

The fatty acid composition of subcutaneous fat in German adults

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Die Fettsäurenkomposition des subkutanen Fettgewebes bei erwachsenen Deutschen

Summary: The fatty acid (FA) composition of subcutaneous fat (SCF) was analyzed in 47 German adults. No influences of sex or age on the FA status of the probands could be detected. SCF consisted mainly of monoenoic fatty acids. Linoleic acid was the major polyunsaturated fatty acid. Linoleic acid metabolites were found in small quantities only. In comparison to the results of studies in the USA and the Netherlands the FA composition of SCF in German probands was characterized by lower levels of linoleic acid and higher contents of palmitic acid. The trans-FA content was similar to that in Dutch women, but lower than in American male probands. However, in evaluating the differences in the FA composition of SCF in different countries, it has to be noted that there are wide ranges in the proportions of the major FA in all populations studied.

Zusammenfassung: In der vorliegenden Studie wurde die Fettsäurenkomposition des subkutanen Fettgewebes (SCF) von 47 deutschen Erwachsenen analysiert. Es zeigte sich kein Zusammenhang zwischen dem Alter bzw. Geschlecht der Probanden und dem Fettsäurenstatus. Monoene bildeten den Hauptanteil der Fettsäuren (FA) im SCF. Linolsäure war die wichtigste mehrfach ungesättigte Fettsäure. Linolsäuremetaboliten wurden nur in geringen Mengen gefunden. Im Vergleich zu den Ergebnissen von Studien in den USA und den Niederlanden ist die FA-Zusammensetzung des SCF bei deutschen Probanden durch geringere Anteile von Linolsäure und höhere Anteile von Palmitinsäure gekennzeichnet. Der Gehalt an trans-FA entspricht dem, der bei Frauen in den Niederlanden gefunden wurde, er ist jedoch deutlich niedriger als bei amerikanischen Männern. In der Beurteilung der Unterschiede in der Fettsäurenkomposition des SCF in verschiedenen Ländern sollte jedoch berücksichtigt werden, daß die Anteile der wichtigsten FA in allen untersuchten Populationen eine große interindividuelle Schwankungsbreite zeigten.

Key words: fatty acids; subcutaneous fat; nutrition; trans fatty acids; atherosclerosis; Germany

Schlüsselwörter: Fettsäuren; subkutanen Fettgewebe; Ernährung; trans-Fettsäuren; Atherosklerose; Deutschland

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Introduction

Analyses of the fatty acid (FA) composition of plasma lipids, erythrocyte lipids, and subcutaneous fat (SCF) are commonly used for the description of the FA status in humans (18, 19). Plasma and erythrocyte lipids contain different proportions of polyunsaturated fatty acids (PUFA) and long-chain polyunsaturated fatty acids (LCP) which may change in a relatively short time, depending on dietary FA-intake (8). The FA composition of SCF, however, well reflects the dietary intake over a period of several months (2). Information about the FA composition of SCF in a population may therefore be of importance in nutritional epidemiological studies.

Of interest are not only quantitatively important FA such as oleic acid (OA; 18:1(n-9)) or linoleic acid (LA; 18:2(n-6)), but also minor FA of physiological importance such as alpha-linolenic acid (LLA; 18:3(n-3)), the eicosanoid precursors dihomo-gamma-linolenic acid (DHLA; 20:3(n-6)), arachidonic acid (AA; 20:4(n-6)) and eicosapentaenoic acid (EPA; 20:5(n-3)), docosahexaenoic acid (DHA; 22:6(n-3)), and also the trans-isomers of major fatty acids. Knowledge about proportions of these FA in SCF is presently available only from a few countries and regions. Studies have been carried out in the Netherlands (3, 4, 22) and in the USA (2, 5, 11, 12, 13). More recently, data from a control group in a clinical study in the USA have been published (14). However, to the best of our knowledge, no detailed data on the FA composition of SCF in healthy German adults have, as yet, been published. The objective of this study, therefore, was to establish reference values for the FA composition of SCF in this population. These values may be used for comparison with other study groups in epidemiological and clinical investigations.

