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Trans fatty acids: Implications for health, analytical methods, incidence in edible fats and intake

(A review)

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Summary

Trans fatty acids (TFA) are supposed to be related to a variety of physiological effects. Numerous studies in this field are gathered and compared, which mainly deal with the influences on lipoprotein levels in plasma and their effects with regard to coronary heart diseases. Furthermore, the analytical accessibility of *trans* fatty acids by different methods is presented. Thus, the most reliable method for an exact quantitation of *trans* fatty acids in edible fats is the combination of Ag-TLC with GC. The contents of TFA, in particular *trans*-octadecenoic acids, in bovine and human milk fat, in partially hydrogenated vegetable fats and oils as well as in processed food from different countries, determined in numerous studies, are summarized. Especially results on the isomeric distribution of positional isomers of *trans*-octadecenoic acid may be of future interest, since negative metabolic activities might only originate from certain isomers. Finally, intake rates of TFA in several countries are presented. It can be concluded that there still is need for further nutritional studies and that the discussion about TFA should not neglect the comparison with the saturated fatty acids C12, C14 and C16.

Zusammenfassung

Trans-Fettsäuren: Gesundheitliche Relevanz, analytische Nachweisverfahren, Vorkommen in Speisefetten und Aufnahme (Übersichtsbericht)

Trans-Fettsäuren (TFA) werden mit einer Vielzahl von physiologischen Wirkungen in Verbindung gebracht. Zahlreiche Studien auf diesem Gebiet werden aufgeführt und gegenübergestellt, die sich überwiegend mit dem Einfluß auf Lipoproteinspiegel im Plasma und deren Wirkung hinsichtlich koronarer Herzerkrankungen befassen. Des weiteren wird die analytische Zugänglichkeit von *trans*-Fettsäuren mit Hilfe verschiedener Methoden dargestellt. Demnach ist die verläßlichste Methode zur exakten Quantifizierung von TFA in Speisefetten die Kombination von Ag-TLC mit GC. Die in zahlreichen Arbeiten bestimmten TFA-Gehalte, insbesondere von *trans*-Octadecensäuren, in bovinem Milchfett und Humanmilchfett, in partiell hydrierten Pflanzenfetten und -ölen sowie in verarbeiteten Lebensmitteln aus verschiedenen Ländern werden zusammengefaßt. Insbesondere die Ergebnisse bezüglich der Isomerenverteilung von Positionsisomeren der *trans*-Octadecensäure könnten von zukünftigem Interesse sein, da negative metabolische Effekte möglicherweise nur von bestimmten Isomeren ausgehen. Schließlich werden Aufnahmeraten von TFA in einigen Ländern aufgelistet. Es kann der Schluß gezogen werden, daß ein Bedarf an weiteren Ernährungsstudien besteht und daß bei der Diskussion über *trans*-Fettsäuren der Vergleich mit den gesättigten Fettsäuren C12, C14 und C16 nicht vernachlässigt werden sollte.

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Introduction

Since decades, the aspects concerning nutritional physiology of *trans* fatty acids (TFA)[1] have controversially been discussed in the literature, and a variety of reviews of this subject have been published [e.g. 1–7]. Some of the earlier studies correlated the intake of TFA with an increase of the cholesterol level, regarding TFA as a risk factor for a premature atherosclerosis [8–13], whereas others could not confirm that [14, 15]. Recent works published since 1990 about dietary studies with females and males especially from MENSINK, KATAN, ZOCK [16–18], WOOD et al. [19] and NESTEL et al. [20], or epidemiological studies from WILLETT et al. [21] and TROISI et al. [22] caused a new worldwide discussion about potential risks in connection with a higher intake of TFA, above all concerning premature atherosclerosis and cardiovascular diseases. Within the last years, these discussions led to numerous efforts to improve the analytical accessibility of TFA by various methods and to enable more precise determinations to be made. Furthermore, the request for quantitative data about TFA contents in different food items, as well as for simple but exact methods for TFA analysis was increasingly expressed.

The present study mainly deals with recently published health aspects related to an increased TFA intake and with current and future ways to measure TFA contents in animal and vegetable fats. In addition, TFA contents in edible fats published up to now and intake rates calculated from such data will be presented.

Implications for health – biochemical aspects

Trans isomeric fatty acids in milk fat and tallow of ruminants are attributable to biohydrogenation in the rumen, which is accompanied by *cis*/*trans*-isomerization, whereas TFA in margarines, shortenings and cooking fats result from the technological process of partial hydrogenation aiming at the production of more solid spreads and the improvement of oxidative and flavour stability. For reasons of nutritional physiology and consumer application a complete saturation of the double bonds is not desirable. Whereas *cis* fatty acids have a bend in their carbon chain (e.g. oleic acid, *cis* Δ9-C18 : 1), *trans* fatty acids are straight and therefore can be packed more closely. Thus, in the *cis* configuration the two parts of the carbon chain are located at the same side of the double bond, while *trans* fatty acids have the corresponding substituents at opposite sides. E.g., elaidic acid (*trans* Δ9-C18 : 1) is a stereo isomer (geometric isomer) of oleic acid with the double bond located at position 9, respectively. However, the different geometric structure leads to an increase of the melting point from 14 °C *(cis)* to 52 °C *(trans)* [5]. Thus, TFA are more similar to saturated fatty acids than to the corresponding *cis* fatty acids as regards their physical properties. This is also reflected in the structure of triglycerides, where TFA as well as saturated fatty acids are primarily found in sn1 and/or sn3 position in the molecule [23]. In contrast, *cis* fatty acids are primarily located in sn2 position of the triglyceride molecule [24].

[1] Abbreviations: FA – fatty acids; FAME – fatty acid methyl esters; Ag-TLC – Argentation thin-layer chromatography; TFA – *trans* fatty acids; C18: 1 – octadecenoic acids; C12, C14, C16, C18 – lauric, myristic, palmitic, stearic acid; LDL/HDL – low density/high density lipoprotein; Lp(a) – lipoprotein(a); CHD – coronary heart disease

Besides the influence on plasma lipoprotein and cholesterol levels various properties are mentioned in the literature, which make a distinction between *cis* and *trans* isomers with regard to metabolism and physiological effects. So, the intake of TFA causes an increased need of essential fatty acids [1]. *Trans* isomers of linoleic acid do not have the desired effects of essential fatty acids [25, 26], but with higher TFA intake even a greater amount of essential linoleic acid is required to achieve a normal growth rate [27].

Furthermore, the rate of mitochondrial β-oxidation is reported probably to be slower compared to *cis* fatty acids accompanied by a reduction of oxidative phosphorylation, i.e. a decrease of adenosine triphosphate (ATP) levels [1, 28]. Oxidation of *cis*-octadecenoic acids is supposed to be inhibited as well [29] and the structure of cellular membranes is said to be altered [30, 31] possibly accompanied by changes in membrane properties. Due to significant incorporation of high-melting TFA into membrane phospholipids membrane fluidity is changed, which may modify the function of membrane-associated enzymes and thereby some cellular reactions [1, 12].

Moreover, inhibitory effects on desaturation and chain elongation of essential fatty acids, reduced arachidonic acid levels in tissue lipids and decreased activity of cyclooxigenase are reported [23, 31 – 33]. In this connection several studies have demonstrated that TFA impair the microsomal desaturation and chain elongation of the essential fatty acids C18 : 2 and α-C18 : 3 to their long chain polyunsaturated metabolites (see ref. in [34]). In 29 premature infants (age: 33.6 $\pm$ 1.4 weeks) KOLETZKO [34] found that TFA in plasma were not related to the precursor essential fatty acids, but correlated inversely to n-3 and n-6 long chain polyunsaturated fatty acids. TFA were also inversely correlated to birth weight, so that intrauterinal growth is likely to be impaired [23].

Several authors pointed out a restriction of biosynthesis of long chain polyenic fatty acids by an inhibition of the Δ6-desaturase caused by *trans*, *trans*-C18 : 2 [1, 35, 36] or by elaidic acid (*trans* Δ9-C18 : 1) [31, 37], which results in an inhibition of prostaglandin synthesis [34, 38, 39]. WILLETT et al. [21] correlated a stronger inhibition of Δ6-desaturase by elaidic acid than by vaccenic acid (*trans* Δ11) [37] to a different impact of TFA from hydrogenated vegetable fats (more elaidic acid) compared to animal fats (more vaccenic acid).

Based among other things upon his studies with infants (age: 5 to 24 months), KOLETZKO [23] thinks that there might be a negative selectivity of cholesterol esterification for *trans*-C18 : 1 with a reduction of lecithin-cholesterol-acyltransferase activity. Thus, it can not be exluded that this leads to an undesirable increase of total and LDL cholesterol levels. Possibly a similar effect could as well be caused by an increase in the activity of the cholesterol ester transferprotein by TFA [40].

Some studies found a decrease of apolipoprotein A-I-levels after TFA intake [16, 19, 41, 42]. At relatively high dietary levels the isomer *trans*-9, *trans*-12-octadecadienoic acid is reported to cause various toxicological and physiological abberrations [1, 43]. Also, some studies suggested an epidemiological relationship between the *trans*-consumption and cancer [44, 45] as to 'Morbus Crohn' [46, 47]. HOGAN et al. [48] found for rats that a high *trans* fatty acid diet somewhat enhances large intestinal carcinogenesis.

However, it should be emphasized that part of the mentioned points are discussed controversely. E.g., the incidence of some human cancers was refuted by several authors [43, 49 – 52].

