European Heart Journal Advance Access published September 22, 2015

European Heart Journal
doi:10.1093/eurheartj/ehv446

Trans fatty acids and mortality in patients referred for coronary angiography: the Ludwigshafen Risk and Cardiovascular Health Study

Marcus E. Kleber[1,2]*, Graciela E. Delgado[1], Stefan Lorkowski[2,3], Winfried März[1,4,5], and Clemens von Schacky[6,7]

[1]Vth Department of Medicine, Medical Faculty Mannheim, Heidelberg University, Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; [2]Competence Cluster of Nutrition and Cardiovascular Health (nutriCARD), Halle-Jena-Leipzig, Germany; [3]Institute of Nutrition, Friedrich Schiller University Jena, Jena, Germany; [4]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria; [5]Synlab Academy, Synlab Services GmbH, Mannheim, Germany; [6]Omegametrix, Martinsried, Germany; and [7]Department of Preventive Cardiology, Medizinische Klinik und Poliklinik I, Munich University, Munich, Germany

Received 10 November 2014; revised 6 July 2015; accepted 17 August 2015

Aims *Trans* fatty acids (TFAs) are generated by the food industry and also occur naturally in trace amounts in dairy products. For the latter, beneficial health effects have been claimed, while there are numerous reports about TFA of industrial origin being hazardous to human health. Therefore, we aimed to investigate the association of TFA with mortality in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.

Methods and results The fatty acid composition of erythrocyte membranes was analysed using the HS-Omega-3 Index® methodology in 3259 participants of the LURIC study at baseline. During a median of 10.0 years of follow-up, a total of 975 (29.9%) study participants died, 614 (18.8%) from cardiovascular causes including 254 (7.8%) sudden cardiac deaths (SCDs). Association of TFA with clinical outcome was investigated with Cox proportional hazards regression. Total TFAs were inversely associated with mortality due to cardiovascular causes or SCD. This was mainly driven by the naturally occurring TFA C16:1n-7t (*trans*-palmitoleic acid). The reduced risk of SCD associated with C16:1n-7t persisted after multivariate adjustment with a hazard ratio of 0.63 (0.46–0.86) for the third tertile compared with the first tertile. There was no association of any TFA subgroup with an increased risk of adverse outcomes.

Conclusions In contrast to previous findings, the low concentrations of total TFAs found in LURIC were inversely associated with adverse cardiac outcomes. While the naturally occurring TFA C16:1n-7t was associated with reduced risk, no increased risk was found for industrially produced TFAs.

Keywords *Trans* fatty acids • *Trans*-palmitoleic acid • Mortality • Cardiovascular mortality • Sudden cardiac death

Introduction

Trans fatty acids (TFAs) are unsaturated fatty acids containing double bonds in *trans* configuration. In animals and plants, fatty acids usually occur in *cis* configuration. However, in milk, dairy products, and meat, some *trans*-isomers occur naturally in small quantities. Other TFAs are 'man-made' by industrial hardening of unsaturated fats. If oils are only partially hydrogenated, a portion of *cis*-isomers is converted into *trans*-isomers (industrially produced TFAs, IP-TFAs). While beneficial health effects have been claimed for the naturally occurring TFA vaccenic acid or *trans*-palmitoleic acid in some studies,[1,2] there are numerous reports suggesting that IP-TFA may be hazardous to human health.[3–7] Industrially produced *trans* fatty acids have been linked to increased risks for a number of diseases that are associated with modern Western lifestyle, such as cardiovascular disease (CVD), stroke, diabetes, infertility, Alzheimer's disease, or certain cancers. Therefore, a range of actions has been taken to reduce the intake of IP-TFA on a population level. These measures are effective as demonstrated by decreasing levels of IP-TFAs in the USA.[8] In Europe, intake of IP-TFA has traditionally been lower compared with the USA where major sources for TFAs have been cakes, cookies, pies and pastries.[9]

* Corresponding author. Tel: +49 621 383 3019, Fax: +49 621 383 3804, Email: marcus.kleber@medma.uni-heidelberg.de

It is unclear whether variations of TFAs at low concentrations are associated with CVD risk and how the highest concentration of TFA that may be harmless should be defined.[10] Equally open today is the question whether the same thresholds should be applied to both IP-TFA and ruminant TFA. Therefore, it has been the aim of our study to investigate the association of total TFA concentrations and individual TFA species measured in the membrane of red blood cells with CVD and mortality in a large and well-characterized clinical cohort, the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.

Methods

Study populations

The LURIC study included 3316 Caucasians hospitalized for coronary angiography between 1997 and 2000 at a tertiary care centre in south-western Germany.[11] Clinical indications for angiography were chest pain or a positive non-invasive stress test suggestive of myocardial ischaemia. To limit clinical heterogeneity, individuals suffering from acute illnesses other than acute coronary syndrome, chronic non-cardiac diseases, and a history of malignancy within the past 5 years were excluded. The study was approved by the ethics committee at the 'Landesärztekammer Rheinland-Pfalz' and was conducted in accordance with the 'Declaration of Helsinki'. Informed written consent was obtained from all participants.

