Eur J Nutr
DOI 10.1007/s00394-016-1218-9

ORIGINAL CONTRIBUTION

Trans-fatty acid levels in erythrocytes in Europe

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Received: 11 December 2015 / Accepted: 25 April 2016

Abstract

Purpose High, but not low levels of trans-fatty acids (TFA) in erythrocytes are associated with increased mortality. Current erythrocyte TFA levels in Europe are not known.

Methods TFA levels in samples submitted by physicians for erythrocyte omega-3 fatty acid analyses are reported, as analysed with a method (HS-Omega-3 Index®) previously used in pertinent prospective epidemiologic studies. From Germany, 6754 samples were included from 2008 through 2015, and 496 samples from 10 other European countries.

Results In Germany, mean levels of C16:1n-7t, a marker for dairy and meat intake, decreased, as did mean levels of industrially produced (IP)-TFA, as did the percentage of individuals with IP-TFA > 1.04 %. Mean levels of IP-TFA in Austria and Switzerland were low before and after measures were taken to reduce them. Average levels of C16:1n-7t were low, and at levels associated with increased risk of death in previous studies. A limitation of our study is that samples were not obtained in a specific or representative manner.

Conclusions Levels of IP-TFA appear to be decreasing, as are those of ruminant-derived C16:1n-7t, a marker for dairy and meat intake. Few individuals had high levels of IP-TFA above a safe range, while many had low levels of C16:1n-7t. Our data argue against further action against TFA in the countries studied. More systematic biomarker-based studies are needed.

Keywords Trans-fatty acidss · Cardiovascular disease · Fatty acid composition · Erythrocyte fatty acids

Abbreviations

TFA Trans-fatty acids
IP Industrially produced

Introduction

Trans-fatty acids (TFA) are thought to be detrimental to health [1–5]. However, in Germany, we recently found erythrocyte levels of C16:1n-7t, a marker of dairy and meat intake, to be inversely associated with total mortality, largely driven by a lower risk of sudden cardiac death [6], while other studies found conflicting data [7–10].

Trans isomers of C18:1 and of C18:2n-6 largely stem from industrial food production [1, 3–5]. In the USA, previous studies found high levels of trans isomers of C18:1 in erythrocytes, and cardiovascular risk to increase by a factor of approx. two from 1.7 to 3.7 % C18t isomers in erythrocytes [11–13]. Total trans isomers of C18:2n-6 were also found to be associated with risk, as was true for the tt and ct but not t c isomers [11–14]. In contrast, in Germany, we recently found that, across the observed tertiles of industrially produced (IP)-TFA, there was no difference in risk of cardiovascular or total mortality, which, together with the data just mentioned, suggested that IP-TFA trans level ≤1.04 % is safe [6].

Although TFA levels from Europe have rarely been reported [15, 16], and it is thus unclear whether reductions are needed, some European countries have taken legislative

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Published online: 05 May 2016

action against TFA (4, 5). Using an identical analytical method as in our previous epidemiologic study in Germany, and in four studies in the USA [6, 13, 17–19], we report the results of TFA from 7250 routine analyses from 11 European countries.

Methods

Data accumulated in the course of consecutive routine analyses of erythrocyte fatty acid compositions from 2008 to 2015 are reported. Samples were sent for analysis of erythrocyte omega-3 fatty acid content by using the HS-Omega-3 Index® method. From Germany, 6754 samples were analysed, while 496 samples were analysed from 10 other European countries. Only results from first, but not from subsequent, determinations were used. The 10 countries, from which 5 samples or more were received, were included in this analysis. Samples for which the country was not specified ($n = 1093$) and from 20 other countries from which less than 5 samples were received were not included. The analysis was performed in an irreversibly anonymized fashion, in compliance with guidance from the ethics committee of the Ludwig Maximilians University of Munich.

Fatty acid compositions were analysed using the HS-Omega-3 Index® methodology as previously described [6]. Fatty acid methyl esters were generated by acid transesterification and analysed by gas chromatography using a GC2010 Gas Chromatograph (Shimadzu, Duisburg, Germany) equipped with an SP2560, 100-m column (Supelco, Bellefonte, PA) using hydrogen as carrier gas. Fatty acids were identified by comparison with a standard mixture of fatty acids, as in previous reports [e.g. 6, 17, 18]. A total of 26 fatty acids were identified and quantified, among them the following TFA: C16:1t, C18:1t, C18:2n-6tt, C18:2n-6ct, C18:2n-6tc. Results are given as percentage of total identified fatty acids after response factor correction. The sum of the five TFA analysed had a coefficient of variation of 7 % [6]. Analyses were quality-controlled according to DIN ISO 15189, including quality assurance similar to clinical chemistry such as constancy checks or proficiency testing among participating laboratories [20, 21].