Publications of the last decades let the National Cholesterol Education Program in the USA (NCEP) 1988 come to the conclusion that especially saturated fatty acids contribute

to an increase of cholesterol levels. Positive correlations with plasma cholesterol concentration and risk of cardiovascular heart disease are described in several studies (e.g. [53–56]). Though the majority of papers is regarding total and LDL cholesterol levels to be the reason for an increased risk of developing atherosclerosis, there are some authors that consider increased cholesterol levels only as an indicator for an existing atherosclerosis. However, today it is predominantly assumed that the amounts of cholesterol in the individual classes of lipoproteins provide a better coronary prediction than the total cholesterol level [57, 58] and that especially a high LDL/HDL-ratio correlates with an increased risk. Numerous epidemiological data or clinical studies suggest a direct relation between the total or the LDL cholesterol level and the incidence of CHD (e.g. [59–63]). However, increased levels of serum cholesterol do not play the only role as regards atherosclerosis, but they should be seen as one of several risk factors.

In contrast to the LDL cholesterol, the HDL cholesterol plays an important part in mobilization and transfer of excess cholesterol from peripheral tissues to the liver. Regarding this aspect, a variety of publications has stated that saturated fatty acids (C12–C16) raise the levels of serum and LDL cholesterol so that these are considered as risk factors for a premature atherosclerosis [56, 64–67]. While especially palmitic acid increases LDL and total cholesterol levels [68], stearic acid is reported to be neutral concerning its effect on lipoprotein levels [58, 69, 70]. On the other hand, polyunsaturated fatty acids in particular [58, 66, 71] and according to newer publications monounsaturated *cis* fatty acids as well are supposed to have a LDL cholesterol-decreasing or HDL cholesterol-increasing (*cis*-C18 : 1) effect [65, 66, 67, 72]. However, compared to saturated fatty acids, some authors [58, 71] did not only observe a reduction of LDL cholesterol by polyenoic fatty acids but of HDL cholesterol as well. Former nutritional studies with respect to the influence of TFA on plasma lipids in rats by GOTTENBOS [73] or in humans by MATTSON et al. [15, 74] and ERICKSON et al. [14] seem to exhibit no difference between *cis* and *trans* fatty acids. In a review KINSELLA et al. [26] also found only little effects in the development of atherosclerosis when evaluating the experimental results available in 1981. However, several further studies from 1961–1975 did show an increase of serum cholesterol by TFA intake in comparison to oleic acid [8–10]. In 1975 THOMAS [75] had reported that for the United Kingdom mortality from atherosclerotic disease is highest in those areas which consume highest amounts of TFA. In 1982 LAINE et al. [76] found out that when compared to lightly hydrogenated soy oil, unhydrogenated soy and corn oil lowered total and LDL cholesterol levels in normocholesterolemic subjects.

Since the beginning of the nineties several new clinical nutritional studies led to an intensive discussion about negative effects on plasma lipids with increased TFA intake. It was shown that *trans*-octadecenoic acids at least shall raise the LDL cholesterol level [16, 17, 19, 20, 42, 67, 77, 78] and on the other hand lower the HDL cholesterol level [16, 42, 67, 77]. Therefore, *trans* fatty acids are said to exert a particular infavourable effect on the LDL/HDL-ratio.

During a three weeks study with 34 females and 25 males, in which 11% of energy intake was given as TFA, MENSINK et al. [16] found a mean increase of LDL cholesterol levels of 14.3 mg/dl and a mean decrease of HDL cholesterol levels of 6.6 mg/dl compared to a diet with equivalent amounts of oleic acid.

In comparison to a linoleic acid-diet, the experiments by ZOCK and KATAN [17, 67] with 7.7% of energy supplied as TFA (56 persons, 3-week-diets, "crossover" pattern) exhibited a mean rise of the total cholesterol level by 6.2 mg/dl as well as of 9.3 mg/dl for the LDL

cholesterol level. The HDL/LDL-ratio was 0.55 for the linoleic acid-diet, 0.50 for the stearic acid-diet and 0.47 for the *trans*-diet. In these studies, it was assumed that, regarding serum lipids, the effects of a moderate intake of oleic and linoleic acid are similar [65, 72, 79]. ZOCK [67] stressed that the results of both studies are comparable, if the different rates of TFA intake are taken into consideration. In conclusion it can be deduced that a TFA intake of 1% of energy raises the LDL cholesterol level by 1.2 mg/dl and lowers the HDL cholesterol level by 0.6 mg/dl.

WOOD et al. [19] performed experiments with 38 healthy males, who got 50% of fat energy intake as soft-margarine with 61.3% C18:2, 13.8% *cis*-monoenoic acids (>98% C18:1) and without any TFA, and in a further diet as hard-margarine (containing hydrogenated vegetable oils) with 3.5% C18:2, 43.4% *cis* monoenoic acids and 29% *trans*-C18:1. In a 7-day dietary record the diets were designed in such a way that 40% of energy were provided from fat of which 60% were from the test fats (24% of energy). The studies resulted in an average increase of total cholesterol by 11 mg/dl and of LDL cholesterol by 8 mg/dl in the *trans*-diet compared to the soft-margarine-diet. The HDL cholesterol level was not altered.

The TFA intake of 47 patients suffering from coronary heart diseases was compared to a healthy control group of 56 persons by SIGUEL et al. [77]. They found increased levels of total and LDL cholesterol as well as a decreased HDL level in the first group exhibiting a higher TFA intake. In this connection, the importance of a sufficient supply with essential FA, such as linoleic acid, was particularly emphasized, which can reduce negative effects resulting from TFA.

LICHTENSTEIN et al. [78] as well reported that the substitution of corn oil for corn oil margarine (4.2% of energy as elaidic acid) within a diet low in total and saturated fat resulted in higher total and LDL cholesterol levels. A change in HDL cholesterol could not be observed.

NESTEL et al. [20] found that relative to oleic acid the consumption of elaidic acid (*trans* Δ9-C18:1, 7% of energy) led to significantly higher total and LDL cholesterol levels in mildly hypercholesterolemic men.

JUDD et al. [41, 42] could confirm the relations between TFA intake and influences on LDL and HDL cholesterol levels, respectively. In experiments with 29 women and 29 men with moderate (3.8% of energy as TFA) and high TFA intake (6.5% of energy as TFA) compared to an oleic acid diet (exchange of oleic acid by TFA, same content of saturated fatty acids) an increase in LDL cholesterol levels of 6% and 7.8%, respectively, was observed. With high TFA intake the HDL level was 2.8% lower than with the oleic acid diet. Comparison of the oleic acid diet with a diet high in saturated fatty acids (the energetical content of the saturated FA C12/C14/C16 was 6% higher than with the high TFA diet) resulted in an increase of 3.5% in HDL levels for the 'saturated' diet.

While RIDKER et al. [80] found no evidence of association between Lp(a) level and risk of future myocardial infarction, numerous other studies are consistent with the concept that an elevated Lp(a) level is an independent risk factor for coronary heart disease (CHD) [e.g. 81–85]. Furthermore, the Lp(a)-specific protein Apo(a) has been detected in atherosclerotic lesions [86]. In a new review from 1994 with a summary on Lp(a) lipoproteins and its clinical implications within the last 20 years DAHLÉN [87] comes to the conclusion that Lp(a) is a factor of outstanding importance in the pathogenesis of atherosclerosis. It is assumed that Lp(a) is affecting enzyme activity of plasmin, which is important for dissolving fibrin fibers after occurence of thrombosis [6].

In several experiments (3-week diets) with 59 participants MENSINK et al. [18] could raise the serum Lp(a) level by 73% through substitution of the cholesterol-increasing saturated FA (C12, C14, C16) by TFA (10% of energy). NESTEL et al. [20] reported about an increase of the Lp(a) level by 30% after changing from a basic diet to a diet rich in elaidic acid.

However, the intake of 11% of daily energy as TFA in the experiments of MENSINK et al. [16] (equivalent to 33 g per day and person) were so high that some scientists questioned, whether the observed effects would also be relevant with lower TFA intake. ZOCK [67] still stresses, that these high intake rates will be reached by persons, who are consuming particular high amounts of hydrogenated fats. Thus, in 1990 ENIG et al. [88] estimated the daily TFA intake in the USA for 13.3 g/person with a range from 1.6 to 38.7 g/person.

APPLEWHITE [89] emphasizes that all the experiments have not shown *trans* isomers to be hypercholesterolemic relative to saturated fatty acids they are intended to replace. Nevertheless, the studies of MENSINK et al. [18] were based e.g. on a substitution of the saturated FA C12/C14/C16 by TFA (see above), which led to a distinct rise of lipoprotein(a) levels.

The studies of MENSINK and KATAN were criticized by REEVES [90], who claimed that the used fat had not been hydrogenated like US-margarines but isomerized catalytically and therefore exhibited an unsual distribution of *trans* positional isomers. KRITCHEVSKY [91] remarked that the fatty acids in that fat had to a greater extent been randomized in the triglycerides and that only because of this cholesterolemic effects could have been caused. In a letter MENSINK et al. [92] could largely refute all these reproaches.

An epidemiological study in the USA by WILLETT et al. [21], in which the TFA intake of 85095 women devided in 5 groups with different intake rates and the incidence of CHD within a period of 8 years has been evaluated, exhibited a risk for CHD being 50% higher for the group 5 (highest TFA intake) in comparison to group 1 (lowest TFA intake). The authors pointed out that TFA intake was positively correlated with CHD death rates, but this was only valid for TFA from hydrogenated vegetable fats such as margarine or cooking fat and not for animal fats. In a further epidemiological study by TROISI et al. [22] the evaluation of TFA intake rates of 748 healthy men resulted in a positive correlation to the LDL cholesterol level as well and a negative correlation to the HDL level. Thus, the risk for a myocardial infarct was raised by 27% with an elevated TFA consumption in that study.

1995 an international study (EURAMIC) evaluated the *trans* contents in tissue lipids from 671 men, who had survived a myocardial infarct, and 717 men, who did not develop an infarct [93]. Here, no relation between high TFA contents and incidence of heart attacks could be established. Only after individuals from Spanish centres, who had markedly lower TFA contents, had been excluded, an insignificant tendency to an increased infarction risk with high TFA values could be seen.

