European Journal of Clinical Nutrition (2014) 68, 517–525

www.nature.com/ejcn

ORIGINAL ARTICLE

Association between erythrocyte membrane fatty acids and biomarkers of dyslipidemia in the EPIC-Potsdam study

S Jacobs[1], K Schiller[1], E Jansen[2], A Fritsche[3,4], C Weikert[5], R di Giuseppe[5], H Boeing[5], MB Schulze[1] and J Kröger[1]

BACKGROUND/OBJECTIVE: Blood proportions of fatty acids (FAs) and FA-ratios reflecting desaturase activity are associated with the risk of chronic diseases like type 2 diabetes mellitus or cardiovascular diseases. Biomarkers of dyslipidemia are considered as potential mediators of this association. We evaluated associations of erythrocyte membrane proportions of individual disease-related polyunsaturated fatty acids (PUFAs), *trans*-FAs, dairy-derived saturated FAs (SFAs) (15:0, 17:0) and FA-ratios with biomarkers of dyslipidemia (high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, non-HDL-cholesterol, triglycerides).
SUBJECTS/METHODS: We conducted a cross-sectional analysis of a subsample ($n = 1759$) of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Associations of individual FAs and FA-ratios with plasma biomarkers of dyslipidemia were evaluated by linear multivariable regression.
RESULTS: Most notably, FA-ratios reflecting activity of Δ6-desaturase (D6D) and stearoyl-coenzyme A-desaturase (SCD) were positively associated with triglyceride and LDL-cholesterol concentrations (adjusted means (95% confidence interval (CI)) of triglycerides (mg/dl) across D6D tertiles: men—102 (94.7–110), 111 (104–120), 144 (134–156) and women—73.5 (70.0–77.2), 82.9 (79.0–86.9), 94.2 (89.7–98.9)); across SCD tertiles: men—99.0 (91.8–107), 115 (107–124), 144 (134–156) and women—72.4 (69.0–76.0), 81.5 (77.8–85.5), 97.2 (92.6–102)), whereas inverse associations with triglycerides were observed for the estimated Δ5-desaturase (D5D) activity (adjusted means (95% CI) of triglycerides (mg/dl) across D5D tertiles: men—128 (119–138), 121 (113–131), 106 (97.9–114) and women—92.0 (87.6–96.6), 82.8 (78.9–86.9), 75.3 (71.6–79.1), *P*-values for trend at least 0.0006). Furthermore, we observed generally weaker and less consistent associations of dairy-derived SFAs (mainly 17:0) with triglycerides and HDL-cholesterol. Individual PUFAs and *trans*-FAs were, if at all, only weakly associated with dyslipidemia markers.
CONCLUSIONS: Our findings suggest that triglyceride and LDL-cholesterol concentrations may be mediators that link intake and metabolism of FAs to metabolic risk.

European Journal of Clinical Nutrition (2014) **68,** 517–525; doi:10.1038/ejcn.2014.18; published online 26 February 2014

INTRODUCTION

The dietary intake and metabolism of fatty acids (FAs) is believed to have an important role in the etiology of chronic diseases such as type 2 diabetes mellitus (T2DM)[1] and cardiovascular diseases (CVDs).[2] Within this context, biomarker FAs may be more valid measures of dietary intake compared with self-reported data, because they are determined in an objective way resulting in reduced information bias as well as a relatively high precision.[3] The FA profile also reflects metabolic processes and therefore integrates the complex interplay between dietary FA intake and endogenous FA metabolism.

The biological mechanisms that link FA profiles with chronic diseases like T2DM and CVD are not completely understood yet. An involvement of dyslipidemia appears plausible in this context because FAs influence transcription factors like peroxisome proliferator-activated receptor-γ (PPARG) or sterol regulatory element-binding protein, which have a pivotal role in regulating genes involved in the control of uptake, transport, storage and disposal of lipids.[4,5] Indeed, intervention studies showed that intake of the CVD-related[6] long-chain n-3 polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), lowers triglycerides[7] and that diets rich in the CVD-[2] and T2DM-related[8–11] n-6 PUFA linoleic acid (LA) markedly lower low-density lipoprotein (LDL)-cholesterol.[12] However, few studies have examined the relations of blood contents of these individual disease-relevant PUFAs to biomarkers of dyslipidemia.[13,14]

Furthermore, recent studies suggest an important role of the activity of desaturase enzymes (as reflected by certain FA product-to-precursor ratios) for the development of T2DM.[15,16] Data from genome-wide association studies (GWAS) suggest dyslipidemia as a potential mechanistic link in this context, because genetic variation in the *FADS1–FADS2* gene cluster, which encode the Δ5-desaturase (D5D) and Δ-6 desaturase (D6D), showed an association with plasma high-density lipoprotein (HDL)-cholesterol and triglyceride concentrations.[17] To the best of our knowledge,

[1]Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany; [2]Center for Health Protection, National Institute for Public Health and the Environment, Bilthoven, The Netherlands; [3]Department of Internal Medicine, Division of Endocrinology, Diabetology, Nephrology, Vascular Disease, and Clinical Chemistry, University Hospital of the Eberhard Karls University, Tübingen, Germany; [4]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Centre Munich at the University of Tübingen (IDM), Partner in the German Center for Diabetes Research (DZD), Tübingen, Germany and [5]Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany. Correspondence: Dr J Kröger, Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, Nuthetal 14558, Germany.
E-mail: kroeger@dife.de
Received 26 April 2013; revised 9 January 2014; accepted 14 January 2014; published online 26 February 2014

the association between FA-based desaturase activities and markers of dyslipidemia has not been studied previously.

Blood concentrations of the odd-numbered saturated FAs (SFAs), pentadecanoic (15:0) and heptadecanoic acid (17:0), have attracted high interest lately because recent studies revealed inverse relations to risk of T2DM[8,9,11] and CVD.[18] Blood or adipose tissue concentrations of these FA are regarded as biomarkers of dairy fat intake.[19] Despite the growing interest, the underlying biological mechanisms explaining the inverse risk relations are largely unexplored.

Trans-FAs are regarded as established risk factor for CVD.[20–22] However, the source of *trans*-FAs is assumed to have a role in the association of TFA with cardiovascular risk. Whereas *trans*-FAs from partially hydrogenated oils unfavorably affect cardiovascular risk,[22] *trans*-FAs derived from naturally occurring foods containing ruminant *trans*-FAs have not been associated with higher cardiovascular risk.[22] Little is known about the relation of blood contents of individual *trans*-FAs with biomarkers of dyslipidemia so far.[23–25]

Therefore, we aimed to study the association between specific disease-relevant individual PUFAs, *trans*-FAs and dairy-derived SFAs, as well as estimated desaturase activity and biomarkers of dyslipidemia.

SUBJECTS AND METHODS

Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study is part of the multicenter prospective cohort study EPIC.[26] EPIC-Potsdam includes 16 644 women mainly aged 35–64 years and 10 904 men mainly aged 40–64 years recruited from the general population of the city of Potsdam, Germany, and surrounding municipalities from 1994 to 1998. Information on education, smoking and physical activity were assessed at baseline with a self-administered questionnaire and a personal PC-guided interview.[26] The baseline assessment included the collection of blood samples. Anthropometric measurement procedures followed a standardized protocol.[26] Follow-up questionnaires were sent out every 2–3 years to identify incident cases of T2D (response rates 93–97%).[26] The verification of self-reports was performed via questionnaires mailed to physicians.

For biochemical measurements, a random sample of 2500 subjects was drawn from all participants of EPIC-Potsdam who provided a blood sample (26 444). We excluded subjects with missing or implausible values for erythrocyte FAs ($n=651$) or biomarkers of dyslipidemia ($n=1$) and participants with lipid-lowering medication ($n=89$). After exclusion, 1759 participants were considered for the cross-sectional analysis. Informed consent was obtained from all participants, and approval was given by the Ethics Committee of the state of Brandenburg, Germany.