Laboratory procedures

Fasting blood samples were obtained by venipuncture in the early morning. Cholesterol and triglycerides (TG) were measured with enzymatic reagents from WAKO (Neuss, Germany) on an Olympus AU640 analyser (Centre Valley, PA). Lipoproteins were separated by a combined ultracentrifugation–precipitation method (β-quantification) as described previously.[11] Erythrocyte fatty acid composition was analysed according to the HS-Omega-3 Index® methodology as described previously.[12] Fatty acid methyl esters were generated from erythrocytes by acid transesterification and analysed by gas chromatography using a GC2010 gas chromatograph (Shimadzu, Duisburg, Germany) equipped with a 100-m SP2560 column (Supelco, Bellefonte, PA) using hydrogen as carrier gas. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of erythrocytes. Results are given as a percentage of total identified fatty acids after response factor correction. The chromatographic conditions allowed to separate the C16:1 *trans*-isomers and three *trans*-isomers of C18:2n6 (C18:2n6tt, C18:2n6ct, and C18:2n6tc). The individual *trans*-isomers of C18:1 (i.e. C18:1δ6 through C18:1δ13) could not be separated, but appeared as two blended peaks, eluting just ahead of oleic acid. The areas of these two peaks were added and referred to as C18:1t. The sum of the five TFAs had a coefficient of variation of 7%. The sum of C18:2n6tt, C18:2n6ct, and C18:2n6tc is referred to as 'C18:2t', whereas the sum of all measured TFAs is referred to as total TFAs.

Clinical definitions

Coronary artery disease (CAD) was defined as the presence of a visible luminal narrowing (>20% stenosis) in at least one of the 15 coronary segments according to the classification of the American Heart Association. Diabetes mellitus was defined according to 2010 guidelines of the American Diabetes Association as increased fasting (≥126 mg/dL) and/or post-challenge (2 h after the 75 g glucose load ≥200 mg/dL) glucose and/or elevated glycated haemoglobin (≥6.5%) and/or history of diabetes. Blood pressure was measured with an automated oscillometric device (Omron MX4, Omron Healthcare GmbH, Hamburg, Germany) while supine for at least 10 min. At least three consecutive measurements of systolic and diastolic blood pressures were taken 30 s apart. Hypertension was defined as a systolic and/or diastolic blood pressure >140 and/or >90 mmHg or a significant history of hypertension. The glomerular filtration rate was estimated by using the 2012 CKD-EPI eGFRcreat-cys equation.[13] Self-reported physical exercise was measured on a scale ranging from −5 (extremely low) to +5 (extremely high, athlete).

Definition of clinical endpoints

Information on vital status was obtained from local registries. Death certificates and medical records of local hospitals and autopsy data were reviewed independently by two experienced clinicians who were blinded to patient characteristics and who classified the causes of death. In cases of disagreement or uncertainty concerning the coding of a specific cause of death, the decision was made by a principal investigator (W.M.). During a median follow-up of 10.0 years (range: 0.1–11.9 years), 995 (30%) participants died. Cardiovascular mortality (CVM) included the following categories: sudden cardiac death [SCD; $n = 259$ (12.8%)], fatal myocardial infarction [$n = 106$ (3.20%)], death due to congestive heart failure [$n = 148$ (4.46%)], death immediately after intervention to treat CAD [$n = 26$ (0.78%)], fatal stroke [$n = 61$ (1.84%)], and other causes of death due to CAD [$n = 19$ (0.57%)]. Information for mortality is complete for all participants. The cause of death of 23 individuals was unknown and these patients were included in calculations of all-cause mortality but not in calculations considering different causes of death. *Trans* fatty acid measurements were available for 3259 study participants.

Statistical analyses

The primary aim of our study was to examine the association of TFA with fatal endpoints, namely all-cause mortality, CVM, and SCD, which we did by building Cox proportional hazard models. As the distribution of individual TFA was slightly skewed to the left, we chose to stratify our patients into tertiles of TFAs. The first tertile of total TFAs included all values ≤0.83% of all fatty acids in the membrane of erythrocytes, the second tertile encompassed all values from 0.84 to 1.08%, and the third tertile included all values ≥1.09%. The functional form of covariates was analysed by calculating Martingale residuals and the proportional hazard assumption was checked by examination of scaled Schoenfeld residuals. Adjustments were carried out including known confounding variables for CVM.

We also adjusted the data distribution by inverse probability weighting so that each tertile's weighted distribution matched that of the whole cohort, thereby balancing the subgroups for the confounding variables. We plotted the reweighted distribution of confounding variables for each C16:1n7t tertile to check whether the balancing worked. A weighted Cox model was calculated and we report the result of the robust score test as implemented in the coxph function in R that corresponds to a log-rank test corrected for weighting.