The epidemiological studies by WILLETT et al. [21, 22] have repeatedly been criticized as regards the methodology [89, 94]. Furthermore, APPLEWHITE [89] and KULLER [94] stated that these studies could not show an independent effect of *trans* isomers on CHD and that there can be no determination of whether the results are related to TFA or to some other measure of dietary intake. Thus, the studies could possibly not establish a relationship between correlation and causation, which can only be demonstrated by experimental studies. HEYDEN [95] criticizes the design of questionnaires, which did not comprise all fats containing TFA as well as other cardiovascular risk factors.

Based on several cited studies MANN [96] concludes that TFA may influence lipoprotein receptors and relates this hypothesis to the increased cholesterol levels or CHD rates established with higher TFA intake.

PFALZGRAF et al. [97] stressed that thermal treatment of vegetable oils, which contain relatively high amounts of polyenoic FA being very sensitive to oxidation, leads in contrast to animal fats to carbonylic compounds, conjugated FA and polymerisates in cooking fats, french fries and other food items. Since the physiological effects of these by-products are almost unknown, a comparison of animal and vegetable fats in epidemiological studies might be complicated. As is well known, oxidized lipoproteins play an important role in the development of atherosclerosis [98, 99].

Analytical aspects of the determination of *trans* fatty acid contents

The possibly increased risk of developing atherosclerosis by consuming TFA as well as further potentially health-damaging effects afford an exact and extensive recording of *trans* fatty acid contents in edible fats. As regards milk fat analyses, samples from diverse milk products should be based upon very different conditions of feeding and lactation as well as breeds of cows. However, the primary source of TFA in food are technologically hydrogenated vegetable fats, which are present in margarines, shortenings, cooking fats and to an increasing extent in processed food like bread, cakes, sweets and snacks.

In the past, it has been shown that studies concerning the determination of TFA contents have led to rather varying and inconsistent results, if different methods, e.g. gas chromatography (GC) with too short columns resp. unsuited column phases or infrared spectroscopy (IR), were applied to identical samples [38, 100]. Particularly the results from IR spectroscopic analyses of fat products with little TFA are biased [101 – 103]. The fourier transform IR technique (FTIR) resulted in some advantages compared with conventional IR, but the fundamental problem of an exact determination of low TFA contents $< 15\%$ is not completely solved [104].

New studies using a spectral subtraction technique by FTIR [104, 105] gave results, that were in close agreement and only a little bit higher than the values gained by the combination of argentation thin-layer chromatography (Ag-TLC) with GC [e.g. 106 – 109], which can be considered as a reference method today. The higher values with FTIR can possibly be explained by a contribution of small amounts of *trans* monoenoic acids, e.g. with 16 C-atoms, as well as amounts of *trans*-C18 : 2 acids.

With GC, it is important to choose the right column material. Regarding the resolution the extreme polar 100 m-capillary columns CP-Sil 88 or SP2560 can today be considered as most progressive in *trans* fatty acid separation (Fig. 1). Their phases of 100% cyanopropyl polysiloxane are clearly better with respect to resolution [107 – 110] than other phases used for TFA analysis as e.g. SP1000 and SP2100 [111], FFAP [112, 113] or SP2340 [112, 114]. In this connection a doubling of column length from 50 to 100 m with the CP-Sil 88 results in a remarkable improvement of resolution leading to a far-reaching separation of almost all positional *trans* isomers of C18 : 1 [108, 109, 115], what is not at all the case with a SP2340- [116] or FFAP-column [112] of 100 m length. After a pre-separation by Ag-TLC, which avoids the partial overlap between *cis* and *trans* isomers (see below), in 1995 MOLKENTIN and PRECHT [107, 108, 115] could separate altogether up to 9 different *cis*- and 10 *trans*-octadecenoic acid peaks on a 100 m-CP-sil 88-column in diverse edible fats. Nearly

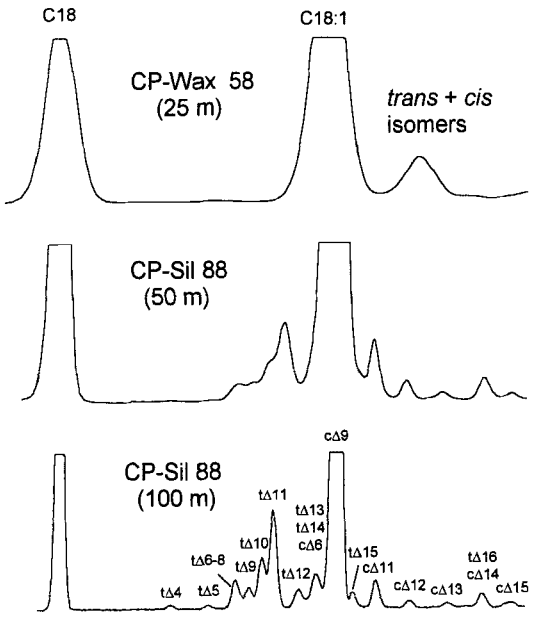

Fig. 1
Chromatograms of C18 : 1-FAME from a typical milk fat obtained with a 25 m-CP-Wax 58, a 50 m-CP-Sil 88 and a 100 m-CP-Sil 88 capillary column

at the same time, WOLFF et al. [109] established a comparable resolution of C18 : 1-isomers (FAME as well as FAIPE) by Ag-TLC/GC. Already in 1991, HUDGINS et al. [118] used a 100 m-SP2560-column for the determination of *trans* isomers in human tissues, but due to the applied GC parameters such a resolution of *trans*-C18 : 1 fatty acids had not been achieved.

With a 100 m-CP-Sil 88-column, derivatisation to isopropyl or methyl esters (FAIPE resp. FAME) leads to nearly the same resolution of C18 : 1-isomers, except that without application of Ag-TLC the use of FAIPE results in an additional overlap of *trans* Δ12 with the intensive oleic acid (*cis* Δ9) [109]. When using Ag-TLC the peaks *trans* Δ4, Δ5, Δ9, Δ10, Δ11, Δ12, Δ15 and Δ16 are well resolved, but due to insufficient differences in the ECL-values (with FAME and FAIPE) the isomers *trans* Δ6 to *trans* Δ8 as well as the peaks *trans* Δ13 and Δ14 are coeluting [107 – 110, 116]. Combination of Ag-TLC [38, 106, 111, 119 – 122] resp. Ag-HPLC [123] with gas chromatography on a 50 m-capillary column or the single use of Ag-HPLC [124], which all avoid the overlap between *cis* and *trans* isomers, only led to a highest separation of 6 *trans*-C18 : 1-peaks [106, 124].

The pattern of individual *trans* isomers has mostly been neglected in the past, but just this isomeric distribution might be of special interest as the negative metabolic activity or the possible differences in the risk for health by TFA from ruminants or hydrogenated plant fats [21] might only originate from certain isomers.

Already in 1977 PARODI [125] could publish the isomeric distribution of *trans* octadecenoic fatty acids of 12 Australian butter fats, but this method is extremely complicated and tedious. At first, preparative GC was used repeatedly to produce a suitable amount of the C18 : 1-fraction, from which the *trans* fatty acids were isolated by Ag-TLC. After reductive ozonolysis the resulting aldehydes and aldesters were analysed gas chromatographically. However, WOLFF [109] stresses that the accuracy of this method could be impaired by the volatility of the shorter fragments and the lack of suitable correction factors for the flame ionization detector.

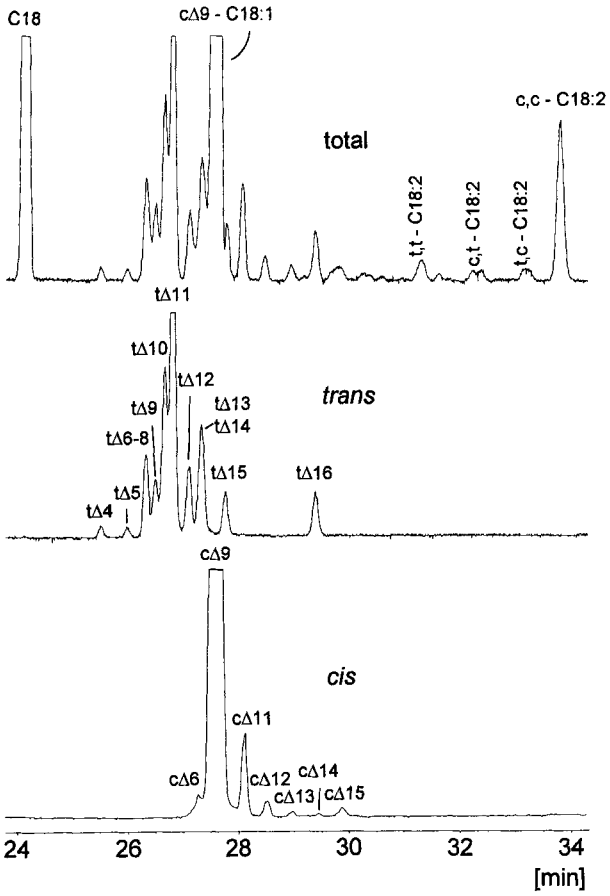

Fig. 2
Partial chromatograms of C18- and C18:1-FAME derived from a milk fat before and after fractionation by Ag-TLC into *cis* monoenoic and *trans* monoenoic acids obtained on a 100 m-CP-Sil 88-capillary column (see [115]) (identification of *trans*, *trans* (*t*, *t*), *cis*, *trans* (*c*, *t*) and *trans*, *cis* (*t*, *c*)-isomers of linoleic acid according to [97, 126])

The exact quantitation of the individual positional isomers of *trans*-C18:1 in edible fats by application of the direct GC method even with a 100 m-column is complicated by the fact that *trans* isomers are partly overlapped by *cis* isomers. Therefore, a prerequisite for the complete quantitation of all *trans* isomers is the pre-fractionation by TLC on silica-gel plates impregnated with $AgNO_3$, as to be seen in Fig. 2.