Measurement of FA composition of erythrocytes and estimation of desaturase activities

Thirty milliliters of blood were obtained from each participant during baseline examination, mostly in the non-fasting status. Plasma, serum, red blood cells and buffy coat were stored at -80 °C. The erythrocyte membrane FAs were analyzed between February and June 2008. Thirty-two FAs were determined by gas chromatography and expressed as the percentage of total FAs present in the chromatogram.[11] Detailed information with respect to the storage conditions of samples, sample preparation and analytical procedures was provided elsewhere.[11] In brief, FA methyl ester were separated on a GC-3900 gas chromatograph (Varian Inc., Middelburg, The Netherlands) equipped with a 100 m × 0.25 mm ID WCOT-fused silica capillary column and flame ionization detector with separation of FA methyl ester peaks based on mixed FA methyl ester standards (Sigma Aldrich, St Louis, MO, USA). The Galaxie software version 1.9.3.2 (Varian Inc.) was used for quantification and identification of peaks.

The desaturase activities were estimated as product-to-precursor ratios of individual FAs in erythrocyte membranes as follows: 16:1n-7/16:0 to reflect stearoyl-coenzyme A-desaturase (SCD) activity, 18:3n-6/18:2n-6 to reflect D6D activity and 20:4n-6/20:3n-6 to reflect D5D activity.[11] Intra-assay coefficients of variation calculated from a total number of 40 FA measurements in a subset of 20 samples were ⩽10% for most FAs, with the exception of 18:3n-6 (18.7%).

Measurement of biomarkers of dyslipidemia

Plasma concentrations of HDL-cholesterol and triglycerides were determined using the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany).[27] Non-HDL-cholesterol was calculated as total cholesterol minus HDL-cholesterol. LDL-cholesterol was calculated using the Friedewald formula.[28]

Genotyping

For genetic analyses, DNA was extracted from buffy coat samples. Participants were genotyped for the Pro12Ala polymorphism in PPARG2 using the TaqMan technology (Applied Biosystems, Foster City, CA, USA) on 384-well plates. The reproducibility of the genotyping method was ⩾99.5%. The allele frequency in the present sample was in Hardy–Weinberg equilibrium ($\chi^2=0.7546$).

Statistical analysis

We performed multivariable linear regression analysis to investigate the relations of erythrocyte FAs to HDL-, LDL-, non-HDL-cholesterol and triglycerides. Categorical variables were entered as binary indicator variables into the models. We tested for interaction with sex by evaluating statistical significance of cross-product terms of the respective FAs/FA-ratios (as a quantitative variable) and sex included in the multivariable-adjusted models.

We used the $\log_e$ transformation of triglycerides to normalize the right skewed distribution. We modeled the individual FA proportions as tertiles to account for nonlinear relations with the outcomes. We estimated geometric means and 95% confidence intervals (CIs) in case of triglycerides and arithmetic means and 95% CIs in case of HDL-cholesterol, LDL-cholesterol and non-HDL-cholesterol by FA tertiles and tested for statistical significance of linear trends across tertiles by modeling the median value of the FA within each tertile as a quantitative variable. We calculated a model adjusted for age at recruitment only and furthermore a multivariable-adjusted model additionally adjusted for smoking status (never; past; current < 20 U per day; current >20 U per day), education (in training, no certificate, part skilled worker; skilled worker; professional school; college of higher education, university), alcohol intake (>0–6; >6–12; >12–24; >24–60; >60–96; >96 g per day), leisure time sports activity (no sports, ⩽4 h per week, >4 h per week), biking (no biking, < 2.5 h per week, 2.5– < 5 h per week, >5 h per week), hormone use (none, oral contraceptive, hormone replacement therapy, in women only), body mass index (BMI) and waist circumference.

In addition, we performed sensitivity analyses to assess the robustness of our results: in a first sensitivity analysis, we examined only fasted participants ($n=248$). Fasting status was defined as time of the last meal >8 h. After exclusion of non-fasted participants, 148 women and 100 men remained. In a second sensitivity analysis, we excluded participants with a history of cancer, diabetes or CVDs, leaving 1535 participants, 571 men and 964 women, for analyses.

We conducted stratified analyses to explore whether the associations of erythrocyte FAs with biomarkers of dyslipidemia varied by PPARγ2 (PPARG2) genotype. We compared homozygous carriers of the Pro12 allele to carriers of the Ala12 allele (dominant model) because the frequency of the homozygous Ala12 allele carriers was low (men: 2.35%; women: 2.65%). We *a priori* restricted exposures to the PPARG ligands long-chain n-3 PUFAs and LA. We tested for interaction by evaluating statistical significance of cross-product terms of the respective PUFA (as a quantitative variable) and genotype included in the multivariable models.

The presence of colinearity among independent variables was tested measuring the variance inflation factor in linear regression analysis.

The type I error for each FA model was set to 5%. All statistical tests were two-tailed and 95% CIs were estimated. A *P*-value for trend of 0.005 and below would satisfy a correction for multiple testing according to Bonferroni (0.05/10 comparisons), if one would consider our analysis as completely exploratory. We performed the statistical analysis with the SAS software, release 9.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Median subcohort proportions of individual erythrocyte membrane FAs as well as FA-ratios reflecting desaturase activity are presented in Table 1, stratified by sex. Compared with men, women had significantly higher median proportions of dairy-derived SFAs, *trans*-FAs, docosahexaenoic acid and LA, whereas median proportions of EPA and estimated D6D activity were lower.

The characteristics of the study population by tertiles of HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and triglycerides, stratified by sex, are shown in Table 2 and 3. Men with high plasma triglycerides, LDL-cholesterol and non-HDL-cholesterol concentrations were significantly more obese. Men with high non-HDL-cholesterol level were significantly less engaged in leisure time sports activity. On the contrary, men with high plasma HDL-cholesterol concentration were significantly less obese, drank more alcohol and had a lower carbohydrate intake compared with men with low HDL-cholesterol concentrations. As expected, women with high plasma HDL-cholesterol concentration were significantly less obese, and more engaged in leisure time sports activity, whereas women with high plasma LDL-cholesterol, non-HDL-cholesterol and triglyceride concentrations were significantly older, more obese and less engaged in leisure time sports activity.

Associations between erythrocyte FAs and biomarkers of dyslipidemia

Table 4 shows multivariable-adjusted means and 95% CIs of HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and triglycerides according to tertiles of erythrocyte FAs, stratified by sex. Age-adjusted means are displayed in Supplementary Table 1. Compared with the age-adjusted model, effect estimates of the multivariable-adjusted model usually indicated the same direction of association; however, the strength of association was weaker.

Regarding dairy-derived SFAs, only weak associations were observed in the multivariable-adjusted model. The proportion of 15:0 in erythrocyte membranes was positively associated with concentrations of HDL-cholesterol in women. An inverse association of the proportion of 17:0 in erythrocyte membranes with plasma concentrations of triglycerides was observed in women only. In men, proportions of 17:0 in erythrocyte membranes were inversely associated with plasma concentrations of HDL-cholesterol. *Trans*-FAs did not show a clear association with biomarkers of dyslipidemia in the multivariable-adjusted model.

The FA-ratio 20:4n-6/20:3n-6, reflecting D5D activity, was inversely associated with triglycerides in both sexes. In contrast, higher FA-ratios reflecting higher SCD and D6D activities were associated with higher triglyceride and LDL-cholesterol concentrations in both sexes. The difference in triglyceride concentration across SCD and D6D tertiles appeared to be more prominent in men compared with women.

The proportion of LA in erythrocytes was not clearly associated with any lipid biomarker in our study. With respect to long-chain n-3 PUFAs, the proportion of EPA was inversely associated with triglyceride concentrations in women. There was no clear association of DHA with any biomarker of dyslipidemia in men or women.

These following results satisfy *P*-values for trend according to correction for multiple testing: associations of D6D and SCD with LDL-cholesterol and triglycerides and associations between D5D and triglycerides in both sexes. We considered these associations as our main findings.