The distribution of all variables was examined visually by viewing histograms and by comparing mean and median values. Continuous data are presented as the mean ± standard deviation when normally distributed or as the median and 25th and 75th percentile for non-normally distributed variables. Categorical data are presented as percentages. Statistical differences between groups and continuous variables were determined using analysis of variance. Non-normally distributed variables were log-transformed before entering analysis in order to achieve an approximately normal distribution. The χ^2 test was used for categorical variables. Correlation and partial correlation adjusted for age and

sex between TFAs and biomarkers were analysed by Spearman's ρ. All tests were two sided and Bonferroni-adjusted thresholds for significance were calculated as indicated for the respective tables to correct for multiple testing. All analyses were carried out using the SPSS 21.0 statistical package (IBM SPSS, USA) and R v3.1.1 (http://www.r-project.org).

Results

Trans fatty acids were measured in 3259 participants of the LURIC study who all underwent coronary angiography. They ranged from 0.27 to 2.40% of total fatty acids in erythrocyte membranes with a mean of 0.96 ± 0.26%. Total TFAs were composed of 61.8 ± 0.1% C18:1t isomers, 22.4 ± 0.1% C18:2t isomers, and 15.8 ± 0.1% C16:1n-7t isomers. Study demographics are shown in *Table 1*.

Correlation of total *trans* fatty acids with biomarkers at study baseline

Study characteristics according to tertiles of total TFAs as well as specific subgroups of TFAs are shown in Supplementary material online, *Tables S1–S4*. Partial correlation coefficients adjusted for age and gender are shown in *Table 2*.

Percentages of total TFAs were directly correlated with LDL-C and inversely correlated with body mass index (BMI), waist-to-hip ratio, physical exercise, blood pressure, TG, and markers of glucose metabolism (fasting glucose, fasting insulin, HbA1c, and homeostasis model assessment (HOMA) index). These correlations were largely similar for the subgroups of C16:1n-7 and C18:1t isomers, but not for the subgroup of C18:2t isomers (Supplementary material online, *Table S5*). About 50% of the LURIC participants were receiving lipid-lowering therapy (mostly statins) at baseline with a lower percentage in the higher tertiles of total TFAs. We, therefore, repeated the analyses restricted to those patients not receiving lipid-lowering therapy (Supplementary material online, *Table S6*). Several associations then turned insignificant, which is likely due to the reduced power following the approximate bisection of the sample. Most notably, however, the strong associations of TFAs with BMI, TG, blood pressure, markers of glucose metabolism, and diabetes mellitus remained almost unchanged.

Table 1 Study demographics

Age (years)	62.7 ± 10.6
Male sex (%)	69.7
BMI (kg/m^2)	27.5 ± 4.07
Waist-to-hip ratio	0.96 ± 0.08
Systolic BP (mmHg)	141 ± 23.6
Diastolic BP (mmHg)	81.0 ± 11.5
LDL-C (mg/dL)	116 ± 34.3
HDL-C (mg/dL)	38.8 ± 10.8
TG (mg/dL)	147 (109–201)
Fasting glucose (mg/dL)	102 (93.6–118)
Coronary artery disease (%)	77.9
Hypertension (%)	72.9
Diabetes (%)	39.8
Lipid-lowering therapy (%)	48.6

Shown are mean ± standard deviation or median (25th–75th percentile).
BMI, body mass index; BP, blood pressure.

Trans fatty acids and mortality

We examined the association of TFA with all-cause mortality, CVM, and SCD by means of Cox regression adjusted for age and gender (Model 1) or additionally adjusted for traditional risk factors and markers significantly associated with TFA, namely BMI, LDL-C, HDL-C, TG, fibrinogen, smoking, hypertension, diabetes, estimated glomerular filtration rate, and lipid-lowering therapy (Model 2). Increasing total TFAs were associated with lower CVM and SCD in Model 1 (*Table 3*). C16:1n-7t was associated with reduced all-cause mortality, CVM as well as SCD in Model 1.

In Model 2, only the association of the highest tertile with decreased risk of SCD remained statistically significant at a hazard ratio (HR; 95% confidence interval) of 0.63 (0.46–0.86), and *P* for trend across tertiles was also significant (*Table 3*). Adjusted survival curves are shown in *Figure 1*. The distribution of confounding variables was balanced by inverse variance weighting. Resulting HRs were similar to the ones obtained by simple adjustment with HR of 0.82 (0.61–1.12) and 0.67 (0.48–0.93) for the second and the third tertile, respectively. The C18:1t and C18:2t isomers did not show any association with endpoints except for an association of the middle tertile of C18:1t with SCD. Of note, we did not observe any increased risk for any of the investigated endpoints for any of the TFAs. This was also true for the individual C18:2t isomers C18:2n6tt, C18:2n6ct, and C18:2n6tc (Supplementary material online, *Table S7*).

