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Higher Content of 18:1 Trans Fatty Acids in Subcutaneous Fat of Persons with Coronarographically Documented Atherosclerosis of the Coronary Arteries

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Key Words
Trans fatty acid isomers · Hardened fats · Atherosclerosis · Ischemic heart disease

Abstract
Aim: To identify the total content of trans fatty acid (TFA) isomers and C18:1 trans isomers in subcutaneous fat samples from persons with atherosclerosis of the coronary arteries, as an indicator of dietary exposure. ***Methods:*** Using capillary gas chromatography, the authors determined total content of TFA isomers and C18:1 trans isomers in the subcutaneous fat of 34 patients with ischemic heart disease who had undergone aortocoronary bypass surgery and in 46 patients with no sign of coronary disease. ***Results:*** On average, the total TFAs in cardiac patients were 2.88 ± 1.19% of all fatty acids, in noncardiac patients 2.56 ± 0.89%. However, the difference is not statistically significant. The average concentration of C18:1 trans in cardiac patients (2.31 ± 1.09%) was statistically significantly higher ($p = 0.05$) than in the noncardiac group (1.95 ± 0.77%). ***Conclusions:*** The results obtained indicate a lower TFA load in comparison with previous studies in other countries. A higher concentration of 18:1 TFAs in the subcutaneous fat of patients with coronary disease might be an impulse to correct the dietary habits of this very high-risk population.

Introduction

Trans fatty acids (TFAs) are unsaturated fatty acids that have at least one double bond in a trans configuration in the molecule. Humans consume TFAs in food as part of ruminant meat fat, milk fat and butter. Hardened fats, however, are unambiguously the main source of TFAs as TFAs are formed in the process of hardened fat production (during catalytic partial hydrogenation).

TFAs are suspected to be one of the ischemic heart disease risk factors. Mann [1] pointed out the chronological connection between the epidemic of ischemic heart disease in the 20th century and a higher content of TFAs in the human diet as a result of the discovery and production of hydrogenated fats. Epidemiological studies on the TFA content in the subcutaneous fat and serum lipoproteins of persons with and without cardiovascular disease have so far only produced ambiguous results [2–11]. However, the

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Table 1. Identified fatty acids

Saturated fatty acids	Cis-monounsaturated fatty acids	Cis-polyunsaturated fatty acids	Trans fatty acids
C12:0	C14:1n–5	C18:2n–6	*C18:1*
C14:0	C16:1n–9	C18:3n–6	C18:1n–7 to n–12
C16:0	C16:1n–7	C18:3n–3	
C18:0	C18:1n–9 to n–12	C18:2conj.	*Other TFA*
C20:0	C18:1n–7	C20:2n–6	C16:1n–9
	C18:1n–6	C20:3n–9	C16:1n–7
	C18:1n–5	C20:3n–6	C18:2n–6tt
	C20:1n–9	C20:4n–6	C18:2n–6ct
		C20:5n–3	C18:2n–6tc
		C22:4n–6	C18:3n–3ttt
		C22:5n–3	C18:3n–3ttc+tct
		C22:6n–3	C18:3n–3ctt+cct
			C18:3n–3ctc+tcc

high intake of TFAs probably has adverse effects on serum lipoprotein values [12–19]. On the basis of a meta-analysis of 20 studies, Zock and Katan [20] claim that replacing butter with soft margarine with a low TFA content favorably effects the blood lipoprotein profile and can lower the risk of cardiovascular diseases, whereas high TFA content (hard) margarine probably does not have any advantages compared with butter. The conclusions of a recent TRANSFAIR study in 8 European countries did not show an association between current intake levels of TFAs and unfavorable serum lipid profiles [21].

The aim of this study was to describe the total content of TFAs in subcutaneous fat as an indicator of dietary exposure, and was performed on 2 groups. The first group involved ischemic heart disease patients who underwent an aortocoronary bypass. The second group consisted of persons with no signs of ischemic heart disease. The purpose of our study was to evaluate the difference in the TFA content in the subcutaneous fat of both groups. We do not claim to confirm TFAs as an etiologic factor for atherosclerosis, but to describe dietary exposure. The results could be used to improve the dietary recommendations for the prevention of cardiovascular diseases.

Patients and Methods

Two groups of patients were studied. Group 1 consisted of 34 cardiac patients (28 men and 6 women) with coronarographically documented atherosclerosis of the coronary arteries. These patients underwent bypass surgery at the Department of Heart Surgery of Prague Vinohrady University Hospital. Group 2 consisted of 46 patients (36 men and 10 women) with no history of cardiac disease. These patients underwent abdominal surgery (herniotomy, appendectomy, cholecystectomy, gut resection) at the Department of Surgery of the same hospital. Both groups did not statistically differ in age (63.8 ± 7.4 years for group 1 and 61.4 ± 8.9 years for group 2). All subjects were informed about the aim of the study and signed the informed consent form. The ethical committee of the 3rd Faculty of Medicine, Charles University, Prague, agreed to our protocol.

During surgery, a sample of subcutaneous fat (approximately 0.5 g) was taken from all patients. The fat samples were hydrolyzed in 3 *M* methanolic KOH solution at laboratory temperature in a nitrogen atmosphere for approximately 20 h. The reaction mixture was then acidified and the liberated components were extracted into hexane. We used preparative thin-layer chromatography to separate the fatty acids from the other components. The separated fatty acids were esterified with methanol during catalysis with concentrated sulfuric acid. Once the reaction mixture had been neutralized with sodium carbonate, the methyl esters created were extracted into hexane and, once dried, they were preserved in a nitrogen atmosphere at –20°C.