Fatty acid results are reported as mean percentages ± standard deviations. One-way ANOVA (http://www.danielsoper.com/statcalc3/calc.aspx?id=43) was used to check for changes in fatty acid concentrations through the years in Germany (Table 1). Then, unpaired t test (http://www.graphpad.com/quickcalcs/ttest1.cfm) was used to compare the years 2008 and 2015 (Table 1). Unpaired t test was also used to compare fatty acid concentrations in samples from Austria and Switzerland before and since 2010 (Table 2).

Results and discussion

In Germany, a significant reduction in mean levels of naturally occurring C16:1n-7t was observed in the years 2008–2015 (Table 1). C16:1n-7t is thought to be representative for dairy- or red meat ("ruminant")-derived naturally occurring TFA [6–9]. In humans, C16:1n-7t arises endogenously from vaccenic acid (18:1t11) [22]. While the consumption of ruminant-derived meat has been stable in Germany from 2008 to 2013 [http://de.statista.com/statistik/daten/studie/177477/umfrage/pro-kopf-verbrauch-von-kalb-und-rindfleisch-in-deutschland/], the consumption of dairy products has increased somewhat [http://

Table 1 Mean erythrocyte trans-fatty acid concentrations in % (of fatty acid methyl esters) in Germany 2008–2015, and percentage of persons with the sum of 18:1t + trans isomers of 18:2n-6 > 1.04 % ("% >1.04 %")

	n	C16:1n-7t	C18:1t	C18:2n-6tt	C18:2n-6ct	C18:2n-6tc	Sum 18:2n-6trans	Sum 18:1t + 18:2n-6trans	% > 1.04 %
2008	511	0.25 ± 0.33	0.52 ± 0.32	0.23 ± 0.16	0.07 ± 0.08	0.18 ± 0.14	0.48 ± 0.45	0.99 ± 0.62	29.5
2009	720	0.15 ± 0.12	0.41 ± 0.22	0.18 ± 0.08	0.04 ± 0.04	0.15 ± 0.12	0.37 ± 0.31	0.78 ± 0.34	9.3
2010	657	0.22 ± 0.20	0.45 ± 0.15	0.16 ± 0.07	0.02 ± 0.01	0.12 ± 0.04	0.30 ± 0.19	0.75 ± 0.25	5.9
2011	812	0.20 ± 0.09	0.40 ± 0.15	0.15 ± 0.08	0.03 ± 0.02	0.12 ± 0.08	0.29 ± 0.23	0.70 ± 0.27	3.8
2012	872	0.18 ± 0.07	0.47 ± 0.15	0.07 ± 0.17	0.03 ± 0.03	0.14 ± 0.06	0.24 ± 0.20	0.71 ± 0.24	5.2
2013	1017	0.15 ± 0.06	0.43 ± 0.16	0.05 ± 0.06	0.04 ± 0.04	0.12 ± 0.06	0.22 ± 0.20	0.65 ± 0.23	4.4
2014	1256	0.15 ± 0.05	0.41 ± 0.17	0.02 ± 0.04	0.02 ± 0.02	0.11 ± 0.06	0.15 ± 0.19	0.56 ± 0.21	1.3
2015 (Jan–Sep)	909	0.15 ± 0.05	0.47 ± 0.15	0.01 ± 0.03	0.02 ± 0.02	0.09 ± 0.04	0.12 ± 0.17	0.59 ± 0.19	1.3

Data from analyses of samples sent for erythrocyte omega-3 fatty acid concentrations; mean ± standard deviation

n number of samples analysed, t trans, c cis

All columns $p < 0.0001$ (one-way ANOVA). Except for C18:2n-6ct (n.s.), all concentrations in 2015 were lower than in 2008 ($p < 0.0001$, t test). Cut-off value of 1.04 % as defined in [6]

Table 2 Mean erythrocyte trans-fatty acid concentrations in % (of fatty acid methyl esters) in some European countries, and percentage of persons with the sum of 18:1t + trans isomers of 18:2n-6 > 1.04 % ("% >1.04 %") in the years 2008–2015