Table 2 Partial correlation of total *trans* fatty acids with biomarkers adjusted for age and sex

	Total TFAs	
	R	*P**
BMI (kg/m^2)	−0.137	<0.001
Waist-to-hip ratio	−0.042	0.018
Physical exercise	−0.057	0.001
Systolic BP (mmHg)	−0.065	<0.001
Diastolic BP (mmHg)	−0.075	<0.001
LDL-C (mg/dL)	0.042	0.016
HDL-C (mg/dL)	−0.012	0.507
TG (mg/dL)	−0.132	<0.001
Fasting glucose (mg/dL)	−0.147	<0.001
Fasting insulin (mmol)	−0.052	0.003
HbA1c (%)	−0.192	<0.001
HOMA index	−0.083	<0.001
High-sensitivity C-reactive protein (mg/dL)	−0.004	0.839
Fibrinogen (mg/dL)	−0.069	<0.001
SAA (mg/L)	0.009	0.589
NT-proBNP (ng/mL)	0.027	0.129

BMI, body mass index; BP, blood pressure; TFAs, *trans* fatty acids; SAA, serum amyloid A; NT-proBNP, N-terminal of the prohormone brain natriuretic peptide.
*After Bonferroni adjustment for 16 tests, a *P* value of 0.0031 could be regarded as significant.

Table 3 **Association of tertiles of *trans* fatty acids with mortality, cardiovascular mortality, and sudden cardiac death**

	N events	Total TFAs				C16:1n-7t				C18:1t				C18:2t			
		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
		HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
All-cause mortality																	
First	292	1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]	
Second	399	0.87 (0.74–1.01)	0.065	0.91 (0.78–1.06)	0.241	0.85 (0.73–0.99)	0.037	0.89 (0.76–1.04)	0.130	0.87 (0.74–1.01)	0.073	0.88 (0.75–1.03)	0.106	0.89 (0.76–1.03)	0.139	0.92 (0.79–1.08)	0.315
Third	284	0.86 (0.73–1.01)	0.072	0.92 (0.78–1.09)	0.345	0.81 (0.69–0.94)	0.005	0.88 (0.76–1.03)	0.116	0.90 (0.77–1.05)	0.186	0.94 (0.81–1.11)	0.476	0.96 (0.81–1.14)	0.630	1.00 (0.84–1.19)	1.000
P_{trend}			0.117		0.473		0.012		0.189		0.176		0.269		0.300		0.463
Cardiovascular mortality																	
First	196	1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]	
Second	240	0.77 (0.64–0.93)	0.008	0.82 (0.68–1.00)	0.051	0.87 (0.71–1.05)	0.140	0.92 (0.76–1.12)	0.398	0.82 (0.67–0.99)	0.042	0.83 (0.68–1.01)	0.069	0.86 (0.71–1.05)	0.139	0.91 (0.75–1.11)	0.344
Third	178	0.79 (0.64–0.98)	0.029	0.87 (0.70–1.08)	0.194	0.75 (0.62–0.91)	0.003	0.85 (0.70–1.04)	0.106	0.89 (0.74–1.09)	0.257	0.95 (0.78–1.16)	0.603	0.88 (0.71–1.08)	0.222	0.93 (0.75–1.15)	0.501
P_{trend}			0.019		0.142		0.013		0.268		0.125		0.174		0.301		0.631
Sudden cardiac death																	
First	100	1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]		1[reference]	
Second	80	0.52 (0.39–0.71)	<0.001	0.56 (0.42–0.76)	<0.001	0.74 (0.55–1.00)	0.050	0.81 (0.60–1.09)	0.157	0.70 (0.51–0.96)	0.027	0.71 (0.52–0.98)	0.034	0.85 (0.63–1.14)	0.270	0.90 (0.67–1.22)	0.490
Third	74	0.68 (0.50–0.92)	0.013	0.74 (0.54–1.02)	0.063	0.55 (0.41–0.76)	<0.001	0.63 (0.46–0.86)	0.004	0.92 (0.70–1.24)	0.594	1.00 (0.74–1.36)	0.981	0.87 (0.63–1.22)	0.426	0.94 (0.67–1.31)	0.703
P_{trend}			<0.001		0.001		0.001		0.015		0.075		0.055		0.532		0.788

Model 1: adjusted for age and gender. Model 2: additionally adjusted for body mass index, LDL-C, HDL-C, logTG, log-fibrinogen, smoking, hypertension, diabetes, lipid-lowering therapy, and estimated glomerular filtration rate. A *P*-value of <0.004 would be regarded as significant after Bonferroni correction for 12 tests (three outcomes and four TFA metrics).
HR, hazard ratio; TFAs, *trans* fatty acids; CI, confidence interval.