Stratification by PPARG2 genotype

Thirty percent of women and twenty-six percent of men carried the PPARG2 Ala12 allele. We repeated our analyses on the association of EPA, DHA and LA with triglycerides stratified by PPARG2 genotype. The interaction test among proportions of these PUFAs and PPARG2 Pro12Ala genotype was not significant, although considerable differences after stratification were observed: in men and women with Pro12Pro genotype, triglycerides were significantly lower across EPA tertiles (Supplementary Figure 1). In contrast, no clear association of EPA with triglycerides was observable among male and female Ala12 carriers. LA and DHA were not significantly associated with triglyceride concentrations in both sexes regardless of PPARG genotype (Supplementary Figure 1).

Sensitivity analyses

We performed sensitivity analyses excluding participants who had not fasted before blood draw or with prior chronic diseases. In general, after restricting analyses to fasted participants (women: $n = 148$; men: $n = 100$), associations were qualitatively comparable; however, only few reached statistical significance owing to the loss of power (Supplementary Table 2). Exclusion of prevalent cases of diabetes, CVD and cancer had no major influence on the results (data not shown).

DISCUSSION

In this cross-sectional study of middle-aged men and women, D6D and SCD activities calculated from erythrocyte FA-ratios were positively associated with triglyceride and LDL-cholesterol concentrations. Higher estimated D5D activity was inversely associated with triglyceride concentrations.

Estimated activities of D6D and SCD were positively and D5D activity was inversely associated with T2DM risk in the EPIC-Potsdam study[11] and other prospective studies.[8–10,29] The biological mechanisms linking desaturase activity with T2DM risk have not been well understood yet. Regarding estimated activity of D6D and SCD, the triglyceride concentration was markedly increased in the higher tertiles compared with the lower tertiles (more than 40% in men, about 30% in women). With respect to D5D, the triglyceride concentration was more than 20% lower in the highest compared with the lowest tertile in both sexes. With respect to clinical relevance of our findings, the magnitude of variation in triglyceride levels between the lower and the upper tertile of estimated D6D, SCD and D5D activities observed in our study was found to be relevant for predicting the risk of T2DM[30] and the coronary heart disease.[31] Our results of strong associations of estimated desaturase activity with triglycerides and LDL-cholesterol do suggest an involvement of an unfavorable lipid profile in this context. However, the cross-sectional design of our study does not allow us to draw conclusions about causality,

Table 1. Proportions of erythrocyte membrane fatty acids and derived ratios in a subcohort of the EPIC-Potsdam study

Erythrocyte fatty acids (% of total fatty acids)	*Men* (n = *655*)	*Women* (n = *1104*)	P-*value*
15:0	0.19 (0.10)	0.22 (0.09)	< 0.0001
17:0	0.31 (0.07)	0.33 (0.06)	< 0.0001
Trans-16:1n-7	0.16 (0.06)	0.17 (0.06)	< 0.0001
Trans-18:1n-9+*trans*-18:1n-7	0.48 (0.13)	0.53 (0.15)	< 0.0001
20:5n-3	0.79 (0.35)	0.76 (0.34)	0.0195
22:6n-3	4.62 (1.41)	4.90 (1.40)	< 0.0001
18:2n-6	10.6 (1.69)	10.8 (1.68)	0.0356
20:4n-6/20:3n-6 ratio (estimated D5D activity)	8.62 (2.59)	8.80 (2.57)	0.0878
18:3n-6/18:2n-6 ratio (estimated D6D activity)	0.005 (0.004)	0.004 (0.003)	< 0.0001
16:1n-7/16:0 ratio (estimated SCD activity)	0.02 (0.010)	0.02 (0.009)	0.9461

Abbreviations: EPIC, European Prospective Investigation into Cancer and Nutrition; SCD, stearoyl-coenzyme A-desaturase. All values are medians; interquartile ranges are within parentheses. *P*-value reflects whether the medians of the erythrocyte fatty acids significantly differ between women and men (two-sample median test).

Table 2. Characteristics by tertiles of HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and triglycerides in men, EPIC-Potsdam study ($n = 655$)[a]

	Tertile of HDL-cholesterol			Tertile of LDL-cholesterol			Tertile of non-HDL-cholesterol			Tertile of triglycerides		
	1	*3*	P-*value*	*1*	*3*	P-*value*	*1*	*3*	P-*value*	*1*	*3*	P-*value*
Age (years)	51.9 (8.1)	51.3 (8.1)	0.49	50.4 (8.1)	52.7 (7.8)	0.003	50.6 (8.2)	53.0 (8.2)	0.002	51.6 (8.2)	51.8 (8.1)	0.88
BMI (kg/m^2)	27.8 (3.7)	25.4 (3.1)	< 0.0001	25.7 (3.7)	27.7 (3.1)	< 0.0001	25.9 (3.9)	27.4 (3.0)	< 0.0001	25.4 (3.1)	27.7 (3.5)	< 0.0001
Waist circumference (cm)	96.8 (10.6)	91.0 (9.1)	< 0.0001	91.1 (10.5)	97.0 (8.7)	< 0.0001	91.8 (11.1)	96.1 (8.4)	< 0.0001	90.4 (9.4)	97.0 (9.8)	< 0.0001
Leisure time sports activity (h per week)	1.1 (2.0)	1.1 (2.0)	0.85	1.1 (1.9)	0.9 (1.6)	0.10	1.2 (2.0)	0.8 (1.6)	0.02	1.1 (1.9)	1.0 (1.7)	0.52
Biking (h per week)	1.7 (2.5)	1.7 (2.4)	0.99	1.9 (2.5)	1.4 (2.3)	0.05	2.0 (2.7)	1.5 (2.4)	0.05	1.8 (2.9)	1.5 (2.3)	0.17
Smoking			0.21			0.10			0.03			0.07
Never smoker (%)	25.7	30.3		29.4	25.2		28.0	24.8		26.8	25.6	
Education			0.56			0.13			0.27			0.27
In training, no certificate, part skilled worker (%)	1.38	1.83		2.29	0.00		2.75	0.46		2.73	0.46	
Professional school (%)	13.8	12.8		14.7	17.4		15.1	15.1		16.8	16.4	
Technical college, university (%)	56.0	50.5		50.9	52.3		51.4	55.1		50.5	54.3	
Prevalent diabetes (%)	4.13	1.38	0.08	2.75	2.75	1.00	3.21	1.83	0.36	2.73	3.20	0.77
Prevalent CVD (%)	12.4	8.26	0.16	8.72	9.17	0.87	10.1	8.72	0.62	6.36	9.13	0.28
Prevalent cancer (%)	2.75	3.21	0.78	3.21	2.29	0.56	3.21	2.29	0.56	3.64	3.20	0.80
Alcohol intake (g per day)	20.2 (33.6)	29.3 (24.8)	0.001	23.2 (21.4)	25.0 (22.7)	0.39	24.6 (35.8)	25.0 (23.2)	0.91	24.9 (23.2)	24.1 (34.6)	0.77
Carbohydrate intake (g/1000 kcal)	101 (14.8)	95.5 (13.6)	< 0.0001	99.7 (15.1)	97.3 (13.8)	0.08	99.0 (15.1)	97.0 (13.4)	0.13	98.9 (14.7)	98.1 (14.8)	0.55
Fasting blood (%)	16.5	12.4	0.22	17.0	10.1	0.04	16.1	12.8	0.34	20.9	7.76	< 0.0001
Erythrocyte fatty acids[b]												
15:0	0.19 (0.10)	0.19 (0.11)	0.09	0.19 (0.11)	0.19 (0.10)	0.72	0.19 (0.10)	0.19 (0.09)	0.59	0.20 (0.10)	0.19 (0.09)	0.32
17:0	0.31 (0.06)	0.31 (0.07)	0.11	0.31 (0.07)	0.30 (0.06)	0.18	0.31 (0.07)	0.30 (0.07)	0.38	0.32 (0.07)	0.30 (0.06)	0.01
Trans-16:1n-7	0.17 (0.06)	0.16 (0.05)	0.03	0.16 (0.05)	0.16 (0.07)	0.83	0.16 (0.05)	0.16 (0.06)	0.97	0.16 (0.05)	0.16 (0.07)	0.45
Trans-18:1n-9+*trans*-18:1n-7	0.48 (0.14)	0.48 (0.14)	0.70	0.47 (0.13)	0.48 (0.12)	0.98	0.48 (0.14)	0.48 (0.12)	0.95	0.49 (0.14)	0.48 (0.13)	0.57
18:2n-6	10.5 (1.66)	10.7 (1.87)	0.31	10.8 (1.85)	10.5 (1.61)	0.02	10.8 (1.90)	10.5 (1.74)	0.02	10.6 (1.81)	10.5 (1.59)	0.36
20:5n-3	0.73 (0.32)	0.86 (0.37)	< 0.0001	0.79 (0.34)	0.80 (0.37)	0.50	0.79 (0.34)	0.81 (0.40)	0.11	0.81 (0.40)	0.79 (0.35)	0.17
22:6n-3	4.64 (1.46)	4.66 (1.32)	0.96	4.73 (1.30)	4.61 (1.46)	0.46	4.66 (1.36)	4.58 (1.48)	0.78	4.68 (1.37)	4.58 (1.47)	0.33
20:4n-6/20:3n-6 ratio (estimated D5D activity)	8.32 (2.40)	9.14 (2.54)	0.0007	8.88 (2.56)	8.36 (2.46)	0.0008	8.82 (2.49)	8.47 (2.43)	0.009	9.19 (2.78)	8.42 (2.59)	< 0.0001
18:3n-6/18:2n-6 ratio (estimated D6D activity)	0.006 (0.004)	0.005 (0.004)	0.46	0.004 (0.003)	0.006 (0.004)	< 0.0001	0.005 (0.003)	0.006 (0.004)	< 0.0001	0.004 (0.003)	0.006 (0.004)	< 0.0001
16:1n-7/16:0 ratio (estimated SCD activity)	0.0202 (0.01)	0.0201 (0.01)	0.91	0.019 (0.01)	0.022 (0.01)	< 0.0001	0.019 (0.01)	0.021 (0.01)	0.002	0.018 (0.008)	0.023 (0.01)	< 0.0001