Probands and methods

Forty-seven healthy probands (26 men, 21 women), undergoing minor and elective surgical interventions, such as herniotomies or varicotomies, were included in the study. Their age ranged from 18 to 67 years (mean \pm SD; 46 ± 9.9 years). All were patients of the surgical department of a local hospital in the region of Düsseldorf and Cologne. The study was carried out during a 9-month period. Only German probands were selected in order to eliminate possible errors caused by special dietary habits. Probands were first interviewed by questionnaire about their diets, preceding diseases and drug intake; statements were confirmed in a second unstructured interview. Careful physical examination and anthropometrical measurements were carried out. The body mass index (BMI) was used as anthropometrical index. The values were in the commonly accepted range (9) in all probands investigated. Probands with obesity, increased blood pressure, special dietary habits (e.g., vegetarian or low-fat diet), relevant diseases within the last 5 years, or metabolic and gastroenterological disturbances were excluded from the study. Additional information on diseases was obtained by preoperative determination of hemoglobin, white blood cell count, liver enzyme activities and serum lipids (cholesterol and triglycerides). Probands with one or more values out of the accepted ranges for these laboratory tests were also excluded from the study. All probands were informed about the study and gave their consent to participate.

A pea-sized piece of SCF was taken intraoperatively from the anterior abdominal wall or the inguinal region. The specimen were prepared by removing visible vessels, cleaned with 0.9 % NaCl, and subsequently frozen. After homogenization extraction of SCF lipids was performed using chloroform/methanol (1/2; vol./vol.).

Transesterification was achieved by heating with methanolic HCl at 85 °C for 60 min. After neutralization the samples were dried under a stream of nitrogen and stored at -18 °C.

Table 1. FA-composition of subcutaneous fat in healthy German adults (n = 47). Values in weight percentages of all FA determined.

FA	Mean	SD	Minimum	Maximum	Median
Saturated fatty acids (SFA)					
15:0	0.38	0.12	0.14	0.72	0.39
16:0	23.96	2.08	18.47	28.18	24.27
17:0	0.46	0.12	0.00	0.70	0.49
18:0	5.86	1.32	2.39	8.49	5.93
19:0	0.05	0.06	0.00	0.28	0.02
20:0	0.24	0.11	0.06	0.56	0.23
21:0	0.39	0.19	0.00	0.82	0.45
22:0	0.08	0.06	0.00	0.21	0.07
24:0	0.01	0.02	0.00	0.05	0.00
Trans and monoenoic fatty acids					
16:1trans	0.41	0.11	0.12	0.72	0.42
16:1cis	5.03	1.65	2.73	10.64	4.75
17:1cis	0.41	0.08	0.25	0.61	0.40
18:1trans	2.42	0.86	0.98	6.15	2.31
18:2trans	0.31	0.08	0.16	0.52	0.32
(n-9)-fatty acids					
18:1(n-9)	44.88	1.92	40.95	48.40	44.95
20:1(n-9)	1.02	0.15	0.77	1.45	1.00
20:3(n-9)	0.02	0.02	0.00	0.11	0.00
22:1(n-9)	0.08	0.05	0.00	0.33	0.07
(n-6)-fatty acids					
18:2(n-6)	11.74	2.02	8.42	17.85	11.68
18:3(n-6)	0.08	0.09	0.00	0.33	0.09
20:2(n-6)	0.29	0.04	0.23	0.40	0.28
20:3(n-6)	0.17	0.07	0.00	0.44	0.17
20:4(n-6)	0.26	0.08	0.00	0.46	0.25
22:4(n-6)	0.13	0.07	0.00	0.28	0.14
22:5(n-6)	0.02	0.03	0.00	0.21	0.01
(n-3)-fatty acids					
18:3(n-3)	0.65	0.14	0.39	1.07	0.65
20:3(n-3)	0.05	0.03	0.00	0.11	0.06
20:5(n-3)	0.02	0.02	0.00	0.08	0.02
22:5(n-3)	0.21	0.08	0.00	0.43	0.21
22:6(n-3)	0.34	0.37	0.05	1.30	0.19
Sums and ratios					
SFA	31.44	2.88	25.20	36.42	31.51
Trans FA	3.15	0.95	1.29	7.03	3.11
Monoenoic FA	54.26	2.73	49.28	59.95	54.20
(n-9)-FA	46.00	1.96	41.93	49.58	46.21
(n-6)-FA	12.70	2.07	9.19	18.73	12.57
(n-3)-FA	1.11	0.26	0.67	1.60	1.08
PUFA total	14.14	2.18	10.14	19.81	14.04
PUFA/SFA	0.46	0.10	0.30	0.75	0.44