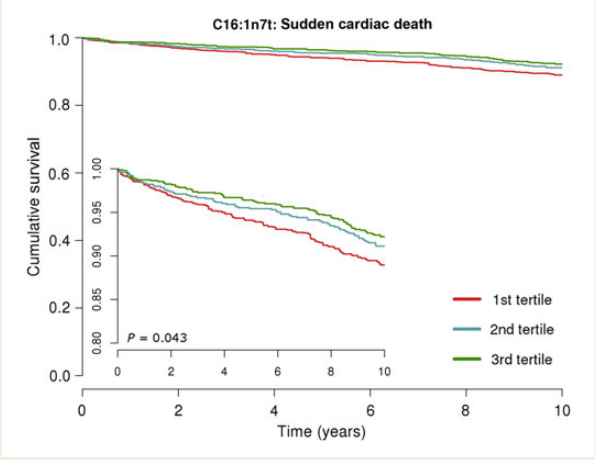

Figure 1 Adjusted survival curves for sudden cardiac death. Tertiles of C16:1n7t were balanced for body mass index, LDL-C, HDL-C, logTG, log-fibrinogen, smoking, hypertension, diabetes, lipid-lowering therapy, and estimated glomerular filtration rate by inverse variance weighting. The inset shows the same data on a truncated *y* axis. Hazard ratios (95% confidence interval) for the second and third tertile compared with the first tertile were 0.82 (0.61–1.12) and 0.67 (0.48–0.93), respectively. The *P*-value of the robust score test was 0.043.

We repeated the analyses for only those patients not on lipid-lowering therapy. While the inverse association of total TFAs with CVM became insignificant, the association of C16:1n-7t with a reduced risk of all-cause mortality and SCD remained statistically significant in Model 2 (Supplementary material online, *Table S8*). Additional adjustment for antihypertensive medication, glycaemic status, and alcohol intake only slightly attenuated the association (Supplementary material online, *Table S9*).

Discussion

Main findings

We analysed the association of the TFA content in erythrocyte membranes with total mortality and a number of biomarkers for cardiovascular risk in the well-characterized LURIC cohort including patients of Caucasian origin scheduled for coronary angiography. Our main findings are as follows: First, the concentrations of TFAs in erythrocyte membranes were low compared with concentrations reported for the USA at a similar time period (e.g. mean 0.96 ± 0.3% in LURIC vs. 2.68 ± 0.8 in Harris *et al.*[14]). Second, none of the TFAs showed any association to adverse outcome. Finally, there were statistically significant inverse associations with the risk of CVM and SCD, especially for *trans*-palmitoleic acid.

Trans fatty acids, coronary heart disease, and mortality

Initially, TFAs were considered as a safe replacement for saturated fat. However, it has soon been noted that TFA increase LDL-C while decreasing HDL-C.[15] Many studies consistently reported associations between TFA consumption and coronary heart disease (CHD).[6,16] A recent meta-analysis confirmed the direct association of dietary TFA intake with coronary outcomes while there was no association for circulating TFA.[17] Consequently, organizations like the World Health Organization recommended reducing TFA dietary intake to <4% and several countries like Denmark introduced legal bans. The German Society for Nutrition went even further by recommending a daily TFA intake of <1% of energy.[18] Furthermore, the FDA has concluded in June 2015 that partially hydrogenated oils are no longer 'Generally Recognized As Safe'.[19]

While TFA concentrations in food products have declined markedly in most industrialized countries over the last decades,[8,14,20,21] the decline was smaller in Eastern Europe[22] and concentrations remain high in several developing countries.[23] Furthermore, many food products labelled as free of TFA still contain significant amounts of TFAs.[24,25]

Most studies suggesting increased risks associated with TFAs have recruited patients decades ago when TFA concentrations were higher than today.[26–28] Furthermore, most studies were conducted in the USA, where TFA concentrations have traditionally been higher compared with Europe,[29] and used questionnaires to assess TFA intake. These estimates may not be accurate because of incomplete or inaccurate nutrient databases and the common under-reporting of unhealthy foods like sugar or fat.[30,31] We, therefore, investigated the association of TFA with all-cause mortality, CVM, and SCD in the German LURIC cohort. *Trans* fatty acids were measured in erythrocyte membranes using the Omega-3 Index technology that allows a more objective way to estimate individual nutrient intake.

We observed rather low concentrations of TFA in our patients and found no association with mortality. On the contrary, we found that total TFAs were inversely associated with CVM and SCD in age- and gender-adjusted models. After adjustment for other cardiovascular risk factors, only the association with SCD remained statistically significant.

Individual *trans* fatty acid and mortality

Looking at specific subgroups of TFAs, we did not find an association with mortality for any of the TFA species that we were able to separate. For the mostly ruminant-derived C16:1n7t, there was a strong inverse association with CVM and SCD with significant *P* for trend values. For the C18:1t isomers, which are a mixture of ruminant-derived and industrially produced TFA, *P* values for trend were almost nominally significant, while for the exclusively industrially produced C18:2t isomers, there was no significant trend. This could point towards an inverse association of ruminant-derived TFA only, with industrially produced TFA showing no association (at least at the concentrations found here).