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; D5D, Δ5-desaturase; D6D, Δ6-desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; SCD, stearoyl-coenzyme A-desaturase. *P*-value reflects whether the values of the variables significantly differ between extreme erythrocyte fatty acid/fatty acid ratio tertiles (Wilcoxon's test for proportions of erythrocyte fatty acids, *t*-test for other quantitative variables and χ^2 test for categorical variables). [a]For quantitative variables, values are arithmetic means (s.d.), and for categorical variables, values are percentages (all such values). [b]For proportions of erythrocyte fatty acids, values are medians (IQR) (all such values).

Table 3. Characteristics by tertiles of HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and triglycerides in women, EPIC-Potsdam study ($n = 1104$)[a]

	Tertile of HDL-cholesterol			*Tertile of LDL-cholesterol*			*Tertile of non-HDL-cholesterol*			*Tertile of triglycerides*		
	1	*3*	P-*value*	*1*	*3*	P-*value*	*1*	*3*	P-*value*	*1*	*3*	P-*value*
Age (years)	49.1 (9.2)	47.8 (9.1)	0.07	44.7 (8.2)	53.0 (8.6)	< 0.0001	44.5 (8.1)	52.8 (8.7)	< 0.0001	46.4 (8.7)	51.5 (9.1)	< 0.0001
BMI (kg/m^2)	27.5 (5.0)	23.9 (3.8)	< 0.0001	23.9 (3.7)	27.8 (4.6)	< 0.0001	24.1 (3.9)	27.3 (4.5)	< 0.0001	23.9 (3.7)	27.9 (4.8)	< 0.0001
Waist circumference (cm)	85.4 (12.0)	75.8 (9.4)	< 0.0001	75.6 (9.3)	86.9 (11.8)	< 0.0001	76.1 (9.7)	85.3 (11.6)	< 0.0001	75.3 (8.8)	87.1 (12.1)	< 0.0001
Leisure time sports activity (h per week)	0.9 (1.6)	1.2 (1.8)	0.02	1.3 (1.9)	0.8 (1.5)	0.0001	1.3 (1.8)	0.8 (1.6)	0.001	1.2 (1.9)	0.8 (1.4)	0.0007
Biking (h per week)	1.9 (3.0)	1.8 (2.9)	0.67	2.1 (3.2)	1.6 (2.6)	0.04	2.1 (3.2)	1.8 (2.8)	0.098	2.0 (2.7)	1.7 (2.9)	0.27
Smoking			0.01			0.0004			0.001			0.09
Never smoker (%)	59.2	54.1		55.2	59.0		55.7	59.5		60.2	57.7	
Education			0.02			< 0.0001			< 0.0001			0.01
In training, no certificate, part skilled worker (%)	4.62	3.53		2.99	6.79		2.99	6.25		3.30	6.50	
Professional school (%)	31.8	28.8		30.4	31.5		30.7	29.9		28.3	29.0	
Technical college, university (%)	24.7	35.3		35.1	20.4		35.1	21.5		34.9	25.5	
Oral contraceptive use (%)	13.3	19.8	0.02	21.5	10.1	< 0.0001	22.6	9.78	< 0.0001	14.8	15.2	0.90
Hormone replacement therapy (%)	22.3	20.9	0.65	18.5	26.4	0.01	17.1	28.0	0.0004	22.0	23.3	0.67
Prevalent diabetes (%)	2.17	0.82	0.13	0.00	2.72	0.002	0.27	2.17	0.02	0.82	2.98	0.03
Prevalent CVD (%)	5.43	3.80	0.29	1.36	9.51	< 0.0001	1.90	9.24	< 0.0001	2.47	8.67	0.0003
Prevalent cancer (%)	7.61	7.07	0.78	5.71	8.15	0.19	5.16	8.70	0.06	4.95	7.86	0.11
Alcohol intake (g per day)	7.0 (9.2)	9.8 (10.9)	0.0002	10.1 (11.1)	6.9 (8.7)	< 0.0001	10.2 (11.2)	7.2 (9.0)	< 0.0001	8.7 (10.5)	7.2 (8.7)	0.03
Carbohydrate intake (g/ 1000 kcal)	103 (12.1)	101 (13.0)	0.01	101 (12.3)	103 (13.0)	0.03	101 (12.0)	103 (12.9)	0.03	102 (12.4)	102 (13.2)	0.70
Fasting blood (%)	12.8	11.1	0.50	11.1	14.7	0.15	11.4	15.8	0.09	15.1	10.6	0.07
Erythrocyte fatty acids[b]												
15:0	0.21 (0.09)	0.23 (0.10)	0.0009	0.22 (0.09)	0.21 (0.09)	0.49	0.22 (0.10)	0.21 (0.10)	0.79	0.22 (0.09)	0.21 (0.09)	0.02
17:0	0.32 (0.06)	0.33 (0.07)	0.04	0.33 (0.06)	0.33 (0.06)	0.28	0.33 (0.06)	0.33 (0.06)	0.47	0.34 (0.06)	0.32 (0.07)	< 0.0001
Trans-16:1n-7	0.18 (0.06)	0.17 (0.06)	0.59	0.17 (0.06)	0.17 (0.07)	0.57	0.17 (0.06)	0.17 (0.07)	0.87	0.18 (0.06)	0.17 (0.07)	0.03
Trans-18:1n-9+*trans*-18:1n-7	0.52 (0.16)	0.52 (0.14)	0.99	0.53 (0.15)	0.52 (0.15)	0.24	0.53 (0.15)	0.52 (0.15)	0.54	0.54 (0.14)	0.51 (0.16)	0.0002
18:2n-6	10.7 (1.67)	10.8 (1.68)	0.24	10.8 (1.62)	10.7 (1.64)	0.04	10.9 (1.61)	10.7 (1.61)	0.03	11.0 (1.71)	10.7 (1.73)	0.03
20:5n-3	0.74 (0.32)	0.78 (0.38)	0.06	0.75 (0.34)	0.78 (0.35)	0.02	0.75 (0.32)	0.78 (0.36)	0.02	0.77 (0.35)	0.76 (0.33)	0.62
22:6n-3	4.94 (1.35)	4.95 (1.47)	0.72	4.92 (1.48)	4.90 (1.43)	0.33	4.92 (1.36)	4.89 (1.46)	0.24	4.84 (1.40)	5.00 (1.42)	0.17
20:4n-6/20:3n-6 ratio (estimated D5D activity)	8.57 (2.31)	9.07 (2.53)	0.0003	9.19 (2.65)	8.51 (2.34)	< 0.0001	9.04 (2.67)	8.64 (2.54)	0.03	9.45 (2.63)	8.24 (2.14)	< 0.0001
18:3n-6/18:2n-6 ratio (estimated D6D activity)	0.0046 (0.003)	0.0044 (0.003)	0.11	0.0040 (0.002)	0.0052 (0.003)	< 0.0001	0.0041 (0.002)	0.0050 (0.003)	< 0.0001	0.0039 (0.002)	0.0053 (0.003)	< 0.0001
16:1n-7/16:0 ratio (estimated SCD activity)	0.020 (0.008)	0.021 (0.009)	0.08	0.019 (0.008)	0.022 (0.009)	< 0.0001	0.019 (0.009)	0.021 (0.01)	0.0002	0.018 (0.007)	0.022 (0.009)	< 0.0001