The FA composition was determined by capillary gas-chromatography using a Dani 6500 HR (Dani Analysentechnik, Mainz, FRG) equipped with a temperature programmable injector (PTV) and a CP Sil 88 capillary column (Chrompack, FRG). Temperature was raised between 100 and 190 °C with 14 °C/min, between 190 and 220 °C with 25 °C/min. These chromatographic conditions provide an excellent separation of FA methyl esters and a high reproducibility of results, also for minor FA (17). However, peak separation of the different trans-18:2-isomers was not consistent. The values for these isomers were, therefore, combined as sum of all trans-18:2-isomers. Substances were identified by comparison with commercially available standards (Nu Chek Prep, Elysian, USA). Values were calculated as relative weight percentages of all FA determined. Normal Gaussian distribution of values was tested by Chi-square-analysis. Since several FA did not show a normal distribution, medians and ranges were calculated in addition to means and standard deviations (SD).

Results

There were no differences in the FA-composition of SCF between males and females. Furthermore, no relation between the age and the FA status could be detected. Therefore, the results of both sexes and all ages were combined (Table 1).

The SCF consisted mainly of monoenoic fatty acids ($54.26\% \pm 2.73$; mean \pm SD), OA being the major FA determined ($44.88\% \pm 1.92$). Saturated FA (SFA) accounted for 31.88% (mean), primarily stearic acid (SA; 16.0 ; $23.96\% \pm 2.08$). PUFA were found only in smaller quantities ($14.14\% \pm 2.18$), with LA being the major PUFA ($11.74\% \pm 2.02$). Linoleic acid metabolites were found only in very small quantities, since DHLA and AA represented only $0.17\% \pm 0.07$ and $0.26\% \pm 0.08$ of the total fatty acids, respectively. Also, metabolites of the (n-9)-series and (n-3)-series were found only in trace amounts.

Trans-FA presented an average of $3.15\% \pm 0.95$. Remarkable, however, was the wide range of values from 1.29% to 7.03% . Major trans-FA was elaidic acid (EA; 18:1 trans) accounting for $2.42\% \pm 0.86$.

Discussion

To the best of our knowledge, this study is the first to provide reference values for the FA composition of SCF, including minor FA such as trans-isomers, in German adults. The study group be defined by the assessment of the anthropometrical status and by excluding probands with unusual dietary habits. As none of the probands was obese or underweight and none of them showed signs of metabolic disturbances, the values from these probands may well be used for comparative purposes.

Possible differences in the FA composition of SCF at various anatomical sites (5, 16) were avoided by standardization of the site of biopsy. The sites chosen in our study enable to gain sufficient amounts of SCF for analytic purposes, even if microsampling techniques (10) are used. Thus, our results may also be used for comparison when these sampling techniques are chosen.

While plasma FA values (especially, those of the cholesteryl ester fraction) reflect the intake of only a short period of some days (19, 20), the FA

status as determined by analysis of SCF provides information about the average FA intake over a period of several hundred days, especially, about the relative proportion of PUFA in the diet (6, 10). Such information is of importance for further epidemiological studies on the dietary intake in groups at risk, e.g., with hyperlipidemia or coronary heart disease, for which it is known that the PUFA status influences the prognosis of the disease.

As in other studies on the FA composition of SCF (1) no significant influence of age or sex on the FA status of adults was found in our study group. It is well established that the FA composition of SCF is mainly influenced by dietary intake (2, 3, 7, 22). Thus, it appears that the dietary fat intake of the elder probands did not differ significantly from the fat intake of the younger ones.

As the physiological function of SCF is mainly directed to the supply of energy-providing FA, saturated fatty acids and monoenic fatty acids form the greatest proportion of all FA. Only 14 % of all FA determined were PUFA. The quantitatively most important PUFA found was linoleic acid, which, being an essential FA, originates exclusively from the diet. Linoleic acid metabolites were found only in small amounts. Further physiologically important PUFA, such as alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid, were present only in small proportions.