	n	C16:1n-7t	C18:1t	C18:2n-6tt	C18:2n-6ct	C18:2n-6tc	Sum 18:2n-6trans	Sum 18:1t + 18:2n-6trans	% > 1.04 %
Austria before 2010[a]	14	0.16 ± 0.08	0.38 ± 0.16	0.25 ± 0.17	0.03 ± 0.03	0.15 ± 0.07	0.43 ± 0.18	0.80 ± 0.08	0
Austria since 2010[a]	135	0.16 ± 0.08	0.37 ± 0.15	0.05 ± 0.07	0.03 ± 0.03	0.12 ± 0.09	0.19 ± 0.12[#]	0.56 ± 0.23[#]	5.2
Belgium	5	0.16 ± 0.06	0.34 ± 0.07	0.11 ± 0.06	0.04 ± 0.03	0.16 ± 0.07	0.31 ± 0.09	0.65 ± 0.15	0
France	38	0.16 ± 0.03	0.52 ± 0.12	0.05 ± 0.09	0.02 ± 0.02	0.09 ± 0.04	0.16 ± 0.14	0.67 ± 0.17	5.3
Great Britain	5	0.16 ± 0.04	0.39 ± 0.08	0.11 ± 0.09	0.02 ± 0.01	0.09 ± 0.04	0.22 ± 0.09	0.61 ± 0.04	0
Greece	19	0.12 ± 0.04	0.44 ± 0.12	0.25 ± 0.14	0.03 ± 0.02	0.13 ± 0.04	0.42 ± 0.14	0.86 ± 0.16	10.5
Luxemburg	5	0.15 ± 0.06	0.57 ± 0.13	0.07 ± 0.07	0.02 ± 0.01	0.14 ± 0.01	0.23 ± 0.06	0.80 ± 0.16	20
Netherlands	5	0.14 ± 0.05	0.47 ± 0.12	0.13 ± 0.06	0.03 ± 0.01	0.15 ± 0.04	0.31 ± 0.08	0.78 ± 0.14	0
Spain	10	0.24 ± 0.16	0.54 ± 0.39	0.16 ± 0.18	0.04 ± 0.03	0.16 ± 0.13	0.36 ± 0.29	0.90 ± 0.41	33.3
Switzerland before 2010[a]	69	0.18 ± 0.09	0.50 ± 0.18	0.19 ± 0.09	0.05 ± 0.05	0.17 ± 0.16	0.41 ± 0.22	0.91 ± 0.31	17.4
Switzerland since 2010[a]	132	0.25 ± 0.20	0.49 ± 0.13	0.10 ± 0.09	0.03 ± 0.03	0.12 ± 0.05	0.25 ± 0.11[#]	0.73 ± 0.18[#]	3.0
Turkey	59	0.17 ± 0.04	0.48 ± 0.13	0.01 ± 0.04	0.01 ± 0.01	0.10 ± 0.04	0.12 ± 0.07	0.61 ± 0.14	3.4

Data from analyses of samples sent for erythrocyte omega-3 fatty acid concentrations; mean ± standard deviation

n number of samples analysed, *t* trans, *c* cis

[#] $p =$ or <0.0002 in comparison with line above (unpaired *t* test). Cut-off value of 1.04 % as defined in [6]

[a] Since 2009, pertinent legislation limits intake of trans-fatty acids in Austria and Switzerland

www.milchindustrie.de/marktdaten/verbrauch-nachfrage/pro-kopf-verbrauch-von-milch-und-milchprodukten/]. Therefore, the decrease cannot be explained by decreased consumption. An alternative explanation would be that the content of C16:1n-7t in ruminant-derived food products has decreased. The content of C16:1n-7t in milk depends on fat content, genes and feeding of cows [23]. Some evidence indicates that milk produced in Germany by organic farming contains more naturally occurring TFA than milk produced by conventional farming [24]. We recently found an inverse association of erythrocyte levels of C16:1n-7t with total mortality [6], which needs to be scrutinized by further research, including intervention trials. While our findings argue against reducing C16:1n-7t in the diet, the advice against dietary fat has been discontinued in current dietary guidelines from the USA (Table 1) [6, 25].

In Germany, levels of C18:1t decreased from 0.52 ± 0.32 (2008) to 0.47 ± 0.15 (2015, Table 1). At levels between 1.7 and 3.7 %, isomers of 18:1t are concentration-dependently associated with increased risk of acute coronary syndrome [13]. However, at low levels (<0.8 %), previously found in Germany, no association with total or cardiovascular mortality was found [6]. In the USA, pertinent legislation and efforts of the food industry led to a decrease of trans isomers of C18:1 from 2.15 ± 0.63 % by 0.47 ± 0.53 % (<0.0001), as reported from 291 participants of the Framingham Offspring group [4, 17]. More recently, in the USA, in 86,012 clinical routine samples analysed largely in 2011, total trans isomers of C18:1 were 0.66 ± 0.24 % [18]. The data from the USA demonstrate that it is feasible to bring levels of trans isomers of 18:1 from a hazardous range into a safe range by legislation and efforts of the food industry.