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; D5D, Δ5-desaturase; D6D, Δ6-desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; SCD, stearoyl-coenzyme A-desaturase. *P*-value reflects whether the values of the variables significantly differ between extreme erythrocyte fatty acid/fatty acid ratio tertiles (Wilcoxon's test for proportions of erythrocyte fatty acids, *t*-test for other quantitative variables and $\chi 2$ test for categorical variables). [a]For quantitative variables, values are arithmetic means (s.d.), and for categorical variables, values are percentages (all such values). [b]For proportions of erythrocyte fatty acids, values are medians (IQR) (all such values).

Table 4. Adjusted arithmetic means (95% CI) of plasma HDL-cholesterol, LDL-cholesterol, non-HDL-cholesterol and adjusted geometric means of plasma triglycerides (mg/dl) by tertiles of erythrocyte fatty acid proportions for men ($n=655$) and women ($n=1104$), EPIC-Potsdam study

	Men				*Women*			
	Tertile of fatty acid			*P-value for trend*	*Tertile of fatty acid*			*P-value for trend*
	1	*2*	*3*		*1*	*2*	*3*	
15:0								
HDL-cholesterol (mg/dl)	44.3 (42.8–45.7)	42.6 (41.2–44.1)	43.1 (41.7–44.6)	0.31	50.5 (49.3–51.7)	51.8 (50.6–53.0)	52.5 (51.3–53.7)	0.03
LDL-cholesterol (mg/dl)	162 (156–168)	164 (158–170)	163 (157–169)	0.86	139 (135–143)	140 (136–144)	141 (137–145)	0.43
Non-HDL-cholesterol (mg/dl)	132 (128–137)	136 (131–140)	136 (131–141)	0.31	120 (116–123)	120 (117–123)	122 (119–125)	0.26
Triglycerides (mg/dl)	120 (111–130)	118 (110–128)	116 (107–125)	0.48	83.9 (79.9–88.2)	83.1 (79.2–87.3)	82.2 (78.3–86.3)	0.55
17:0								
HDL-cholesterol (mg/dl)	44.5 (43.0–46.0)	43.4 (42.0–44.8)	42.1 (40.6–43.6)	0.04	52.4 (51.1–53.7)	50.7 (49.4–51.9)	51.7 (50.5–53.0)	0.59
LDL-cholesterol (mg/dl)	165 (159–172)	162 (156-168)	161 (155–167)	0.34	141 (137–145)	139 (135–143)	140 (136–144)	0.68
Non-HDL-cholesterol (mg/dl)	134 (129–139)	135 (131–140)	135 (130–140)	0.79	121 (117–124)	120 (116–123)	121 (118–125)	0.77
Triglycerides (mg/dl)	125 (115–136)	116 (107–125)	113 (105–123)	0.10	87.8 (83.4–92.4)	82.7 (78.8–86.8)	79.0 (75.1–83.1)	0.006
Trans-*16:1n-7*								
HDL-cholesterol (mg/dl)	43.5 (42.0–45.0)	44.2 (42.8–45.7)	42.3 (40.8-43.7)	0.21	52.9 (51.7–54.2)	50.2 (48.9–51.4)	51.7 (50.4–52.9)	0.28
LDL-cholesterol (mg/dl)	164 (158–170)	159 (153–165)	165 (159–171)	0.65	141 (137–145)	135 (131–139)	143 (139–147)	0.33
Non-HDL-cholesterol (mg/dl)	135 (131–140)	133 (129–138)	136 (131–140)	0.90	122 (118–125)	116 (113–120)	124 (120–127)	0.24
Triglycerides (mg/dl)	118 (109–128)	111 (103–120)	125 (116–135)	0.26	84.5 (80.4–88.7)	80.6 (76.8–84.7)	84.2 (80.1–88.5)	0.96
Trans-*18:1n-9*+trans-*18:1n-7*								
HDL-cholesterol (mg/dl)	43.5 (42.0–45.0)	43.1 (41.7–44.6)	43.4 (42.0-44.9)	0.96	52.2 (51.0–53.4)	51.1 (49.8–52.3)	51.5 (50.3–52.8)	0.50
LDL-cholesterol (mg/dl)	158 (152–165)	167 (161–173)	163 (157–169)	0.39	138 (134–142)	140 (136–144)	142 (138–146)	0.15
Non-HDL-cholesterol (mg/dl)	131 (126–136)	138 (134–143)	135 (130–140)	0.27	118 (115–122)	120 (117–124)	123 (119–126)	0.08
Triglycerides (mg/dl)	111 (102–119)	125 (115–134)	119 (110–129)	0.23	84.3 (80.3–88.6)	83.0 (79.0–87.1)	82.0 (78.0–86.1)	0.44
20:5n-3								
HDL-cholesterol (mg/dl)	42.3 (40.8–43.7)	43.2 (41.8–44.7)	44.5 (43.1–46.0)	0.03	51.0 (49.7–52.3)	51.9 (50.7–53.1)	51.9 (50.6–53.1)	0.40
LDL-cholesterol (mg/dl)	162 (156–168)	164 (158–170)	162 (156–168)	0.90	140 (136–145)	142 (138–146)	137 (133–142)	0.27
Non-HDL-cholesterol (mg/dl)	134 (129–138)	135 (130–139)	136 (131–140)	0.56	121 (117–124)	121 (118–125)	119 (116–123)	0.50
Triglycerides (mg/dl)	121 (112–131)	121 (112–131)	112 (104–121)	0.15	86.0 (81.7–90.4)	85.6 (81.5–89.9)	78.0 (74.1–82.0)	0.007
22:6n-3								
HDL-cholesterol (mg/dl)	43.0 (41.6–44.5)	44.0 (42.6–45.5)	43.0 (41.5–44.4)	0.99	53.2 (52.0–54.4)	51.2 (49.9–52.4)	50.4 (49.2−–51.6)	0.002
LDL-cholesterol (mg/dl)	163 (157–169)	161 (155–167)	164 (158–170)	0.89	142 (138–146)	140 (136–144)	138 (134–142)	0.24
Non-HDL-cholesterol (mg/dl)	135 (131–140)	133 (128–138)	136 (131–141)	0.87	122 (119–126)	121 (117–124)	119 (115–122)	0.13
Triglycerides (mg/dl)	119 (111–129)	117 (108–126)	118 (109–127)	0.81	81.6 (77.7–85.8)	83.4 (79.4–87.6)	84.2 (80.2–88.5)	0.39
18:2n-6								
HDL-cholesterol (mg/dl)	43.1 (41.7–44.6)	43.6 (42.1–45.0)	43.3 (41.8–44.8)	0.89	51.9 (50.7–53.2)	51.8 (50.5–53.0)	51.1 (49.8–52.3)	0.36
LDL-cholesterol (mg/dl)	162 (156–168)	166 (161–172)	160 (154–166)	0.57	140 (136–144)	140 (136–144)	140 (136–144)	0.79
Non-HDL-cholesterol (mg/dl)	133 (128–138)	138 (134–143)	133 (128–138)	0.91	121 (118–124)	121 (117–124)	120 (117–123)	0.76
Triglycerides (mg/dl)	122 (112–131)	120 (111–129)	113 (105–122)	0.20	81.6 (77.7–85.8)	83.0 (79.1–87.2)	84.6 (80.5–88.9)	0.32
20:4n-6/20:3n-6 ratio (estimated D5D activity)								
HDL-cholesterol (mg/dl)	43.0 (41.5–44.4)	43.1 (41.6–44.5)	44.0 (42.5–45.4)	0.35	50.9 (49.6–52.1)	52.1 (50.9−–53.3)	51.8 (50.5–53.1)	0.32
LDL-cholesterol (mg/dl)	168 (162–174)	162 (156–168)	158 (152–164)	0.02	142 (138–146)	140 (136–144)	137 (133–141)	0.06
Non-HDL-cholesterol (mg/dl)	138 (133–142)	134 (129–138)	133 (128–137)	0.13	120 (117–124)	121 (118–124)	120 (117–123)	0.83
Triglycerides (mg/dl)	128 (119–138)	121 (113–131)	106 (97.9–114)	0.0006	92.0 (87.6–96.6)	82.8 (78.9–86.9)	75.3 (71.6–79.1)	< 0.0001
18:3n-6/18:2n-6 ratio (estimated D6D activity)								
HDL-cholesterol (mg/dl)	43.5 (42.0–44.9)	43.0 (41.6–44.4)	43.6 (42.1–45.0)	0.89	50.0 (48.8–51.3)	52.0 (50.8–53.2)	52.7 (51.5–54.0)	0.005
LDL-cholesterol (mg/dl)	155 (149–161)	160 (154–166)	174 (168–180)	< 0.0001	134 (130–138)	140 (136–144)	146 (142–150)	0.0001
Non-HDL-cholesterol (mg/dl)	131 (126–135)	134 (129–138)	140 (135–145)	0.005	117 (114–121)	121 (118–124)	123 (120–127)	0.02
Triglycerides (mg/dl)	102 (94.7–110)	111 (104–120)	144 (134–156)	< 0.0001	73.5 (70.0–77.2)	82.9 (79.0–86.9)	94.2 (89.7−–98.9)	< 0.0001

