | 1·35 | 1·53 | 1·81 | 1·51 | | 13·5 | 13·1 | 12·2 | 13·2 | | 9·93 | 8·59 | 7·09 | 9·02*** | |
| High | 944 | 1·38 | 1·56 | 1·78 | 1·48 | | 12·3 | 11·7 | 11·2 | 11·9 | | 9·12 | 7·64 | 6·25 | 8·16 | |
| Dietary fatty acids | | | | | | | | | | | | | | | | |
| LA | | | | | | 0·14 | | | | | 0·46 | | | | | 0·15 |
| Low | 1035 | 1·37 | 1·53 | 1·75 | 1·48 | | 12·9 | 12·3 | 11·8 | 12·5 | | 9·57 | 8·15 | 6·91 | 8·63 | |
| High | 1031 | 1·38 | 1·57 | 1·83 | 1·51 | | 13·0 | 12·6 | 11·7 | 12·6 | | 9·54 | 8·17 | 6·53 | 8·62 | |
| AA | | | | | | 0·94 | | | | | 0·62 | | | | | 0·55 |
| Low | 1030 | 1·37 | 1·56 | 1·82 | 1·50 | | 12·8 | 12·4 | 11·8 | 12·5 | | 9·42 | 8·06 | 6·69 | 8·53* | |
| High | 1036 | 1·37 | 1·53 | 1·78 | 1·49 | | 13·1 | 12·5 | 11·7 | 12·7 | | 9·69 | 8·26 | 6·73 | 8·72 | |
| ALA | | | | | | 0·37 | | | | | 0·12 | | | | | 0·86 |
| Low | 1022 | 1·34 | 1·54 | 1·77 | 1·48 | | 12·8 | 12·5 | 11·7 | 12·5 | | 9·58 | 8·25 | 6·78 | 8·64 | |
| High | 1044 | 1·40 | 1·56 | 1·82 | 1·51 | | 13·1 | 12·4 | 11·7 | 12·7 | | 9·54 | 8·06 | 6·64 | 8·61 | |
| EPA | | | | | | 0·79 | | | | | 0·75 | | | | | 0·60 |
| Low | 1028 | 1·40 | 1·58 | 1·83 | 1·52 | | 13·2 | 12·7 | 12·0 | 12·8 | | 9·60 | 8·14 | 6·68 | 8·65 | |
| High | 1038 | 1·34 | 1·52 | 1·76 | 1·47 | | 12·7 | 12·2 | 11·5 | 12·3 | | 9·51 | 8·18 | 6·74 | 8·60 | |
| DHA | | | | | | 0·95 | | | | | 0·91 | | | | | 0·77 |
| Low | 1035 | 1·39 | 1·58 | 1·82 | 1·52 | | 13·1 | 12·7 | 11·8 | 12·8 | | 9·56 | 8·12 | 6·63 | 8·61 | |
| High | 1031 | 1·35 | 1·52 | 1·77 | 1·47 | | 12·8 | 12·2 | 11·6 | 12·4 | | 9·55 | 8·20 | 6·78 | 8·64 | |
| SFA | | | | | | 0·71 | | | | | 0·19 | | | | | 0·09 |
| Low | 1026 | 1·38 | 1·55 | 1·81 | 1·50 | | 13·1 | 12·5 | 11·5 | 12·6 | | 9·59 | 8·14 | 6·49 | 8·63 | |
| High | 1040 | 1·36 | 1·54 | 1·78 | 1·49 | | 12·8 | 12·4 | 11·9 | 12·5 | | 9·52 | 8·18 | 6·93 | 8·62 | |
| *n*-6:*n*-3 ratio | | | | | | 0·002 | | | | | 0·73 | | | | | 0·008 |
| Low | 1032 | 1·37 | 1·53 | 1·72 | 1·48 | | 12·8 | 12·2 | 11·6 | 12·4 | | 9·47 | 8·08 | 6·92 | 8·59 | |
| High | 1034 | 1·38 | 1·56 | 1·86 | 1·51 | | 13·1 | 12·7 | 11·8 | 12·8 | | 9·65 | 8·24 | 6·51 | 8·67 | |

CC, homozygote carrier of the major C-allele; CT, heterozygote carrier of the minor T-allele; TT, homozygote carrier of the minor T-allele; TFA, *trans*-fatty acid; LA, linoleic acid (18 : 2*n*-6); ALA, α-LA (18 : 3*n*-3).