It has to be pointed out that the analysis of the FA composition of SCF in individuals under common diets only provides data for the evaluation of the intake of major FA (e.g., linoleic acid) on an average. The contents of minor FA in SCF are, in most cases, not related to those found in membranes (18). Therefore, more detailed information concerning the metabolism of eicosanoid precursors or structurally important FA may hardly be obtained by SCF analyses. They have to be gained by additional analyses of plasma or membrane lipid fractions.

The mean trans-FA content of SCF in our study group was similar to that found in Dutch females (15, 22), but clearly lower than in US male

Table 2. Linoleic acid, alpha-linolenic acid, and oleic acid contents in adipose tissue as determined in different populations. Values in weight percentages of all FA determined (*Mean \pm SEM).

Study	Alpha-linolenic acid	Linoleic acid	Oleic acid
Leichsenring et al. 1992 (Germany; n = 47)	0.65 \pm 0.14	11.74 \pm 2.02	44.88 \pm 1.92
Ito et al. 1991; (14) (USA; n = 76)	0.62 \pm 0.15	14.44 \pm 2.34	40.27 \pm 1.98
Berry et al. 1986; (2) (USA; n = 413)	1.91 \pm 0.27	16.32 \pm 2.89	46.12 \pm 2.19
Phinney et al. 1991; (21) (USA; n = 12; obese females)	0.67 \pm 0.03*	16.00 \pm 0.69*	46.88 \pm 0.47*
van Staveren et al. 1986; (22) (The Netherlands; n = 59)	0.87 \pm 0.16	14.21 \pm 2.10	41.64 \pm 2.16

probands (12). As there is no specific accumulation of trans-FA in SCF (12), the proportions measured directly reflect the dietary preferences of the probands. Information about trans-FA in various populations may thus be used as an indicator of the amounts of hydrogenated fats consumed (15). However, the *in vivo* effects of small proportions of trans-FA in human lipids are still under discussion. Recent studies could not demonstrate a strong correlation between risk factors of cardiovascular diseases and the trans-FA content of SCF (12).

Table 2 provides a comparison of the values for some important FA determined in our study with results from previous studies. The linoleic acid content of SCF appears to be similar in all studies. In the study from the Netherlands higher amounts of LLA were found (22). The mean LLA content reported by Berry et al. (2) (derived from analyses of 413 specimen) is out of the range of those contents found in other studies. As LLA is only a minor compound of SCF and difficult to separate from other adjacently eluting FA in gas-chromatography, especially when packed columns are used, it may be assumed that the different results published by Berry et al. are mainly caused by methodological factors.

In comparison with probands investigated in other countries, the SCF composition of the German probands was characterized by lower levels of LA and higher proportions of palmitic acid. This probably reflects different dietary habits in the German population, when compared with Americans. The latter have markedly increased their average intake of vegetable fats and PUFA in the past years; this was probably a consequence of campaigns designed to reduce the incidence of atherosclerotic disease by increasing the average PUFA intake (12). It may be inferred from our results that the PUFA intake in Germany is not as high as in the USA. It should be noted, however, that in all populations studied wide ranges of values were present, e.g., LA values from 8.52 % to 20.89 % in the USA (12) and from 8.42 % to 17.85 % in our study, respectively. It might be of interest to follow those probands with lowest and highest proportions of LA in SCF, i.e., lowest and highest LA intake longitudinally, in order to characterize the physiological consequences of such a widely differing PUFA status.

A high intake of PUFA may have desirable effects, such as a decrease in plasma cholesterol levels, but also undesirable effects, such as increased susceptibility of PUFA-rich lipid fractions and membranes to oxygen-induced free radical damage. It is not yet clear which FA compositions in the various lipid classes reflect a physiological optimum. By further studies in different regions of the world the variability of the FA composition in human SCF may be elucidated. This may help to identify possible relations to risk factors of cardiovascular or neoplastic diseases more precisely.

Acknowledgements

The authors are grateful to Dr. W. Hölter, Head Department of Surgery, St. Josefs Hospital, Monheim, FRG, for his support of the clinical part of the study.

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Received February 1, 1992

accepted March 28, 1992

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