Table 4. (Continued)

	Men				Women			
	Tertile of fatty acid			*P-value for trend*	*Tertile of fatty acid*			*P-value for trend*
	1	*2*	*3*		*1*	*2*	*3*	
16:1n-7/16:0 ratio (estimated SCD activity)								
HDL-cholesterol (mg/dl)	42.9 (41.4–44.3)	42.8 (41.3–44.2)	44.4 (42.9–45.9)	0.16	50.2 (49.0–51.4)	51.2 (50.0–52.4)	53.4 (52.1–54.6)	0.0005
LDL-cholesterol (mg/dl)	155 (149–161)	162 (156–168)	171 (165–177)	0.0004	136 (132–140)	139 (135–143)	145 (141–149)	0.0008
Non-HDL-cholesterol (mg/dl)	132 (127–137)	135 (131–140)	137 (132–142)	0.19	119 (116–123)	120 (117–124)	122 (119–125)	0.31
Triglycerides (mg/dl)	99.0 (91.8–107)	115 (107–124)	144 (134–156)	< 0.0001	72.4 (69.0–76.0)	81.5 (77.8–85.5)	97.2 (92.6–102)	< 0.0001

Abbreviations: BMI, body mass index; CI, confidence interval; D5D, Δ5-desaturase; D6D, Δ6-desaturase; EPIC, European Prospective Investigation into Cancer and Nutrition; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SCD, stearoyl-coenzyme A-desaturase. The model was adjusted for age at recruitment, smoking status (never, past, current smoker < 20 U per day, current smoker ⩾ 20 U per day), alcohol intake (>0–6; >6–12; >12–24; >24–60; >60–96; >96 g per day), leisure time sports activity (h per week), biking (h per week), education status (in training, no certificate, part skilled worker; skilled worker; professional school; college of higher education, university), total carbohydrate intake (g per day), BMI (kg/m^2) and waist circumference (cm). We estimated geometric means and 95% CIs in case of triglycerides and arithmetic means and 95% CIs in case of HDL-cholesterol, LDL-cholesterol and non-HDL-cholesterol by fatty acid tertiles and tested for statistical significance of linear trends across tertiles by modeling the median value of the fatty acid within each tertile as a quantitative variable. *P*-value for trend reflects whether the mean of the biomarker significantly decreases or increases across the FA tertiles.

but should rather be considered as hypothesis-generating. Still, our hypothesis is supported by results from genome-wide association studies, which identified variants in the *FADS* gene region to be associated with triglyceride concentrations.[17]

Intake of long-chain n-3 PUFAs had clear triglyceride-lowering effects in intervention studies.[7] Similarly, cross-sectional studies on blood long-chain n-3 PUFA levels found inverse associations with plasma triglycerides.[14] In our study, we found an overall weak inverse association of the proportion of EPA in erythrocytes and triglyceride concentrations in women. Stratification for PPARG2 Pro12Ala genotype revealed an inverse association in Pro12Pro carriers, but no apparent association among Ala12 carriers, although this interaction did not reach statistical significance. In contrast, in a controlled intervention study, Ala12 carriers presented a greater decrease in serum triglycerides in response to n-3 PUFA supplementation than Pro12Pro carriers when the intake of SFA was below 10% of energy.[32] In a randomized controlled trial with obese patients, the Pro12Ala polymorphism was not associated with the n-3 long-chain PUFA treatment response on triglycerides.[33] The results from both studies are in contrast to each other and to our findings. The heterogeneity of these studies complicates the comparison of their results. Of note, measurement error may also have influenced our observations for EPA. While the intra-assay coefficient of variation, calculated from 40 measurements in a subset of 20 samples, was only 3.1% for EPA, there is some indication of poor long-term reproducibility of measured EPA proportions in our study. In a sample of 250 participants, we measured the FA concentration of the same samples repeatedly in 2008 and 2012. In general, an acceptable reproducibility was obtained with a correlation coefficient >0.7 for dihomo-γ-linolenic acid, DHA and LA and ⩾ 0.3 for the other FAs, despite EPA. Differences in FA profile between the two measurements may be owing to changes in analytical procedures and, to a lesser extent, also to changes in FA composition over time (e.g. due to FA oxidation[34]). As the measurement error is expected to be random and unrelated to the outcome, an attenuation of the observed associations would be the consequence.