† Cut-points for 'high' were age > 50 years; waist circumference, men ≥ 94 cm and women ≥ 80 cm; sport activity > 1 h/week; alcohol consumption ≥ 12 g/d; glucose: fasting plasma glucose ≥ 5·6 mmol/l, non-fasting plasma glucose ≥ 11·1 mmol/l, or self-reported diabetes; TAG > 1·7 mmol/l; HDL-cholesterol, men ≥ 1·03 mmol/l and women ≥ 1·29 mmol/l; non-HDL-cholesterol, ≥ 4·1 mmol/l; EPA (erythrocyte), > 0·72 % of total FA; *n*-3 PUFA (erythrocyte) > 8·0 % of total FA; TFA (erythrocyte) > 0·71 % of total FA; SFA (erythrocyte) > 43·9 % of total FA; *n*-6:*n*-3 ratio (erythrocyte) ≥ 3·68; LA (dietary) > 14·9 % of total fat intake; AA (dietary) > 0·208 % of total fat intake; ALA (dietary) > 1·82 % of total fat intake; EPA (dietary) > 0·122 % of total fat intake; DHA(dietary) > 0·212 % of total fat intake; SFA (dietary) > 40·6 % of total fat intake; *n*-6:*n*-3 ratio (dietary) > 6·48; hypertension, with medication or systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg; lipid-lowering medication, use of statins or fibrates.

‡ Mean values of estimated D5D activity were compared between the two strata of the total population using *t* test or Welch-test, as appropriate: **P*< 0·05, ***P*< 0·01, ****P*< 0·001.

contents of desaturase products among the minor T-allele carriers in the present study and earlier studies suggest less efficient desaturase activities for these subjects.

Although the differences in erythrocyte membrane *n*-6 PUFA between rs174546 genotypes might seem small, they may have important metabolic consequences. The magnitude of variation in LA and DGLA levels between the genotypes observed in our study was found to be relevant for predicting the risk of type 2 diabetes[28,29] and the metabolic syndrome[30]. Furthermore, variations in estimated D5D activity in a similar range as detected in our study between the genotypes were related to the risk of type 2 diabetes[29], the metabolic syndrome[30] and cardiovascular mortality[31].

There was no significant association between rs174546 genotype and most of the general characteristics including sex, age, lifestyle variables, and certain biochemical and medical characteristics. This observation is in agreement with the concept of Mendelian randomisation that genetic variants are generally not associated with a wide range of behavioural, social and physiological factors[32]. In accordance with other studies[33,34], we found associations between *FADS1* polymorphism and TAG and HDL-cholesterol concentrations; however, these associations were weak, and there was no clear trend across the genotypes. Furthermore, dietary fatty acid intakes were not significantly related to rs174546 genotype, except ALA intake which was slightly higher in the subjects with the CC genotype. However, this is likely to be a chance finding and cannot explain the genotype differences in ALA in erythrocytes. CC carriers had lower ALA contents in erythrocytes, despite a higher dietary intake which suggests that the efficient desaturation associated with this genotype led to relatively low ALA contents in erythrocyte membranes. Rzehak *et al.*[13] did not observe an effect of adjustment for total energy intake, physical activity and BMI on the association between the genotypes and fatty acids in erythrocytes, and we can confirm this independence of the associations for a much larger sample of variables.

Significant interactions with estimated D5D activity were found only for alcohol consumption and non-HDL-cholesterol; however, after correcting for multiple testing, these results became non-significant. Stratified analyses indicated that risk factors for chronic diseases like hypertension, high concentrations of TAG and non-HDL-cholesterol may modify the association between *FADS1* polymorphism and DGLA, but not AA and the AA:DGLA ratio. The effect of the minor allele on DGLA concentrations appears to be less pronounced among persons with these risk factors. Interestingly, the lipid-lowering medication modified the association between *FADS1* genotype and DGLA, AA and DTA concentrations, but did not modify the association of genotypes with estimated D5D activity in our study. While the effect of simvastatin – a lipid-lowering drug – on serum fatty acids has been investigated in randomised trials[35,36], modifications by *FADS* genotypes have not been considered so far. Harris *et al.*[36] found a significant increase of AA (% of total fatty acids) and a significant decrease of LA (% of total fatty acids) after a 6-month treatment with simvastatin. GLA, EDA, DGLA and DTA were not significantly altered by the treatment. A significant increase of AA, DGLA and GLA and a significant decrease of LA were observed by Jula *et al.*[35]. The present results suggest that the use of statins or fibrates may not be associated with increased AA and DGLA concentrations among individuals with the TT genotype of rs174546. Since higher concentrations of AA in adipose tissue have been associated with a greater risk of myocardial infarction and the metabolic syndrome[4] and higher proportions of DGLA in cholesterol esters predicted increased diabetes risk[37], individuals with the TT genotype may have higher benefit from treatment with lipid-lowering medication than the individuals carrying the major C-allele.

A limitation of our analyses is the low number of homozygote carriers of the minor allele who were on lipid-lowering medication. However, while it might have resulted in a low power to detect effect modification for other fatty acids, this does not explain the significant interactions observed for DGLA, AA and DTA. Furthermore, *in vitro* studies on THP-1 cells demonstrated a complex mechanism regarding mRNA expression of D5D and activity of the enzyme depending on the medication chosen[38]. While simvastatin increased the activity (evaluated as product:precursor ratio) and the mRNA levels of D5D after treating the THP-1 cells, fenofibrate did not affect calculated D5D activity even after up to 48 h of incubation, although it increased the D5D mRNA levels[38]. In our study, the AA:DGLA ratio was 8·44 for persons who were taking medication *v.* 8·64 for persons who were not taking medication, an observation that does not support increased D5D activity for persons taking lipid-lowering drugs. However, we were not able to investigate the use of statins (about two-thirds of all users) and of fibrates separately.