Depending on the type of fat there are more or less great overlaps of the octadecenoic acid isomers *cis* Δ7 (mainly in hydrogenated fats), *cis* Δ6, *cis* Δ10 and *cis* Δ15 with the isomers *trans* Δ12, *trans* Δ13/14, *trans* Δ15 and *trans* Δ16, respectively. According to a newly published paper the cumbersome pre-fractionation of FAME is not necessary with milk fats as direct GC underestimates the true TFA content of the combined reference method (Ag-TLC/GC) with a constant error of 0.367 g/100 g fat and a relative error of 8.3% with a correlation coefficient of r = 0.989 [122]. In a further study [108] even more precise equations are presented:

Total *trans*-C18:1 = $1.20911 \cdot$ *trans* $\Delta 6-11_{(50\,\text{m-column})} + 0.927791$, with $r = 0.9929$ and a standard error of estimation (see) of 0.17%. By this way, only the group of *trans* Δ6−11, easy to measure on a 50 m-column, has to be considered. A still better formula for estimating the TFA content in milk fats by direct GC on a 100 m-column is given with:

Total *trans*-C18:1 = 1.05676 ($\pm$0.00381) · $trans_{(100\,m\text{-column})}$ − 0.001876 ($\pm$0.000514) C16:0. Because of the very high correlation coefficient of r = 0.99988 and a see-value of 0.065% Ag-TLC is absolutely unnecessary in this case [115]. The term $trans_{(100\,m\text{-column})}$ in the equation comprises all quantified *trans* peaks except *trans* Δ15, independent from overlaps between some *trans* peaks with *cis* peaks. Furthermore, the formula involves the content of palmitic acid (C16:0) into the correlation. This study was based upon 61 German milk fats from very different conditions of feeding and lactation.

Moreover, by analysis of TFA using Ag-TLC/GC and of triglycerides (resolution restricted to triglycerides of different carbon number) of 100 different milk fats as well as statistical methods formulae consisting of different triglycerides could be developed, which allow every positional isomer of *trans*-C18:1 in milk fat to be determined individually with high precision [107, 115]. As well, a formula with 13 different triglycerides for the exact quantitation of the total *trans*-C18:1 content has been developed:

trans-C18:1 = 3.7190 · C26 + 2.4439 · C28 − 8.1505 · C30 + 3.5187 · C32 − 0.4819 · C34 − 0.3737 · C36 + 0.3514 · C40 + 1.3285 · C42 − 1.3429 · C44 − 0.2689 · C46 + 0.9557 · C48 − 0.7356 · C52 + 1.0775 · C54,

with r = 0.9977 and see = 0.29%.

After a rapid analysis of triglycerides [127], which can be performed within 10 − 20 min, inserting of the corresponding triglyceride contents into the equation results in an exact determination of the total *trans*-C18:1 content in milk fats. Portions of *cis* isomers eluting under the *trans*-octadecenoic acids (and v.v.), that can be detected by TLC, were taken into consideration for the evaluation of the formula.

However, in the moment an exact determination of the isomeric distribution of *trans*-octadecenoic acids in hydrogenated vegetable fats still affords the application of the combined Ag-TLC/GC method.

Sources of *trans* fatty acids

Bovine milk fat

Mostly, GC analyses of TFA are focussed on *trans*-octadecenoic acids, which comprise more than 97 − 98% of all *trans* isomers in milk fats. In Table 1 analytical results of TFA contents in milk fats from various countries are given. In the case of small numbers of samples the variation ranges of feeding and lactation will probably not be representative. Thus, the corresponding mean values can not be regarded as characteristic for these countries and should not be used for evaluations of the mean TFA intake by milk products.

In extensive new studies not only the total content of *trans*-C18:1 acids but as well the contents of all individual positional isomers in 1756 German milk fats have been determined [115]. To that end, besides argentation thin-layer chromatography (Ag-TLC) in combination with GC methods, triglyceride combinations have been applied. The results are summarized in Table 2.

Further isomeric distributions of *trans*-C18:1 acids in milk fats from 10 EU-countries are listed in Table 3 [115].

In Table 4 the mean contents of the individual positional isomers of C18:1 expressed as percentage of the total amount of C18:1-TFA given by different authors are shown. All results were obtained with the combined reference method (Ag-TLC/GC), except the values

Table 1
Mean values (mean), standard deviations (sd), minimal (min) and maximal (max) content of *trans* fatty acids in (n) milk fats from different countries [wt% of fatty acids]

mean	sd	min	max	n	Analysis	Year	Country	*trans* FA	Ref.
6.01		4.27	7.64	116	IR	1971	Australia	total	[128]
5.53		4.52	7.31	17	TLC/densitometer	1973	New Zealand	monoenoic	[129]
2.7				1	Ag-TLC/GC	1981	Sweden	C18:1	[130]
		4.0	5.7	13	IR*	1983	Canada	total	[100]
3.4				3	GC**	1983	USA	C18:1	[131]
3.33	0.99	1.75	5.20	31	Ag-TLC/GC	1994	Austria	C18:1	[122]
3.4				10	GC**	1982	Germany	C18:1	[132]
2.75		1.5	6.3	10	GC**	1993	Germany	C18:1	[97]
3.62	1.22	1.29	6.75	1756	Ag-TLC/GC/TR***	1995	Germany	C18:1	[115]
3.83	1.34	1.91	6.34	100	Ag-TLC/GC	1995	Germany	C18:1	[115]
3.19				1	Ag-TLC/GC/TR	1995	Belgium	C18:1	[115]
4.21	0.60			4	Ag-TLC/GC/TR	1995	Denmark	C18:1	[115]
4.04	0.30			10	Ag-TLC/GC/TR	1995	Spain	C18:1	[115]
3.8		2.46	5.10	24	Ag-TLC/GC	1994	France	C18:1	[115]
4.47	0.92			10	Ag-TLC/GC/TR	1995	France	C18:1	[115]
4.01	0.15			4	Ag-TLC/GC/TR	1995	Greece	C18:1	[115]
4.14	1.30			12	Ag-TLC/GC/TR	1995	Italy	C18:1	[115]
5.91	0.92			22	Ag-TLC/GC/TR	1995	Ireland	C18:1	[115]
3.51				1	Ag-TLC/GC/TR	1995	Luxembourg	C18:1	[115]
4.09	0.91			24	Ag-TLC/GC/TR	1995	Netherlands	C18:1	[115]
4.78	0.91			23	Ag-TLC/GC/TR	1995	United Kingdom	C18:1	[115]

* wt% of methyl esters
** Direct GC without TLC; values probably too small
*** Derived from triglyceride formulae, which were based on Ag-TLC/GC analyses

Table 2
trans-Octadecenoic acid isomers [wt% of fatty acids] in 1756 German milk fats based on very different conditions of feeding and lactation (mean: mean value, median: median value, sd: standard deviation, min: minimal content, max: maximal content) [115]

trans Position	mean	sd	min	max	median
Δ4	0.05	0.007	0.02	0.08	0.05
Δ5	0.05	0.008	0.00	0.11	0.05
Δ6–8	0.16	0.040	0.07	0.27	0.15
Δ9	0.23	0.026	0.16	0.30	0.23
Δ10	0.17*	0.031	0.03	0.30	0.17
Δ11	1.72*	0.976	0.35	4.43	1.42
Δ12	0.21	0.031	0.10	0.31	0.21
Δ13/14	0.49	0.088	0.00	0.85	0.48
Δ15	0.28	0.081	0.04	0.48	0.27
Δ16	0.33	0.060	0.11	0.52	0.33

* Based upon 1707 milk fats

Table 3
Positional isomers of *trans*-octadecenoic acid [wt%] in milk fats from different EU-countries (mean: mean value, sd: standard deviation) [115]

	B	DK		E		F		GR		I		IR		LUX	NL		UK	
n =	1	4		10		10		4		12		22		1	24		23	
	mean	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	mean	sd	mean	sd
Δ4	0.04	0.05	0.01	0.04	0.00	0.06	0.01	0.05	0.00	0.05	0.01	0.06	0.00	0.06	0.05	0.01	0.06	0.01
Δ5	0.04	0.04	0.00	0.03	0.00	0.04	0.01	0.04	0.00	0.04	0.01	0.05	0.01	0.05	0.05	0.01	0.04	0.01
Δ6−8	0.16	0.19	0.02	0.20	0.01	0.20	0.03	0.20	0.00	0.20	0.03	0.23	0.02	0.16	0.17	0.03	0.21	0.03
Δ9	0.22	0.25	0.02	0.25	0.01	0.24	0.01	0.24	0.00	0.24	0.02	0.26	0.01	0.23	0.25	0.02	0.25	0.01
Δ10	0.17	0.17	0.01	0.20	0.01	0.19	0.02	0.19	0.02	0.21	0.04	0.16	0.02	0.16	0.17	0.03	0.18	0.02
Δ11	1.47	2.00	0.44	2.11	0.20	2.36	0.75	1.91	0.09	2.10	0.90	3.54	0.80	1.48	1.98	0.82	2.51	0.79
Δ12	0.18	0.24	0.02	0.23	0.01	0.24	0.02	0.24	0.01	0.23	0.04	0.25	0.02	0.21	0.24	0.02	0.25	0.02
Δ13/14	0.38	0.57	0.07	0.56	0.04	0.54	0.04	0.55	0.03	0.52	0.12	0.58	0.05	0.49	0.57	0.06	0.61	0.05
Δ15	0.23	0.31	0.04	0.31	0.03	0.33	0.05	0.32	0.01	0.29	0.10	0.44	0.05	0.30	0.31	0.06	0.37	0.05
Δ16	0.29	0.37	0.01	0.35	0.01	0.38	0.04	0.34	0.01	0.34	0.07	0.43	0.03	0.32	0.39	0.03	0.40	0.04

Table 4
Distribution of positional isomers of *trans*-octadecenoic acid in milk fats [% of C18:1-TFA] with specification of mean values (mean), standard deviations (sd), minimal and maximal values (min, max) and number of samples (n)