Serum esterified LA proportions have been inversely associated with the risk of CVD[2] and T2DM.[8–11] In our study, the proportion of LA in erythrocytes was not clearly associated with lipid markers. In contrast, intervention studies showed that LA-rich diets lower LDL-cholesterol with marginal effects on HDL-cholesterol.[12] However, blood FAs reflect both dietary intake of FAs and endogenous FA metabolism, such as the activity of D6D that converts LA to higher unsaturated PUFAs. In a cross-sectional study, the LA proportion in total serum was inversely associated with triglycerides in US whites, Japanese and Japanese-Americans.[14] The discrepancies to our results might be owing to different study characteristics of study participants, for example, with respect to ethnicity. Furthermore, LA levels in erythrocytes differ from those in plasma lipids. In addition, lower LA concentrations in total plasma at higher triglyceride concentrations may also reflect the fact that the LA content of the plasma triglyceride fraction is generally lower than the LA content of plasma phospholipids or cholesterol esters.[3]

SFA intake has been widely shown to be positively associated with CVD risk factors such as elevated LDL-cholesterol, blood pressure, insulin resistance and inflammation.[35–38] However, individual SFAs differ in their physiologic effects,[36] for example, blood 15:0 and 17:0, that have been verified as relative and objective estimates of dietary ruminant fat intake in a Western population,[19] and that have been associated with lower risk of T2DM[8,9,11] and myocardial infarction.[39] Consistent with these findings, we found an inverse association of erythrocyte membrane 17:0 with triglycerides in women. Our results support those of two previous studies that found inverse correlations of blood 15:0 and 17:0 with triglycerides.[18,39] However, these correlation coefficients were only adjusted for BMI and smoking, while our study suggests that these associations are largely independent of various other demographic, anthropometric and lifestyle factors.

We found no clear associations of erythrocyte *trans*-FAs with lipid markers. Intervention studies demonstrated adverse effects of increased *trans*-FA consumption on blood lipid profiles with increased LDL- and decreased HDL-cholesterol.[20] Similar observations were made in studies investigating the blood content of total *trans*-FAs.[40–43] However, three studies on blood contents of *trans*-FAs revealed important differences between individual *trans*-FAs.[23–25] These differences seem to depend on the source of *trans*-FAs, for example, whether they are derived from ruminant fats or industrially hardened vegetable oil. Circulating *trans*-16:1n-7, mainly derived from consumption of ruminant fats,[23,25] and total *trans*-18:1 FAs, in the middle-aged Chinese study population presumably mainly derived from consumption of dairy products,[24] showed overall beneficial associations with lipid markers (e.g. positively associated with HDL-cholesterol and inversely with triglycerides). On the contrary, erythrocyte total *trans*-18:2 FAs mainly derived from partially hydrogenated vegetable oils or transformed LA during frying[44] were adversely associated with lipid markers (e.g. positively with triglycerides and LDL-cholesterol).[24] In our study, *trans*-18:1n-9, mainly derived from industrially hardened vegetable oil,[45] and *trans*-18:1n-7, mostly reflecting consumption of ruminant fat,[46] were detected in the analytical method as a sum. Therefore, beneficial and adverse effects of the two *trans*-FA isomers might have neutralized each other. However, we found no association of *trans*-16:1n-7 with lipid markers.

A limitation of our study lies in the fact that many of our participants had not fasted before blood drawal. It is well known that the plasma triglyceride level depends on food intake. However, on the population level, triglyceride levels increased only modestly in response to normal food intake[47] and non-fasting triglycerides and non-fasting calculated LDL-cholesterol predicted risk of cardiovascular events.[47] Because the fasting status should not affect the erythrocyte FA profile, we expect—if anything—an attenuation of the effect estimates. In our sensitivity analysis restricted to fasted participants, associations were generally qualitatively comparable, although only few reached statistical significance owing to the loss of power. A further limitation of this study is that our results may not be generalizable to other ethnicities. Owing to the cross-sectional design, it cannot be determined whether the erythrocyte FAs were the cause or the result of a change in biomarkers of dyslipidemia (reverse causation). Still, in genome-wide association studies, genetic variants in *FADS* genes, which encode D5D and D6D, were associated with triglyceride concentrations[17] and experimental studies have proposed plausible biological mechanisms.[4,5] Erythrocyte FAs were expressed as percentages of total FAs. Thus, it is difficult to interpret results for individual FAs independent of the other FAs. Random measurement error in erythrocyte FAs might have led to an attenuation of the effect estimates. This is especially relevant for FAs with a high coefficient of variation, such as 18:3n-6, and consequently the calculated FA-ratio reflecting D6D activity. LDL-cholesterol was calculated with the Friedewald formula. The reliability of the LDL-cholesterol estimation decreases with increasing triglyceride concentrations.[48] Desaturase activities were measured indirectly as FA-ratios. However, direct measures are difficult to obtain in large epidemiological studies. In a recent intervention study of isotope-labeled α-linolenic acid, acceptable correlations of labeled ALA and EPA as direct indicators of D5D and D6D activity with the conventionally used plasma FA-ratios were observed.[49] Two studies indicate an acceptable correlation between gene expression and calculated FA-ratio for SCD derived from plasma very low-density lipoprotein triglyceride composition and adipose tissue FA composition, whereas the correlations for D5D and D6D were moderate to low.[50,51] However, it remains unclear to which extent these findings are transferable to FA-ratios derived from erythrocyte membranes. Still, we have previously reported that variation in *FADS1* and *FADS2* genes was associated with the estimated desaturase activities from erythrocyte membranes.[52] Regarding stratification for PPARG genotype, our study may be underpowered for a statistical test on interaction.

In conclusion, we observed unfavorable lipid profiles with higher estimated D6D and SCD activity, whereas higher estimated D5D activity was related to more favorable lipid profiles. Our results are supported by results from genome-wide association studies[17] and findings from experimental studies that propose plausible biological mechanisms for the association between FAs and biomarkers of dyslipidemia.[4,5] Our findings suggest that triglyceride and LDL-cholesterol concentrations may be mediators linking the intake and metabolism of FAs to metabolic risk.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all EPIC participants for their contribution to the study and Ellen Kohlsdorf for data management. We are indebted to Hans Cremers for the excellent performance of the FA analysis. The recruitment phase of the EPIC-Potsdam Study was supported by the Federal Ministry of Science, Germany (01EA 9401) and the European Union (SOC 95201408 05F02). The present study was also supported by a grant from the German Research Foundation (DFG, SCHU 1516/5-1).

REFERENCES

1 Riserus U, Willett WC, Hu FB. Dietary fats and prevention of type 2 diabetes. *Prog Lipid Res* 2009; **48**: 44–51.
2 Erkkila A, de Mello VD, Riserus U, Laaksonen DE. Dietary fatty acids and cardiovascular disease: an epidemiological approach. *Prog Lipid Res* 2008; **47**: 172–187.
3 Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* 2008; **47**: 348–380.
4 Bordoni A, Di Nunzio M, Danesi F, Biagi PL. Polyunsaturated fatty acids: from diet to binding to ppars and other nuclear receptors. *Genes Nutr* 2006; **1**: 95–106.
5 Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. *J Nutr* 2005; **135**: 2075–2078.
6 Delgado-Lista J, Perez-Martinez P, Lopez-Miranda J, Perez-Jimenez F. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *Br J Nutr* 2012; **107**(Suppl 2): S201–S213.
7 Jacobson TA, Glickstein SB, Rowe JD, Soni PN. Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: a review. *J Clin Lipidol* 2012; **6**: 5–18.
8 Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA *et al.* Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes: interpreting the role of linoleic acid. *Am J Clin Nutr* 2007; **86**: 189–197.
9 Krachler B, Norberg M, Eriksson JW, Hallmans G, Johansson I, Vessby B *et al.* Fatty acid profile of the erythrocyte membrane preceding development of Type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis* 2008; **18**: 503–510.
10 Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ *et al.* Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. *Am J Clin Nutr* 2010; **92**: 1214–1222.
11 Kroger J, Zietemann V, Enzenbach C, Weikert C, Jansen EHJM, Doring F *et al.* Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Am J Clin Nutr* 2011; **93**: 127–142.
12 Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003; **77**: 1146–1155.
13 Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F *et al.* Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* 2006; **91**: 439–446.
14 Motoyama KR, Curb JD, Kadowaki T, El-Saed A, Abbott RD, Okamura T *et al.* Association of serum *n*-6 and *n*-3 polyunsaturated fatty acids with lipids in 3 populations of middle-aged men. *Am J Clin Nutr* 2009; **90**: 49–55.