C16:1n-7t, or *trans*-palmitoleic acid, is relatively specific for dairy products, although it can be produced by the partial hydrogenation of vegetable oils as well. Mozaffarian *et al.*[1,2] have recently reported an inverse association of C16:1n-7t with incident diabetes, which is consistent with our findings for prevalent diabetes (Supplementary material online, *Table S2*), and animal models showed that the *cis*-isomer C16:1n-7c is a major signalling lipid associated with improved insulin sensitivity.[32] Similarly, the *trans*-isomer may act as a signalling molecule, but so far no physiologic function has been shown for C16:1n-7t.

A number of reports examined the effect of dairy fats on CHD (reviewed in 33). Animal studies have shown beneficial effects of *trans*-vaccenic acid (C18:1n-11t), which constitutes 50–80% of TFAs in ruminant-derived fats,[34] on post-prandial lipid metabolism and dyslipidaemia.[35] Interestingly, a recent report has shown that C16:1n-7t may be synthesized in humans from C18:1n-11t with conversion rates of ~17%.[36] This conversion may explain the similar albeit mostly weaker associations for the C18:1t isomers compared with C16:1n-7t because C18:1n-11t constitutes one of the major TFA summarized in this variable. Alternatively, these associations could be due to direct effects of C18:1n-11t or another isomer.

A recent study has shown that circulating C18:2n-t/t was associated with higher total mortality, CVM, and total CHD, whereas C18:2n-t/c was positively related to total mortality, and nonfatal CHD only after mutual adjustment.[37] Another study showed each 1 SD increase of plasma *trans* 18:2 to be associated with a 22% lower risk of heart failure.[38] The differing results for TFA subspecies were exploited by Liu *et al.*[39] to calculate a TFA index by dividing the sum of industrially derived TFA by the sum of ruminant-derived TFA. This TFA index was associated with 10-year CHD risk, but only when the TFAs were measured in erythrocyte membranes.

Implications for public health

The TRANSFAIR study reported that the average TFA intake is 2.4 g/d for Germany, which is below 1% of total energy intake, and that 79% of TFA intake was derived from milk and ruminant fat.[40] The data had been collected in the 1990s and might, therefore, be comparable with our LURIC patients, although the methodology used in this study has been questioned.[41] Nevertheless, we also observe rather low TFA levels of only 0.96% of total fatty acids of erythrocyte membranes. Our results are also in line with findings by Kröger *et al.*[42] who reported very similar erythrocyte concentrations of C16:1n-7t and C18:1t isomers in the EPIC-Potsdam study. A large percentage of total TFAs seems to be of natural origin and is likely not derived from industrial processes. We observe no direct association with mortality or CVM for any of the investigated TFA, suggesting no need for efforts to further decreasing TFA in food in Germany. Based on our data, it seems that an upper limit for the sum of the *trans*-isomers of 18:1 and 18:2 of the mean of the upper tertile, i.e. 1.04%, might be regarded as safe. However, while well within the framework of our data, this concept also needs to be substantiated by more research.

In contrast, the beneficial associations of C16:1n-7t with biomarkers and long-term cardiovascular outcome in our patients might allow speculating about the potential benefit of interventions to increase the C16:1n-7t content of milk products or perhaps direct supplementation. Of course, this concept has to be investigated in more detail.

Strengths and limitations

All LURIC participants were of German origin and were recruited at a tertiary referral centre. Therefore, our findings may not be representative for a random population sample or applicable to other ethnicities. Furthermore, TFAs were only measured once in baseline samples and concentrations may vary over time due to dietary changes, lifestyle changes, or diseases. As TFA concentrations in our study were rather low, we could not examine the effect of higher concentrations (like they have been reported by previous studies) on mortality. Data on the dietary intake of fatty acids were not available; we, therefore, cannot exclude that low TFA concentrations are caused by changed dietary patterns on physician advice in patients with severe disease. Some potential confounding variables like socio-economic status, lifestyle, or drug treatment during follow-up were also not available. The major strengths of the LURIC cohort are, however, the precise clinical and metabolic characterization of the participants including the availability of coronary angiograms and its cross-sectional and prospective design. Furthermore, fatty acids were measured in erythrocyte cell membranes that represent fatty acid intake over the last months, as erythrocytes have a lifespan of ~120 days.[43]

Conclusion

We examined the association of TFA measured in erythrocyte membranes with plasma biomarkers and long-term outcome in the LURIC cohort. In contrast to previous findings, we observed that at generally low concentrations, TFAs were inversely associated with adverse cardiac outcomes. Especially ruminant-derived TFAs were associated with a reduced risk of CVM and SCD. Furthermore, high TFA concentrations in our study (which are low relative to other populations) were associated with mostly favourable metabolic profiles with lower TG, lower fasting glucose, and lower blood pressure.