PRECHT, MOLKENTIN 1995 [115]					LUND, JENSEN 1983 [111]	WOLFF 1994 [106]	WOLFF 1994 [106]	PARODI 1976 [125]
trans Position	mean $n = 1765$	sd	min	max	mean $n = 5$	$\text{mean}_{\text{spring}}$ $n = 12$	$\text{mean}_{\text{autumn}}$ $n = 12$	mean $n = 18$
Δ4	1.6	0.6	0.5	4.9				
Δ5	1.5	0.6	0.0	5.2				0.3
Δ6–8	4.7	0.6	3.0	8.4				1.8
Δ9	6.9	1.6	3.8	12.7		7.2	9.6	8.8
Δ10	4.7	1.2	0.4	44.4				5.5
Δ11	43.2	14.3	14.0	71.7		58.2	50.4	60.5
Δ12	6.3	1.4	3.5	12.5	72.4	6.2	7.2	4.1
Δ13/14	14.2	3.1	0.0	28.1	15.3	15.4	17.2	9.6
Δ15	7.9	1.2	1.7	15.9	5.4	5.7	6.6	3.9
Δ16	9.8	2.0	5.3	17.3	7.0	7.3	9.0	5.5

of PARODI [125], who used reductive ozonolysis and GC after fractionation by Ag-TLC. The studies demonstrate that elaidic acid (*trans* Δ9), in contrast to vaccenic acid (*trans* Δ11), is present in relative small amounts, with a vaccenic/elaidic acid ratio of approx. 7:1 (16:1–2:1) [125].

Compared to earlier data on Australian butter fats from PARODI [125], Table 4 shows lower contents of vaccenic acid (43.2 as against 60.5%) for recent measurements of 1756 milk fats by PRECHT et al. [115], while for *trans* Δ4, Δ5 and Δ6–8 higher values have been found.

PARODI [125] stated that fat from perirenal and subcutaneous adipose tissue of a dairy cow had a distribution of *trans* positional isomers similar to that of butter samples.

Fig. 3 shows frequency distributions of the total content of C18:1-TFA as well as of every individual positional isomer in milk fats [115]. In these studies distinctly higher TFA contents were found during pasture feeding. The same effect, expressed by two relative maxima, is to be seen with *trans* Δ11 and *trans* Δ15. The left relative maximum is attributable to milk fats from barn feeding, and the right is caused by milk fats from pasture feeding.

In recent studies [107, 110] the seasonal variations of TFA contents in milk fats from a large milk collection area were determined, i.e. butter samples from bulk milk of always the same herds were analysed weekly.

Fig. 4 shows the values obtained weekly for oleic acid and *trans*-octadecenoic acids. The contents of TFA and oleic acid apparently have a similar course. Compared to pasture feeding during the summer the TFA contents in winter are half as high. Its variation range over the year was 1.97–4.37 wt% with a mean value of 3.18 wt% [107]. When analysing 12 autumn and 12 spring samples of butter from France by Ag-TLC/GC values of 3.22 wt% and 4.28 wt%, respectively, were found [106]. New studies by WOLFF et al. [133] with French butter samples exhibited the highest levels of TFA in May–June (4.3 wt%) and the lowest in January–March (2.4 wt%).

RENNER et al. [134] reported that the content of total C18:2-isomers in milk fat may be four times as high in summer than in winter.

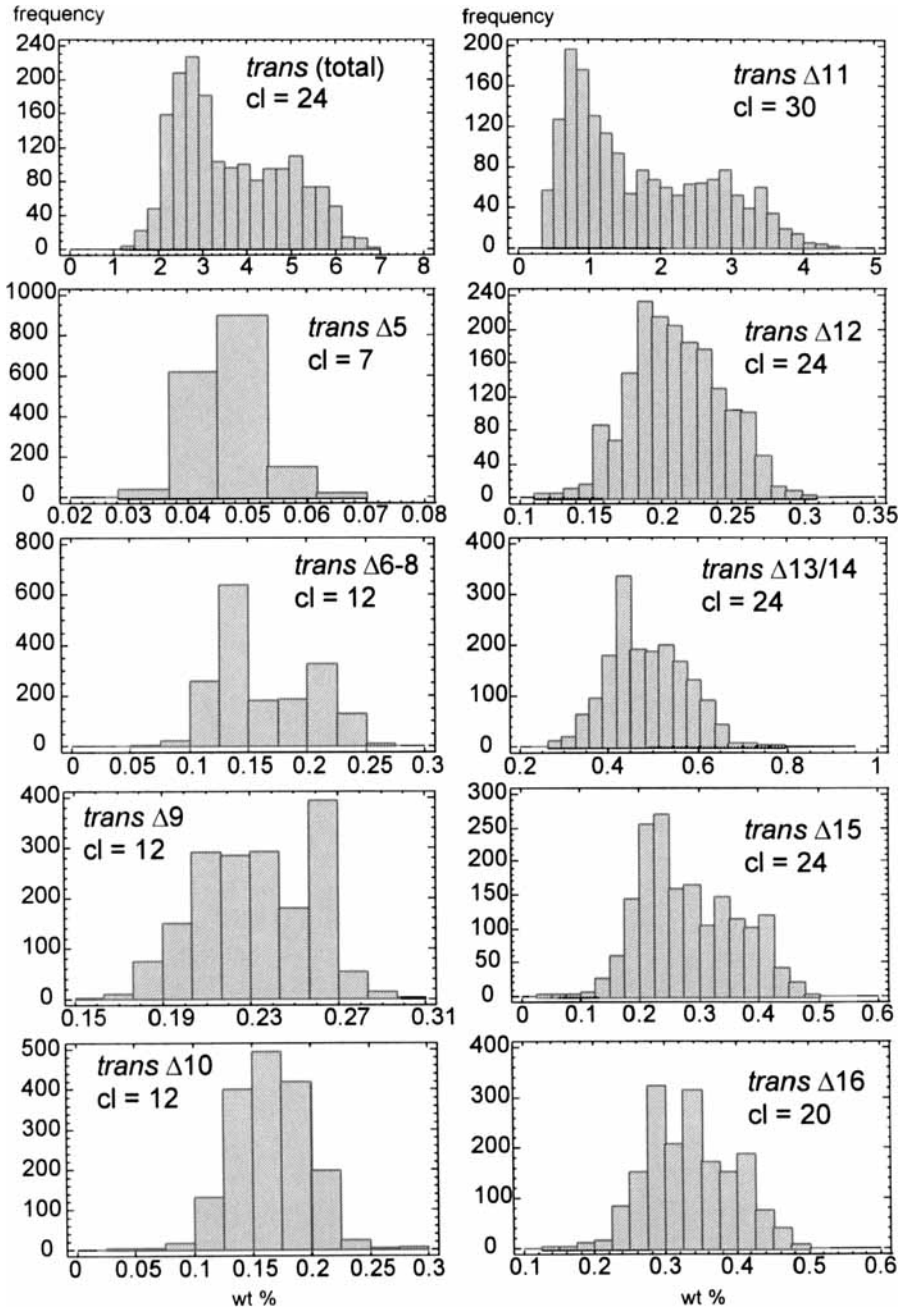

Fig. 3
Frequency distributions of total *trans*-C18:1 contents as well as of the individual *trans* positional isomers of C18:1 (wt% FA) in 1756 different German milk fats (cl: number of classes)

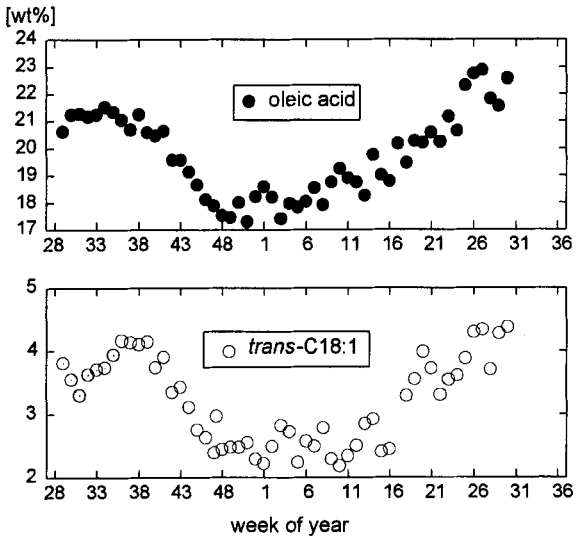

Fig. 4
Weekly variation of the contents of oleic acid and the total contents of *trans*-C18:1 acids in a large German milk collection area [107]

Due to the high intake of polyunsaturated fatty acids during pasture feeding and their partially hydrogenation in the rumen the content of isomers of oleic and linoleic acid in milk fat is especially high under these conditions. Thus, the above introduced studies as well show that only a few analyses of a milk product, such as butter, cheese, cream, etc., may lead to wrong conclusions as regards the mean TFA content, since due to influences of feeding and lactation the milk fat in these products is varying strongly by season [135].