15 Kroger J, Schulze MB. Recent insights into the relation of Delta5 desaturase and Delta6 desaturase activity to the development of type 2 diabetes. *Curr Opin Lipidol* 2012; **23**: 4–10.
16 Bjermo H, Riserus U. Role of hepatic desaturases in obesity-related metabolic disorders. *Curr Opin Clin Nutr Metab Care* 2010; **13**: 703–708.
17 Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE *et al.* Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet* 2009; **41**: 56–65.
18 Warensjo E, Jansson JH, Berglund L, Boman K, Ahren B, Weinehall L *et al.* Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case–control study. *Br J Nutr* 2004; **91**: 635–642.
19 Smedman AEM, Gustafsson IB, Berglund LGT, Vessby BOH. Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. *Am J Clin Nutr* 1999; **69**: 22–29.
20 Mozaffarian D, Aro A, Willett WC. Health effects of *trans*-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* 2009; **63**(Suppl 2): S5–S21.
21 Mozaffarian D, Clarke R. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *Eur J Clin Nutr* 2009; **63**: S22–S33.
22 Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. *Trans* fatty acids and cardiovascular disease. *N Engl J Med* 2006; **354**: 1601–1613.
23 Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS *et al. Trans*-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. *Ann Intern Med* 2010; **153**: 790–799.
24 Yu DX, Sun Q, Ye XW, Pan A, Zong G, Zhou YH *et al.* Erythrocyte *trans*-fatty acids, type 2 diabetes and cardiovascular risk factors in middle-aged and older Chinese individuals. *Diabetologia* 2012; **55**: 2954–2962.
25 Mozaffarian D, de Oliveira Otto MC, Lemaitre RN, Fretts AM, Hotamisligil G, Tsai MY *et al. Trans*-palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr* 2013; **97**: 854–861.
26 Boeing H, Wahrendorf J, Becker N. EPIC-Germany—a source for studies into diet and risk of chronic diseases. *Ann Nutr Metab* 1999; **43**: 195–204.
27 van Dieren S, Nothlings U, van der Schouw YT, Spijkerman AM, Rutten GE, van der AD *et al.* Non-fasting lipids and risk of cardiovascular disease in patients with diabetes mellitus. *Diabetologia* 2011; **54**: 73–77.
28 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.
29 Chow LS, Li S, Eberly LE, Seaquist ER, Eckfeldt JH, Hoogeveen RC *et al.* Estimated plasma stearoyl co-A desaturase-1 activity and risk of incident diabetes: the Atherosclerosis Risk in Communities (ARIC) study. *Metabolism* 2013; **62**: 100–108.
30 Gupta AK, Dahlof B, Dobson J, Sever PS, Wedel H, Poulter NR. Determinants of new-onset diabetes among 19,257 hypertensive patients randomized in the Anglo-Scandinavian Cardiac Outcomes Trial—Blood Pressure Lowering Arm and the relative influence of antihypertensive medication. *Diabetes Care* 2008; **31**: 982–988.
31 Sarwar N, Danesh J, Eiriksdottir G, Sigurdsson G, Wareham N, Bingham S *et al.* Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies. *Circulation* 2007; **115**: 450–458.
32 Lindi V, Schwab U, Louheranta A, Laakso M, Vessby B, Hermansen K *et al.* Impact of the Pro12Ala polymorphism of the PPAR-gamma2 gene on serum triacylglycerol response to *n*-3 fatty acid supplementation. *Mol Genet Metab* 2003; **79**: 52–60.
33 Itariu BK, Zeyda M, Hochbrugger EE, Neuhofer A, Prager G, Schindler K *et al.* Long-chain *n*-3 PUFAs reduce adipose tissue and systemic inflammation in severely obese nondiabetic patients: a randomized controlled trial. *Am J Clin Nutr* 2012; **96**: 1137–1149.
34 Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. *Eur J Clin Nutr* 2000; **54**: 367–372.
35 Hall WL. Dietary saturated and unsaturated fats as determinants of blood pressure and vascular function. *Nutr Res Rev* 2009; **22**: 18–38.
36 Astrup A, Dyerberg J, Elwood P, Hermansen K, Hu FB, Jakobsen MU *et al.* The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: where does the evidence stand in 2010? *Am J Clin Nutr* 2011; **93**: 684–688.
37 Kennedy A, Martinez K, Chuang CC, LaPoint K, McIntosh M. Saturated fatty acid-mediated inflammation and insulin resistance in adipose tissue: mechanisms of action and implications. *J Nutr* 2009; **139**: 1–4.
38 Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis* 2008; **197**: 12–24.
39 Warensjo E, Jansson JH, Cederholm T, Boman K, Eliasson M, Hallmans G *et al.* Biomarkers of milk fat and the risk of myocardial infarction in men and women: a prospective, matched case–control study. *Am J Clin Nutr* 2010; **92**: 194–202.
40 Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ *et al.* A prospective study of trans fatty acids in erythrocytes and risk of coronary heart disease. *Circulation* 2007; **115**: 1858–1865.
41 Kabagambe EK, Tsai MY, Hopkins PN, Ordovas JM, Peacock JM, Borecki IB *et al.* Erythrocyte fatty acid composition and the metabolic syndrome: a National Heart, Lung, and Blood Institute GOLDN study. *Clin Chem* 2008; **54**: 154–162.
42 Counil E, Julien P, Lamarche B, Chateau-Degat ML, Ferland A, Dewailly E. Association between *trans*-fatty acids in erythrocytes and pro-atherogenic lipid profiles among Canadian Inuit of Nunavik: possible influences of sex and age. *Br J Nutr* 2009; **102**: 766–776.
43 Kabagambe EK, Ordovas JM, Hopkins PN, Tsai MY, Arnett DK. The relation between erythrocyte trans fat and triglyceride, VLDL- and HDL-cholesterol concentrations depends on polyunsaturated fat. *PLoS One* 2012; **7**: 15.
44 Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH *et al.* Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study. *Circulation* 2006; **114**: 209–215.
45 Thiebaut AC, Rotival M, Gauthier E, Lenoir GM, Boutron-Ruault MC, Joulin V *et al.* Correlation between serum phospholipid fatty acids and dietary intakes assessed a few years earlier. *Nutr Cancer* 2009; **61**: 500–509.
46 Mendis S, Cruz-Hernandez C, Ratnayake WMN. Fatty acid profile of Canadian dairy products with special attention to the < i>trans < /i>-octadecenoic acid and conjugated linoleic acid isomers. *J AOAC Int* 2008; **91**: 811–819.
47 Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. *Circulation* 2008; **118**: 2047–2056.
48 Warnick GR, Knopp RH, Fitzpatrick V, Branson L. Estimating low-density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints. *Clin Chem* 1990; **36**: 15–19.
49 Gillingham LG, Harding SV, Rideout TC, Yurkova N, Cunnane SC, Eck PK *et al.* Dietary oils and FADS1–FADS2 genetic variants modulate [^{13}C]alpha-linolenic acid metabolism and plasma fatty acid composition. *Am J Clin Nutr* 2013; **97**: 195–207.
50 Peter A, Cegan A, Wagner S, Lehmann R, Stefan N, Konigsrainer A *et al.* Hepatic lipid composition and stearoyl-coenzyme A desaturase 1 mRNA expression can be estimated from plasma VLDL fatty acid ratios. *Clin Chem* 2009; **55**: 2113–2120.
51 Sjogren P, Sierra-Johnson J, Gertow K, Rosell M, Vessby B, de Faire U *et al.* Fatty acid desaturases in human adipose tissue: relationships between gene expression, desaturation indexes and insulin resistance. *Diabetologia* 2008; **51**: 328–335.
52 Zietemann V, Kroger J, Enzenbach C, Jansen E, Fritsche A, Weikert C *et al.* 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. *Br J Nutr* 2010; **104**: 1748–1759.

Supplementary Information accompanies this paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)