In Germany, levels of tt and tc, but not ct, trans isomers of 18:2n-6 decreased from 2008 to 2015 (Table 1). The sum of trans isomers of 18:2n-6 decreased from 0.48 ± 0.45 to 0.12 ± 0.17, driving the continuous reduction in total IP-TFA from 2008 to 2015 (Table 1). The measures taken in the USA just mentioned also reduced trans isomers of C18:2 from 0.35 ± 0.09 % by 0.09 ± 0.10 (both $p < 0.0001$) from 1999 to 2006, as reported from 291 participants from the Framingham Offspring study [17]. Analyses in the USA, mostly in 2011, found total trans isomers of C18:2 to be 0.25 ± 0.06 %, practically identical [18].

In Germany, from 2008 to 2015, the mean sum of IP-TFA was always below 1.04 % and the percentage individuals with total IP-TFA > 1.04 decreased from 29.5 to 1.3 % (Table 1). In our previous epidemiologic study, we did not see an increase in risk of total or cardiovascular mortality in individuals with total IP-TFA below 1.04 % [6]. It is not known whether levels >1.04 % and approximately <2.0 % are associated with elevated risk of total or cardiovascular mortality [6, 13, 17–19]. It is, however, possible to identify individuals with IP-TFA in a dangerous range by the use of fatty acid analysis using the HS-Omega-3 Index methodology. Our data indicate that risk associated with IP-TFA has declined in Germany 2008–2015 without legislation.

In Austria and Switzerland, legislation to curb TFA was introduced in 2009 [5]. In Austria and Switzerland, levels of IP-TFA found since 2010 were significantly lower than levels found before 2010, but levels before 2010 were below 1.04 % (Table 2) [6]. The percentage of individuals with total IP-TFA > 1.04 % decreased in Switzerland, but inexplicably increased in Austria. While our data demonstrate the effectiveness of the measures taken in Austria and Switzerland, they do not support a need for the measures taken (Table 2). From our perspective, legislative or other action against IP-TFA in the countries studied seemed and seems unfounded.

The number of samples was lower from other European countries, which makes our data less reliable and impossible to break down by years (Table 2). However, while mean levels of total IP-TFA were below 1.04 % in all European countries studied, the percentage of individuals with total IP-TFA > 1.04 % varied from 0 to 33.3 % (Table 2) [6].

In the TRANSFAIR study, intake of individual TFA in adults and/or the total population was calculated, using the best available national food consumption data set [26]. Trans isomers of C18:1 were the predominant TFA in the diet. The authors of TRANSFAIR concluded that "The current intake of TFA in most Western European countries does not appear to be a reason for major concern." [26]. Based on our biomarker data, we concur with this conclusion for IP-TFA, but not for ruminant-derived TFA. Clearly, our findings need to be substantiated by further research.

Limitations of the present cross-sectional study are many: our approach did not allow for assessment of demographics, diet, health status, vital parameters, etc. Sampling was not systematic, but depended on the discretion of patient and physician. Thus, we did not observe changes of fatty acids in one group of individuals followed over time, but changes on a group level. However, in the USA, cardiometabolic risk markers from a clinical laboratory were recently found to be broadly similar to those of the population [27]. Samples were taken in order to assess omega-3 fatty acid status, and not trans-fatty acid status, which can be considered an argument against a systematic bias in the trans-fatty acid data. While our analytical method identified and quantified TFA associated or not with cardiovascular risk (total C16:1t, C18:1t and C18:2n-6 isomers), it did not separate ruminant-derived C16:1n-7t from industrially produced C16:1n-9t, or isomers of C18:1t [12]. Only 5 samples each could be analysed from Belgium, Great Britain, Luxemburg and the Netherlands, and are reported for completeness only. Numbers of samples of most European countries were too low to be broken down by years of sampling (Table 2). No samples from countries with high trans-fatty acids intakes, such as Hungary, Poland and Czech Republic, could be included [5]. Strengths of the present study are the large number of samples, the use of objective and informative biomarkers of TFA, independent of the uncertainties of assessments of food or diet and bioavailability issues [28, 29], and a standardized analytical method, which made the present data well comparable to the data previously reported [6, 13, 17–19].

In the future, a systematic analysis of TFA status across countries is needed, including European if not more countries, and providing large and representative numbers, preferably using the analytical methodology used in this and other studies [6, 13, 17–19]. This would require a large and costly multicentre cross-sectional study, but could be a good topic for a European Framework Program. The results would identify countries with a need for action for or against TFA.

In conclusion, in 11 European countries, levels of both dairy- and meat-derived TFA and industrially produced TFA were low. Levels of ruminant-derived TFA decreased in Germany from 2008 to 2015. All mean and most individual levels of IP-TFA were below 1.04 %, and thus at levels we consider safe. Although our findings need to be substantiated by further research, our findings do not support further actions against TFA in Europe.

Acknowledgments This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Compliance with ethical standards

Conflict of interest C.v.S. has founded Omegametrix, a laboratory performing fatty acid analysis, and declares no other conflict of interest in the field of TFA. The other authors declare no conflict of interest.

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