In further experiments with underfed cows particular low TFA contents were found in the milk fat [136]. At the top of lactation high-output cows normally have a negative energy

Table 5
Further *trans* fatty acids in milk fats ([wt% of fatty acids], mean: mean value)

Fatty acid	mean		n	Analytics	Year	Country	Ref.
C15:1	0.03		1	Ag-TLC/GC	1983	Denmark	[111]
C16:1	0.18		1	Ag-TLC/GC	1983	Denmark	[111]
C16:1*	0.60	0.5–0.8	10	GC	1993	Germany	[97]
C16:1	0.08	0.04–0.13	12 (autumn)	Ag-TLC/GC	1994	France	[106]
C16:1	0.13	0.10–0.16	12 (spring)	Ag-TLC/GC	1994	France	[106]
C18:2**	0.5	0.1–2.2	10	GC	1982	Germany	[134]
C18:2**	0.53	0.3–0.8	10	GC	1993	Germany	[97]
*t*Δ11, *c*Δ15-C18:2***	0.29	0.11–0.60	12	Ag-TLC/GC	1992	Austria	[105]
*c*Δ9, *t*Δ13-C18:2***	0.22	0.16–0.28	12	Ag-TLC/GC	1992	Austria	[105]
trans, *trans* C18:2***	0.59	0.27–0.90	31	Ag-TLC/GC	1994	Austria	[122]
cΔ9, tΔ11-C18:2***	0.85	0.33–1.40	31	Ag-TLC/GC	1994	Austria	[122]

* Measured in 5 milk and 5 butter samples
** Total content of *trans* isomers of C18:2
*** [g TFA/100 g milk fat]

balance, which is due to the limited ability of food intake. The lower TFA content related to an energy deficit is attributable to the mobilization of C18 acids from adipose tissues. These are not hydrogenated as fatty acids taken up with the fodder. Furthermore, stearic acid is converted to oleic acid by means of desaturase without production of isomers.

Besides positional isomers of octadecenoic acid, milk fats contain several other *trans* fatty acids, which altogether hardly comprise more than 2–3%, however. In Table 5 mean contents of these minor *trans* fatty acids are summarized.

In a butter fat sample STROCCHI et al. [137] found 0.3% *trans*-C16 : 1, 0.1% of the *trans* isomers of C17 : 1, C19 : 1, C20 : 1, C23 : 1, respectively, as well as traces of TFA of C21 : 1, C22 : 1 and C24 : 1 by GC/MS.

The results concerning the contents of *trans*-C18 : 1 fatty acids in milk fat allow an estimation of TFA intake by milk products for the different countries to be made. Here, the total intake of TFA will not differ essentially from the intake of the sum of all positional isomers of *trans*-octadecenoic acid. The mean intake rates of TFA per day and person listed in Table 6 are related to newer studies of WOLFF [16] as well as of PRECHT et al. [115].

The values calculated by WOLFF [106] are based on the assumption that the mean *trans*-C18 : 1 content of 3.8% evaluated from 24 French milk fats can be applied to the other European countries too. Therefore, an exact determination of the average *trans*-C18 : 1 content could lead to small changes in the daily intake of C18 : 1-TFA by milk products. So, according to Table 3 the *trans*-C18 : 1 content in Irish milk products will surely be higher. Due to the pasture feeding prevailing in Ireland the mean milk fat composition is more likely to be similar to a milk fat e.g. from a typical German summer feeding.

Up to now, only few knowledge about the intake of single TFA isomers is available. However, in recent studies an estimation of the intake of individual *trans* positional isomers of octadecenoic acid by milk products could be made. For these positional isomers Δ4, Δ5, Δ6–8, Δ9, Δ10, Δ11, Δ12, Δ13/14, Δ15 and Δ16 values of 18.2, 18.2, 58.2, 83.7, 61.8, 625.8, 76.4, 178.3, 101.9 and 120.1 mg/day and person were calculated. The data [115] are based upon at least 1707 different milk fats as well as the 'Statistisches Jahrbuch über Ernährung, Landwirtschaft und Forsten (1994)' [138]. Such informations are important with respect to comparative studies on hydrogenated vegetable fats and milk fats, since

Table 6
Estimated intake of *trans*-octadecenoic acids from milk products for some countries [g/day and person]

Country	[g *trans*-C18 : 1/day]	Analytics	Ref.
France	1.46	Ag-TLC/GC	[106]
Germany	1.37	Ag-TLC/GC	[106]
Germany	1.32	Ag-TLC/GC	[115]
Italy	1.08	Ag-TLC/GC	[106]
Netherlands	1.14	Ag-TLC/GC	[106]
Belgium/Luxembourg	1.35	Ag-TLC/GC	[106]
United Kingdom	1.13	Ag-TLC/GC	[106]
Ireland	1.34	Ag-TLC/GC	[106]
Denmark	1.66	Ag-TLC/GC	[106]
Greece	0.71	Ag-TLC/GC	[106]
Spain	0.61	Ag-TLC/GC	[106]
Portugal	0.57	Ag-TLC/GC	[106]
EU (12 countries)	1.16	Ag-TLC/GC	[106]

certain *trans* isomers such as elaidic acid, which in contrast to animal fats is occuring increased in hydrogenated vegetable fats, possibly cause particularly negative effects [21]. Of further interest are informations about the TFA intake, that are only related to the consumption of butter or margarine. Thus, in Germany the average daily intake of elaidic and vaccenic acid by butter amounts to 28 resp. 208 mg per person and day, whereas for margarine the values are 285 resp. 195 mg [139]. Cooking fats and hydrogenated fats in bread, cakes and pastries are not included in this calculation.

Human tissues and milk fat

The content of *trans* fatty acids in human tissues as well as the transfer to mother's milk are related to the intake of TFA with food. LEICHSENRING et al. [140] examined the TFA content in subcutaneous fat of 47 German adults by gas chromatography. These investigations resulted in an average of 3.15% TFA with a wide range of variation of 1.29% – 7.03%. The major *trans* fatty acid was elaidic acid. The TFA content was similar to that found in Dutch females [141, 142], but lower than in US male probands [118]. In comparison, HECKERS et al. [143] analysed various depot fats of 16 men by means of gas chromatography and found a mean of 2% *trans*-octadecenoic acids, with a minimal value of 1.03%

Table 7
TFA-contents in fat from human milk ([wt% of fatty acids], mean: mean value, min: minimal content, max: maximal content)

mean	min	max	n	Analytics	Year	Country	Component	Ref.
	2.1	4.0	3	GC	1977	USA	C18:1	[146]
4.48			11	GC	1980	USA	C18:1	[147]
3.38			10	GC	1983	USA	C18:1	[148]
	2.1	4.1	14	GC	1985	Canada	C18:1	[149]
	1	4	5	IR	1961	Germany	total	[150]
2.94			15	GC	1987	Germany	C18:1	[3]
0.21			15	GC	1987	Germany	C14:1	[3]
0.56			15	GC	1987	Germany	C16:1	[3]
0.15			15	GC	1987	Germany	C18:2*tt**	[3]
0.15			15	GC	1987	Germany	C18:2*ct***	[3]
0.07			15	GC	1987	Germany	C18:2*tc***	[3]
0.48			15	GC	1987	Sudan	C18:1	[3]
0.08			15	GC	1987	Sudan	C14:1	[3]
0.10			15	GC	1987	Sudan	C16:1	[3]
0.04			15	GC	1987	Sudan	C18:2*tt***	[3]
0.05			15	GC	1987	Sudan	C18:2*ct***	[3]
1.99	1.20	3.17	10	Ag-TLC/GC	1995	France	C18:1	[151]
4.5			1	Ag-TLC/GC	1995	Germany	C18:1	[152]
4.14	3.18	5.43	6	Ag-TLC/GC	1977	USA	total***	[153]
3.56	2.68	4.31	5	Ag-TLC/GC	1977	USA	total****	[153]
3.1	2.1	4.0	3	GC	1977	USA	C18:1	[146]
4.76			8	GC	1984	USA	C18:1	[154]

* Linolelaidic acid (C18:2n-6 *trans, trans*)
** Conjugated dienes (C18:2n-6, *trans, cis* and C18:2n-6, *cis, trans*)
*** Morning milk
**** Morning and evening milk

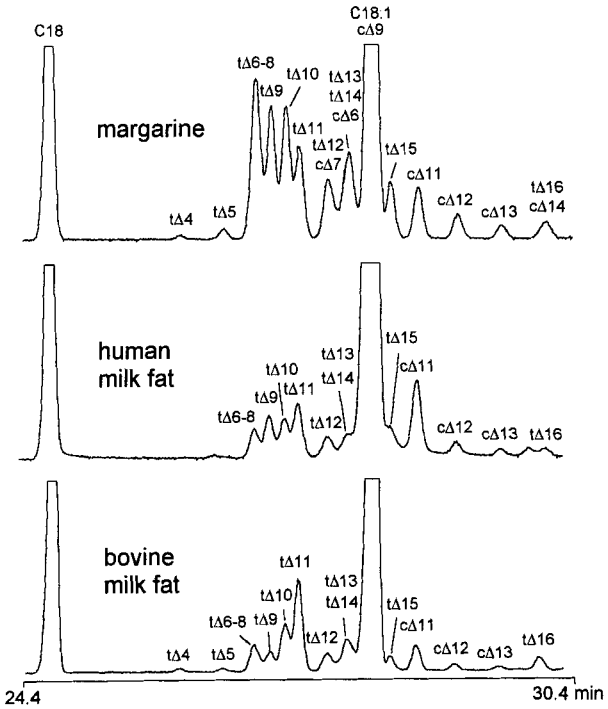

Fig. 5
Comparison of *trans*-octadecenoic acid profiles of margarine, human milk fat and bovine milk fat [152]

(subepicardial) and a maximum of 4.3% (mesenterial). However, these results are likely to be too low due to the applied analytics. *Trans* isomers of linoleic acid have not been found in human tissues yet [144, 145].

HUDGINS et al. [118] assume that there is no specific accumulation of TFA in the subcutaneous fat. Therefore, the analysed contents of TFA directly reflect the dietary preferences of the probands. Since in all studies only the total content of TFA has been measured, it can not be excluded that there is a preferential accumulation of certain isomers.

Up to now, only few data about the TFA content in human milk fat have been published. The values listed in Table 7 are in more or less good agreement with the TFA contents of bovine milk fat shown in Table 1. Moreover, the TFA contents of human milk fat as well as of bovine milk fat exhibit a similar level as TFA contents in adipose tissues.

Fig. 5 shows partial chromatograms of human milk fat in comparison to margarine and bovine milk fat in the range of C18 : 0 to C18 : 2. The content of *trans*-Δ9-Δ18 : 1 (elaidic acid) in human milk fat appears to be remarkably higher than in bovine milk fat. In this way, the pattern of positional isomers looks more like the composition of margarine and reflects its consumption by an overlay of the isomeric pattern of hydrogenated vegetable fats to the usual milk pattern. Thus, the TFA content in human milk fat should be affected by a change in the diet.