The analyses themselves were carried out on a capillary gas chromatograph, Chrompack CP 9001 (Chrompack, Middelburg, The Netherlands) equipped with a split/splitless injector and a flame-ionizing detector. Column CP-Sil 88 (Chrompack) had the following parameters: 100 m long, internal diameter 0.25 mm, and stationary phase thickness 0.25 µm. The injector and detector were heated to 250°C, the temperature program of the thermostat was 80–230°C, 2°C/min, and then isothermally for 40 min. Hydrogen with an entrance pressure of 80 kPa was used as the carrier gas. Under the same analytic conditions, we differentiated the fatty acid critical pairs on column CP-WAX 52 CB (Chrompack), 25 m long, ID 0.25 mm and d.f. 0.25 µm.

In total 35 fatty acids were identified in the individual samples, including unsaturated fatty acids containing one or several double bonds in a trans configuration (table 1).

Table 2. Content of trans fatty acids in subcutaneous fat

Group	n	x, %	SD, %	p	Minimum, %	Maximum, %
Σ Total trans fatty acids						
Cardiac group	34	2.88	1.19	n.s.	1.06	5.84
Noncardiac group	46	2.56	0.89		0.88	4.90
Σ C18:1 trans						
Cardiac group	34	2.31	1.09	0.05	0.73	5.16
Noncardiac group	46	1.95	0.77		0.52	4.19

The selection of 34 cardiac patients and 46 patients with a negative cardiologic history can be considered as two independent selections. To statistically analyze parameters monitored in individual groups we used the t test to compare means.

Results

The contents of total TFAs and C18:1 trans in the subcutaneous fat of both groups are shown in table 2.

Having determined the TFA content in subcutaneous fat, the proportion of TFAs in dietary fat was estimated. The following conversion was used: TFA% in dietary fat = 2 × TFA% in subcutaneous fat [22]. For patients with documented cardiovascular disease, TFAs made up approximately 5.76% (SD = 2.38%) of the total dietary fat; persons with no history of cardiovascular disease had approximately 5.12% (SD = 1.78%) TFAs. A statistically significant difference between both values has not been proved.

Using the same calculation, C18:1 trans isomers made up approximately 4.62% (SD = 2.18%) of dietary fat in the cardiac patient group and 3.90% (SD = 1.54%) in the noncardiac patient group. This shows a statistically significant difference (p = 0.05) in the representation of C18:1 trans in dietary fat between ischemic heart disease patients and noncardiac patients.

Discussion

Our absolute values of TFA content in subcutaneous fat and their estimated proportion in dietary fat are lower than those of earlier reports. For example, in the US the TFA content in adipose tissue is approximately 4% and, on the basis of this figure, it is estimated that TFAs make up approximately 8% of total fat in the American diet (t C18:1 + ct, ts and tt C18:2 from partially hydrogenated vegetable oils 3.5% in subcutaneous fat, i.e. 7% of total dietary fat) [22]. In the UK, Thomas et al. [5] found 3.1% of TFAs (2.4% C18:1 trans) in subcutaneous fat taken postmortem from cardiac patients and 3.3% in a control group (2.5% C18:1 trans). In the Netherlands, Katan et al. [23] and Van Staveren et al. [24] found a 4.9% average TFA content in subcutaneous fat, 3.1% C18:1 trans and 0.9% C18:2 trans. In the US, Hudgins et al. [25] found 4.1% of total TFA in human adipose tissue, and of that 2.7% C18:1 trans. Similarly, London et al. [26] found 4.3% of total TFA, and of that 2.9% C18:1 trans. Aro et al. [8] presented lower values than those found in the Czech Republic (1.6% TFAs, both in cardiac patients and patients with no sign of ischemic heart disease). In our study, the lower values might be due to changes in the technology of edible fat production in the Czech Republic in the last years. In the first half of the 1990s there was a very high TFA isomer content in most shortenings and margarines including spread margarines [27]. Currently, the situation is much better. Most spread margarines now have only a trace or a very low TFA content. However, in half-hard margarines the average TFA content is still high. Six of nine products from various producers had more than 20% of TFAs in fat and only 2 of them had a TFA content of <2%. In shortenings, analysis revealed that in 1 of 4 products the TFA content in fat was lower than 1%, and the remaining 3 products had 27.0–38% TFAs in fat [28]. The TFA content in other food products (waffles, biscuits, instant soups, etc.) might also be a problem as hardened fat is used in their production. The persistently increased TFA content in some products was an incentive for this study.

To estimate dietary exposure we used the TFA determination in subcutaneous fat which reflects the dietary intake of TFAs [22–24, 29]. We are aware that it would be advantageous to compare our results with dietary intake data. This would be quite problematic because the TFA content in most food products is not available.

Conclusion

The results obtained indicate a lower TFA load in comparison with previously carried out foreign studies. Still, we found a significantly higher concentration of 18:1 trans fatty acids in the subcutaneous fat obtained from patients with coronary disease, in comparison with a group of patients without signs, symptoms and a history of coronary heart disease. This result may be an impulse to correct the dietary habits of high-risk population groups.

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