Authors' contributions

M.E.K. and G.E.D. performed statistical analysis; C.v.S. and W.M. handled funding and supervision; C.v.S. acquired the data; C.v.S. and W.M. conceived and designed the research; M.E.K. drafted the manuscript; and G.E.D., S.L., W.M., and C.v.S. made critical revision of the manuscript for key intellectual content.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

We extend our appreciation to the participants of the LURIC study and thank the LURIC study team who were either temporarily or permanently involved in patient recruitment as well as sample and data handling, in addition to the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg and Ulm, Germany.

Funding

This work was supported by the 7th Framework Program (AtheroRemo, grant agreement number 201668 and RiskyCAD, grant agreement number 305739) of the EU and by the INTERREG-IV-Oberrhein-Program (Project A28, Genetic mechanisms of cardiovascular diseases) with support from the European Regional Development Fund (ERDF) and the Wissenschaftsoffensive TMO. M.E.K., S.L., and W.M. are supported by the German Federal Ministry of Education and

Research as part of the Competence Cluster of Nutrition and Cardiovascular Health (nutriCARD).

Conflict of interest: C.v.S. has founded Omegametrix, a laboratory performing fatty acid analysis, which analysed LURIC samples free of charge. W.M. is employed with synlab Services GmbH and holds shares of synlab Holding GmbH. The other authors declare no conflict of interest.