Extensive studies on the TFA content in milk fat and plasma lipids of nursing mothers and their infants were performed by KOLETZKO [23]. The average content of TFA in plasma of new-born infants (n = 47) amounting to 1.50 ± 0.11% was lower than of their mothers (2.00 ± 0.11%). There was no difference between smoking and nonsmoking mothers. However, with breast-fed infants (n = 11, TFA of mother's milk = 4.1%) a distinct

increase of plasma TFA levels between the 4th and 21st day post partum from 1.18 to 1.90% was found. Thus, the increase was higher than with the reference group (n = 10) fed with adapted milk (TFA = 1.9%).

Margarines, shortenings, cooking fats, dietary/reformatory fats and plant oils

The major TFA in edible fats of vegetable origin are *trans*-octadecenoic acids (Table 8) as well as the geometrical isomers of linoleic acid (Table 9). The origin of these is attributable to partial hydrogenation of native oils. The total content of TFA depends on the degree of hardening as well as on the amount of polyunsaturated fatty acids in the raw materials. Thus, cooking fats as a rule contain more TFA than margarines [97, 131, 155–157], among which soft-margarines exhibit lower contents than hard-margarines [158].

Table 8
trans-monoenoic acids in margarines, shortenings, cooking and dietary fats from vegetable oils ([wt% of fatty acids], mean: mean values, min: minimal content, max: maximal content, M: margarine, S: shortening, C: cooking fat, D: dietary fat, Sf: sunflower margarine)

mean	min	max	n/kind	Analytics	Year	Country	TFA	Ref.
7.9	1.0	18.8	20 M	IR	1963	France	total	[160]
24.1	0.0	36.0	10 M	IR	1973	USA	total	[161]
16.8	10.8	22.1	47 M	IR	1976	Australia	total	[162]
18.5	9.9	28.7	30 M	IR*	1977	USA	total	[163]
18.0	6.9	31.4	12 M	GC	1977	USA	C18:1	[164]
20.4	6.3	33.6	7 M	Ag-TLC/GC	1977	USA	C18:1	[165]
16.2	12.9	20.9	5 M	Ag-TLC/GC	1978	USA	C18:1	[38]
8.1	0.1	34.7	83 M, D	GC	1978	Germany	C18:1	[155]
8.6	0.1	53.2	18 S, Br, D					[155]
30.3	12.0	64.8	8 M	IR	1979	Canada	total	[166]
21.3	8.7	32.9	82 M	GC	1979	Canada	total	[167]
14.2	10.0	20.7	8 M	Ag-TLC/GC	1981	Sweden	C18:1	[130]
20.8	9.4	39.3	47 M	O_3/GC	1982	Canada	C18:1	[172]
18.4	6.8	31.0	40 M	Ag-TLC/GC	1983	USA	C18:1	[131]
21.7	8.7	35.4	7 S					[131]
24.2			24 M	IR*	1984	Netherlands	total	[156]
42.1			7 S, C					[156]
25.2	13.4	34.2	5 M	GC	1984	Israel	total	[168]
22.2	4.1	42.3	18 M	IR	1984	UK	total	[169]
19.89	10.74	30.06	84 M	GC**	1985	USA	C18:1	[170]
7.3	0.7	17.7	30 M	GC	1985	Denmark	C18:1	[171]
22.93	10.82	44.42	11 M, S	Ag-TLC/GC	1992	Austria	C18:1	[120]
10.2	3.50	23.0	12 M	GC***	1993	Germany	C18:1	[97]
10.8	0.1	30.2	5 S, C					[97]
1.1	0.1	3.5	4 D					[97]
9.32	0.17	25.90	46 M	Ag-TLC/GC	1995	Germany	C18:1	[157]
9.79	0.04	32.51	16 S, C					[157]
0.65	0.03	2.94	31 D					[157]
20.71	12.93	25.90	11 Sf					[157]

* [wt% of product]
** [wt% of triglycerides]
*** [wt% of FAME]

Furthermore, the TFA contents in margarines from sunflower oil generally are higher than in any other margarine (Table 8 bottom) [157], which results from the fact that the exclusively underlying sunflower oil is very rich in unsaturated fatty acids, in particular linoleic acid. Studies on the relations between TFA amounts produced during hydrogenation and the degree of hydrogenation as well as other process variables were performed by PURI [159].

For American [131, 163, 170] and Canadian [167, 172] margarines representative studies with higher numbers of samples led to mean values between 18.4 and 21.3 wt% TFA, while European studies [97, 130, 155–157, 169, 171] exhibited a variation range of mean values from 7.3 to 24.4 wt% with most values being clearly lower than the American, however. In an extensive investigation from 1982 by RENNER et al. [132] comprising 66 margarines and 24 cooking fats data about the average content of elaidic acid (3.7 resp. 2.5%) as well as of the remaining *trans*-C18 : 1-isomers (5.6 resp. 6.3%) are given, but these values do not allow to draw conclusions as regards the mean content of total *trans*-C18 : 1. Moreover, it should be mentioned that, due to the applied GC column (FFAP), the peak identified as elaidic acid (*trans* Δ9) [113] probably contains further positional isomers such as *trans* Δ10 and Δ11.

The *trans* isomers of linoleic acid (Table 9) occuring besides *trans*-octadecenoic acids are to be found in American margarines to approx. 1.9% [131, 170] and in German margarines to approx. 0.6% [97, 134] on average. The major isomers of these are *t*Δ9–*c*Δ12 and *c*Δ9–*t*Δ12 [170]. In various French dietary margarines WOLFF et al. [145] additionally analysed up to 1% of the *trans*-linolenic acid isomers *c*Δ9–*c*Δ12–*t*Δ15, *c*Δ9–*t*Δ12–*c*Δ15 and *t*Δ9–*c*Δ12–*c*Δ15, with the major isomers having always the configuration *c*, *c*, *t* and *t*, *c*, *c*. In margarine TFA of other chain length (C14 : 1, C16 : 1) are only occuring in traces [38, 97, 171]. Thus, the difference between the total TFA content and the *trans*-C18 : 1 content is relatively small, what approximately allows a comparison between corresponding studies to be made.

The contents of *trans*-octadecenoic acids in German margarines and shortenings/cooking fats published in 1978 in the study of HECKERS et al. [155] exhibit especially low minimal values of 0.1%, since here dietary fats, which are not allowed to contain any hydrogenated

Table 9
Mean contents and variation ranges of octadecadienoic acids in margarines, shortenings, cooking and dietary fats ([wt% of fatty acids], M = margarine, S = shortenings, C = cooking fat, D = dietary fat)

tc, *ct*, *tt*	*cc*	*n*/kind	Analytics	Year	Country	Ref.
3.2 (0.7–8.6)	28.2 (9.0–44.0)	12 M	GC	1977	USA	[164]
1.6 (0.4–2.5)	31.5 (22.6–41.4)	5 M	Ag-TLC/GC	1978	USA	[38]
0.7 (0–5.1)		66 M	GC	1982	Germany	[134]
0.9 (0–9.9)		24 S				[134]
1.94 (0–5.2)	29.4 (8.2–48.4)	40 M	Ag-TLC/GC	1983	USA	[131]
1.92 (0.17–11.56)	27.14 (6.06–46.39)	84 M	GC*	1985	USA	[170]
0.5 (0.1–1.7)	23.9 (13.3–33.1)	12 M	GC**	1993	Germany	[97]
1.9 (0.0–7.0)	7.3 (1.5–14.2)	5 S, C				[97]
0.5 (0.1–0.7)	41.6 (27.5–58.8)	4 D				[97]

* [wt% of triglycerides]
** [wt% of FAME]

vegetable fats at all, were included as well. Futhermore, the resulting mean values of approx. 8%, which appear to be rather low for that time, are attributable to the used GC method without application of a pre-fractionation by Ag-TLC [120, 164]. The results of current studies about C18:1-TFA contents in a representative choice of diverse German edible fats [157], that were obtained by the combination of Ag-TLC with GC, are to be seen at the bottom of Table 8. Excluding dietary fats, mean TFA contents of 9.32% and 9.79% for margarines (n = 46) resp. shortenings/cooking fats (n = 16) were found. The low minimal values analysed here as well as the great number of dietary/reformatory fats show that the public discussion on TFA has prompted several manufacturers to produce fats low in TFA. However, even dietary and reformatory fats (n = 31) are not completely free from TFA. Their mean content of 0.65% could be attributable to heat treatment during the production process.

Moreover, by combination of Ag-TLC with GC using a 100 m-column [108, 117] a gas chromatographical separation into 10 single peaks of *trans*-octadecenoic acids could be achieved for the first time [139]. In the meantime, comparable results have been achieved by WOLFF et al. [173], too. In former studies a resolution of 6–7 peaks never has been exceeded [106, 124, 170]. Therefore, in earlier papers elaidic acid has frequently been regarded as the most important isomer of *trans*-C18:1 in hydrogenated vegetable fats [7] and several nutritional studies were primarily based in that acid [e.g. 20]. From Table 10 [139] one can see that besides elaidic acid margarines and cooking fats also contain similar amounts of *trans* Δ6–8 (as sum), *trans* Δ10 and *trans* Δ11. Comparable relations were found in 1976 for Australian margarines by PARODI [162], who used a tedious ozonolysis method, and later in the same way by MARCHAND [172] for Canadian and SAMPUGNA et al. [174] for American margarines.