Gene expression of PPARα, SREBP-1c and D5D and thus the conversion of LA and ALA to long-chain fatty acids may depend on the relative proportion of *n*-6 and *n*-3 precursors[39]. A study on the formation of EPA found the highest conversion at an administered LA:ALA ratio of 1:1, although gene expression of D5D was higher in the presence of ALA only[39]. An increased concentration of DGLA as a result of an inhibition of the activity of D5D by EPA was stated by Das[1]. We found higher AA:DGLA ratios for persons with higher EPA concentrations in erythrocyte membranes, while there was no significant association for dietary EPA. There was no evidence for an effect modification by the *n*-6:*n*-3 ratio in erythrocyte membranes with regard to the associations between the genotype and AA:DGLA ratio. However, the differences in estimated D5D activity and DGLA concentrations between the genotypes were larger in the subjects with a high dietary *n*-6:*n*-3 ratio. This observation appears to be plausible as *n*-6 and *n*-3 PUFA compete for D5D and D6D. As argued in a recent study, the more efficient desaturation associated with the CC genotype in combination with a high dietary intake of *n*-6 PUFA may have led to larger differences in the proportions of longer-chain *n*-6 PUFA between CC and TT genotypes compared with a situation when *n*-6 PUFA intake is low[40]. The exact mechanisms by which genetic variants of the *FADS1 FADS2* gene cluster and PUFA influence expression or activity of the desaturase enzymes remain unknown so far[4].

In controlled dietary intervention studies, substituting PUFA for SFA has been shown to alter blood fatty acid composition towards higher concentrations of PUFA at the expense of SFA[18]. Moreover, PUFA-rich diets resulted in reduction of D6D activity, but increased D5D activity compared with diets rich in SFA[18]. In our study, the estimated

D5D activity did not differ significantly between the participants with a low or high dietary intake of SFA. Similarly, dietary intakes of LA, AA, ALA, EPA and DHA were not significantly related to the estimated D5D activity in our study, although modulations of the activities of D5D and D6D by intakes of these fatty acids have been detected in controlled intervention studies[7,18]. However, it has been noted that D6D is more sensitive towards dietary changes than D5D[7]. Furthermore, assessing the dietary intake using traditional dietary assessment methods (here: FFQ) in large-scale observational studies is usually associated with a considerable measurement error resulting in a lower ability to detect true associations[41]. Although a validation study showed that correlations between fatty acid intakes from twelve 24 h recalls and the FFQ were reasonably well in our study[26], we cannot rule out the possibility that measurement error obscured true associations between dietary fatty acid intakes and estimated D5D activity.

More studies are needed to understand the mechanisms by which *FADS* polymorphisms influence the regulation and composition of *n*-6 PUFA and which other factors influence the complex feedback regulation mechanisms of the whole PUFA pathway. Functional analyses of the polymorphisms are rare, and a significant impact on promoter activity was found only for rs968567, and not for rs3834458[42]. The consequence of this increased promoter activity on desaturase expression or activity for persons carrying the minor allele is unclear as well[2]. At least, there was no evidence of a strong modification of the association between the rs174546 genotype and estimated D5D activity in our study by factors discussed to influence the activity of desaturases.

Some limitations of our findings should be noted. We only investigated one SNP (rs174546) in *FADS1,* and we therefore cannot generalise the present results for other genetic variants. LD between several SNP of *FADS1* and *FADS2* is high[9], and we cannot determine the causal nature of the observed association. Activity of the D5D was investigated by the AA:DGLA ratio as well as by the product (AA) and the precursor (DGLA). It remains unclear from our analysis whether the present results reflect altered desaturase expression or activity. The choices of cut-points for stratified analyses were made before performing all the analyses. As expected, we observed significant differences in the estimated D5D activity between subgroups of most variables (e.g. waist circumference, hypertension, TAG, HDL-cholesterol, EPA in erythrocyte membranes, SFA in erythrocyte membranes and *n*-6:*n*-3 ratio in erythrocyte membranes). However, the choice of a cut-point may affect the ability to detect effect modifications by these characteristics when evaluating the associations between genotype and *n*-6 PUFA. Additionally, an absence of statistical interaction may arise from a balance between synergistic and antagonistic responses, rather than an absence of biological interaction[27]. Consequently, we cannot exclude that we may have missed some biological interaction.

In conclusion, rs174546, which was used as a measure of genetic variation in the *FADS1 FADS2* gene cluster, was associated with a distinct difference in *n*-6 PUFA concentrations in erythrocyte membranes, in particular DGLA. No clear departure from the addition of the separate effects was found for the genotype–D5D relationship for most of the factors that are related to D5D activity. However, the dietary *n*-6:*n*-3 ratio may modify the association between the *FADS1 FADS2* genotype and the estimated D5D activity. Furthermore, the associations between the *FADS1 FADS2* genotype and the DGLA and AA concentrations may be modified by lipid-lowering medication.

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