References

1. Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, Hotamisligil GS. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. *Ann Intern Med* 2010;**153**:790–799.
2. Mozaffarian D, de Oliveira Otto MC, Lemaitre RN, Fretts AM, Hotamisligil G, Tsai MY, Siscovick DS, Nettleton JA. 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.
3. Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA, Hennekens CH. Intake of trans fatty acids and risk of coronary heart disease among women. *Lancet* 1993;**341**:581–585.
4. Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ, Rexrode KM, Willett WC, Hu FB. A prospective study of trans fatty acids in erythrocytes and risk of coronary heart disease. *Circulation* 2007;**115**:1858–1865.
5. Chien KL, Lin HJ, Hsu HC, Chen PC, Su TC, Chen MF, Lee YT. Comparison of predictive performance of various fatty acids for the risk of cardiovascular disease events and all-cause deaths in a community-based cohort. *Atherosclerosis* 2013; **230**:140–147.
6. Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med* 2006;**354**:1601–1613.
7. Laake I, Pedersen JI, Selmer R, Kirkhus B, Lindman AS, Tverdal A, Veierod MB. A prospective study of intake of trans-fatty acids from ruminant fat, partially hydrogenated vegetable oils, and marine oils and mortality from CVD. *Br J Nutr* 2012;**108**: 743–754.
8. Dyer O. Bans and labelling helped to reduce Americans' trans fat levels by 58%. *BMJ* 2012;**344**:e1084.
9. Kris-Etherton PM, Lefevre M, Mensink RP, Petersen B, Fleming J, Flickinger BD. Trans fatty acid intakes and food sources in the U.S. population: NHANES 1999–2002. *Lipids* 2012;**47**:931–940.
10. Nestel P. Trans fatty acids: are its cardiovascular risks fully appreciated? *Clin Ther* 2014;**36**:315–321.
11. Winkelmann BR, Marz W, Boehm BO, Zotz R, Hager J, Hellstern P, Senges J, Group LS. Rationale and design of the LURIC study—a resource for functional genomics, pharmacogenomics and long-term prognosis of cardiovascular disease. *Pharmacogenomics* 2001;**2**(Suppl. 1):S1–S73.
12. Köhler A, Bittner D, Löw A, von Schacky C. Effects of a convenience drink fortified with n-3 fatty acids on the n-3 index. *Br J Nutr* 2010;**104**:729–736.
13. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS, CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med* 2012;**367**:20–29.
14. Harris WS, Pottala JV, Vasan RS, Larson MG, Robins SJ. Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans. *J Nutr* 2012;**142**:1297–1303.
15. Mensink RP, Katan MB. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med* 1990;**323**: 439–445.
16. Willett WC. Trans fatty acids and cardiovascular disease-epidemiological data. *Atheroscler Suppl* 2006;**7**:5–8.
17. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, Franco OH, Butterworth AS, Forouhi NG, Thompson SG, Khaw KT, Mozaffarian D, Danesh J, Di Angelantonio E. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med* 2014;**160**:398–406.
18. Deutsche Gesellschaft für Ernährung ÖGfE. Schweizerische Gesellschaft für Ernährungsforschung, Schweizerische Vereinigung für Ernährung Referenzwerte für die Nährstoffzufuhr. Frankfurt am Main: Umschau Braus; 2000.
19. FDA News Release. The FDA takes step to remove artificial trans fats in processed foods. June 16, 2015. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm451237.htm (3 July 2015).
20. Downs SM, Thow AM, Leeder SR. The effectiveness of policies for reducing dietary trans fat: a systematic review of the evidence. *Bull World Health Organ* 2013;**91**: 262–269H.
21. Leth T, Jensen HG, Mikkelsen AA, Bysted A. The effect of the regulation on trans fatty acid content in Danish food. *Atheroscler Suppl* 2006;**7**:53–56.
22. Stender S, Astrup A, Dyerberg J. A trans European Union difference in the decline in trans fatty acids in popular foods: a market basket investigation. *BMJ Open* 2012;**2**: e000859.
23. Karn S, Abraham R, Ramakrishnan L. Assessment of trans fatty acid content in widely consumed snacks by gas chromatography in a developing country. *Food Nutr Sci* 2013;**4**:1281–1286.
24. Clapp J, Curtis CJ, Middleton AE, Goldstein GP. Prevalence of partially hydrogenated oils in US packaged foods, 2012. *Prev Chronic Dis* 2014;**11**:E145.
25. Stender S, Astrup A, Dyerberg J. Tracing artificial trans fat in popular foods in Europe: a market basket investigation. *BMJ Open* 2014;**4**:e005218.
26. Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT. Estimated intakes of trans fatty and other fatty acids in the US population. *J Am Diet Assoc* 1999;**99**:166–174; quiz 175–6.
27. Doell D, Folmer D, Lee H, Honigfort M, Carberry S. Updated estimate of trans fat intake by the US population. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 2012;**29**:861–874.
28. Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC. Trans-fatty acids intake and risk of myocardial infarction. *Circulation* 1994;**89**:94–101.
29. Craig-Schmidt MC. World-wide consumption of trans fatty acids. *Atheroscler Suppl* 2006;**7**:1–4.
30. Archer E, Hand GA, Blair SN. Validity of U.S. nutritional surveillance: National Health and Nutrition Examination Survey caloric energy intake data, 1971–2010. *PLoS One* 2013;**8**:e76632.
31. Heitmann BL, Lissner L, Osler M. Do we eat less fat, or just report so? *Int J Obes Relat Metab Disord* 2000;**24**:435–442.
32. Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. *Cell* 2008;**134**:933–944.
33. Gebauer SK, Chardigny JM, Jakobsen MU, Lamarche B, Lock AL, Proctor SD, Baer DJ. Effects of ruminant trans fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies. *Adv Nutr* 2011;**2**:332–354.
34. Lock AL, Bauman DE. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* 2004;**39**:1197–1206.
35. Wang Y, Jacome-Sosa MM, Vine DF, Proctor SD. Beneficial effects of vaccenic acid on postprandial lipid metabolism and dyslipidemia: impact of natural trans-fats to improve CVD risk. *Lipid Technol* 2010;**22**:103–106.
36. Jaudszus A, Kramer R, Pfeuffer M, Roth A, Jahreis G, Kuhnt K. trans Palmitoleic acid arises endogenously from dietary vaccenic acid. *Am J Clin Nutr* 2014;**99**:431–435.
37. Wang Q, Imamura F, Lemaitre RN, Rimm EB, Wang M, King IB, Song X, Siscovick D, Mozaffarian D. Plasma phospholipid trans-fatty acids levels, cardiovascular diseases, and total mortality: the cardiovascular health study. *J Am Heart Assoc* 2014;**3**: e000914.
38. Tokede OA, Petrone AB, Hanson NQ, Tsai MY, Weir NA, Glynn RJ, Gaziano JM, Djousse L. Plasma phospholipid trans fatty acids and risk of heart failure. *Am J Clin Nutr* 2013;**97**:698–705.
39. Liu XR, Deng ZY, Hu JN, Fan YW, Liu R, Li J, Peng JT, Su H, Peng Q, Li WF. Erythrocyte membrane trans-fatty acid index is positively associated with a 10-year CHD risk probability. *Br J Nutr* 2013;**109**:1695–1703.
40. Hulshof KF, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C, Hermann-Kunz E, Kesteloot H, Leth T, Martins I, Moreiras O, Moschandreas J, Pizzoferrato L, Rimestad AH, Thorgeirsdottir H, van Amelsvoort JM, Aro A, Kafatos AG, Lanzmann-Petithory D, van Poppel G. Intake of fatty acids in western Europe with emphasis on trans fatty acids: the TRANSFAIR Study. *Eur J Clin Nutr* 1999;**53**:143–157.
41. Wolff RL, Precht D. A critique of 50-m CP-Sil 88 capillary columns used alone to assess trans-unsaturated FA in foods: the case of the TRANSFAIR study. *Lipids* 2002;**37**:627–629.
42. Kröger J, Zietemann V, Enzenbach C, Weikert C, Jansen EH, Doring F, Joost HG, Boeing H, Schulze MB. 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.
43. Harris WS, Thomas RM. Biological variability of blood omega-3 biomarkers. *Clin Biochem* 2010;**43**:338–340.