Fig. 6 illustrates the content of *trans*-C18:1 fatty acids as well as their isomeric distribution in different fats. This isomeric pattern present in all hydrogenated plant fats in almost identical distribution but different quantity clearly differs from bovine milk fat, which

Table 10
Isomeric distribution of *trans*-octadecenoic acids in diverse edible fats ([wt% of fatty acids], mean: mean value, sd: standard deviation, min: minimal content, max: maximal content) [139]

	Margarines (n = 46)				Shortenings/cooking fats (n = 16)				Dietary/reformatory fats* (n = 31)			
	mean	sd	min	max	mean	sd	min	max	mean	sd	min	max
tr. C18:1 (total)	9.32	7.35	0.17	25.90	9.79	8.51	0.04	32.51	0.65	0.65	0.03	2.94
Δ4	0.03	0.03	0.00	0.11	0.03	0.03	0.00	0.12	0.02	0.02	0.00	0.09
Δ5	0.06	0.05	0.00	0.16	0.08	0.08	0.00	0.29	0.02	0.02	0.00	0.06
Δ6–8	1.63	1.13	0.02	3.90	1.54	1.41	0.00	5.32	0.09	0.08	0.00	0.34
Δ9	2.04	1.43	0.05	4.84	2.28	1.73	0.01	6.52	0.12	0.11	0.01	0.43
Δ10	1.93	1.57	0.04	5.30	1.98	1.86	0.01	6.74	0.12	0.10	0.01	0.37
Δ11	1.38	1.32	0.03	4.50	1.45	1.46	0.01	5.19	0.08	0.07	0.00	0.26
Δ12	1.12	1.08	0.02	4.08	1.19	1.04	0.00	4.06	0.08	0.12	0.00	0.63
Δ13/14	0.92	0.86	0.01	3.56	0.97	0.84	0.00	3.25	0.09	0.15	0.00	0.81
Δ15	0.13	0.10	0.00	0.45	0.18	0.17	0.00	0.67	0.01	0.02	0.00	0.07
Δ16	0.09	0.09	0.00	0.53	0.10	0.09	0.00	0.35	0.02	0.03	0.00	0.11

* Term restricted to margarines of special composition in Germany

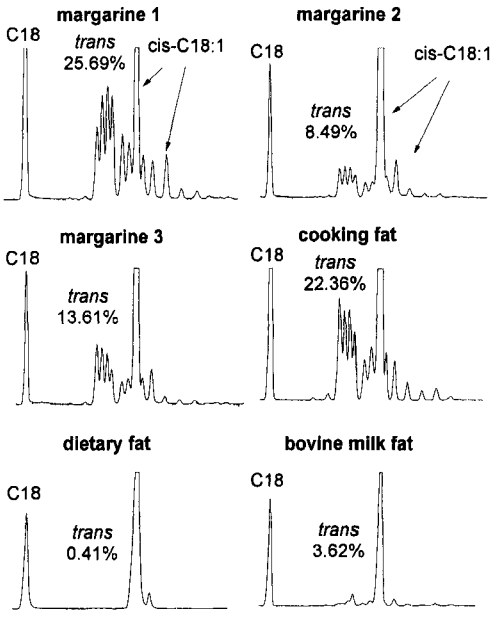

Fig. 6
Comparison of octadecenoic acids in different edible fats [107]

besides high amounts of Δ10 and Δ11 only contains small amounts of Δ6 – Δ9 (see Fig. 5) [108, 110].

Native plant oils do not contain any TFA. However, processing and refining can lead to low TFA contents [97, 169]. Occasionally, edible oils are hydrogenated slightly, e.g. to improve the oxidative stability by reduction of the linolenic acid content (C18 : 3), what may result in increased TFA contents of up to 13.4% [131, 175]. However, according to HUNTER et al. [43] the TFA content in such hydrogenated oils is declining: from 1963 to 1984 the average content changed from 15 to 8%. A survey is given in Table 11. It should be emphasized that the content of *trans* isomers of C18 : 2 (C18 : 2i) in these oils may be relatively high compared to *trans*-C18 : 1. Thus, a sample analysed by ENIG [131] contained 5.6% C18 : 2i besides 7.0% *trans*-C18 : 1.

Table 11
Mean contents and variation ranges of *trans* fatty acids in edible oils ([wt% of fatty acids], n_{TFA} = samples with detectable TFA contents)

Total TFA	n	n_{TFA}	Year	Country	Ref.
0	9	0	1976	USA	[175]
9.7 (5.6 – 13.3) hydr.	5	5	1976	USA	[175]
0.05 (0 – 0.4)	14	2	1983	USA	[131]
12.4 (11.0 – 13.4) hydr.	4	4	1983	USA	[131]
0.5 (0.2 – 1.0) raw	11	11	1984	United	[169]
1.6 (0.2 – 6.7) refin.	7	7	1984	Kingdom	[169]
0.25 (0 – 1.5)*	6	1	1993	Germany	[97]

* [wt% of FAME]

Animal fats and fish oils

Animal fats contain relatively few TFA, e.g. the content of *trans*-C18 : 1 in beef tallow amounts to 4.39% [152] or 4.6% [151] and so is comparable to the content in bovine milk fat. In lard even only 0.2 – 0.3% TFA were found [131, 176].

In oils from several species of fish 0 – 1.8% of *trans*-C18 : 1 fatty acids have been detected [145]. Besides isomers of C16 : 1 und C18 : 1 hydrogenated fish oils primarily contain a mixture of geometrical and positional isomers of mono-, di- und triunsaturated C20 – C22 fatty acids [177]. In hydrogenated herring oil OJANPERÄ [178] found 2.0% *trans* ω8-C16 : 1, 2.3% *trans* ω7-16 : 1, 1.8% *trans* ω6-C16 : 1, 1.2% *trans* ω5-C16 : 1, 2.8% *trans* ω10-C18 : 1. 3.1% *trans* ω9-C18 : 1, 2.9% *trans* ω8-C18 : 1, 6.6% *trans* ω9/10-C20 : 1, 2.4% *trans* ω7-C20 : 1 and 6.9% *trans* ω9/10/11-C22 : 1.

Other food

Apart from the fats mentioned above, *trans* fatty acids occur in numerous fat-containing foodstuffs, such as meat and sausages, bread, cakes and pastries, chocolate bars, nut-nougat creams, pommes frites and instant soups and sauces [88, 97, 131]. Especially high TFA contents (related to total fatty acids) are to be found in hydrogenated vegetable fats present in instant soups and sauces (19.8% [97]), nut-nougat creams (6.6 [97] – 7.2% [179]) and in pommes frites (13.1% [180] – 20.8% [97]).

Intake of TFA

A survey on TFA intake rates in different western countries has been given by SCHAAFSMA [181], which in Table 12 was completed by some newer data.

According to HUNTER and APPLEWHITE [43] the TFA content in American cooking fats has been decreasing from 27 to 17% on average between 1960 and 1984. Between 1986

Table 12
Estimated total intake of TFA in some countries [g/day and person] with specification of references and year of publication

Country	TFA	Year	Ref.
Canada	9.6	1982	[185]
Finland	5.6	1983	[186]
Finland	3.0	1991	[187]
Finland	1.5 – 1.9	1991	[188]
Germany	4.5 – 6.4	1979	[143]
Germany	3.4*; 4.1**	1992	[189]
Netherlands	17.4	1986	[190]
Netherlands	10.0	1989	[191]
Sweden	7.0	1991	[192]
United Kingdom	12.0	1983	[193]
USA	8.3	1985	[194]
USA	7.6	1986	[43]
USA	13.3	1990	[88]
USA	8.1	1991	[179]

* Women
** Men

and 1991 the authors [182] did not find changes in the average TFA intake in the USA and they also do not expect a substantial increase in future. The data concerning the amount of this average TFA intake in the USA are controversial. While HUNTER and APPLEWHITE [182] assumed 7.6 g/day (1986) resp. 8.3 g/day (1991) to be realistic, ENIG et al. [88] in 1990 suggested a value of 13.3 g/Tag. Anyway, the TFA intake in the USA is higher than e.g. in Germany, what probably is due to other habits in consumption and the usually higher TFA contents in US margarines [183, 184].

Conclusions

The major difference between the numerous recent studies and many earlier studies is that former experiments mainly were performed with animals (mostly rats), while now several results from human studies are available. The transferability of results from animal studies on men is not free of doubt.

To draw final conclusions with respect to an increased risk of developing atherosclerosis related to high intake of TFA, several long-term studies with human beings still have to be performed. These studies should confirm the assumed alterations in cholesterol levels, in particular also with a lower intake of TFA with the diet. In this connection studies should be of top priority that are based on a substitution of saturated fatty acids by TFA, since the exchange of *cis*-C18 : 1 against TFA carried out in most former studies could have caused the observed effects only because of the reduction of *cis* unsaturated FA. When comparing the few data available up to now, the LDL-cholesterol-raising effect of TFA seems to be similar to that of corresponding amounts of saturated FA. However, very high TFA intake seems to cause a particular unfavourable increase of the LDL/HDL-cholesterol ratio in plasma. Furthermore, regarding TFA the influence of individual positional isomers such as elaidic acid (*trans* Δ9-C18 : 1) or vaccenic acid (*trans* Δ11-C18 : 1) should be examined, what partly is equivalent to a comparison of TFA from ruminant fats (high contents of *trans* Δ11) with TFA from partially hydrogenated fats (in contrast to milk fat high contents of *trans* Δ6–9). On the other hand, the manufacturers should try to achieve the desired texture of margarines, shortenings and cooking fats by increasing the content of C18 : 0 instead of TFA or the saturated FA C12–C16, as it has been proposed by GRUNDY [195] as well. This way, serum cholesterol levels would not be increased. So far, a reduced content of TFA in margarine was nearly always associated with an increase of the saturated FA C12–C16 [139].

There is no doubt that *cis* and *trans* fatty acids can not be considered as equivalent FA as regards nutritional physiology.

Of major importance is the probably negative effect of TFA on lipoprotein(a) levels e.g. compared to saturated FA.

Finally, it can be concluded that numerous research gaps especially with respect to the physiological effects of individual positional isomers of TFA afford further studies.

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