Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA) – a review

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The present review provides an outline of the current knowledge of trans fatty acids (TFA) including their structure and formation, analysis, occurrence in foods, estimation and evaluation of daily intake, contents in human adipose tissues and fluids, and physiological properties. Special emphasis is put on conjugated linoleic acids (CLA), which are related to unique beneficial physiological properties. The effects of CLA on carcinogenesis in *in vivo* and human cancer cell culture studies, on cancer inhibition via the immune system, and further physiological properties are briefly reported. Ways to affect the CLA contents in foods, e. g. influence of feeding regimens or processing conditions, are also discussed.

Analyse, Vorkommen und physiologische Eigenschaften von trans-Fettsäuren mit besonderem Hinblick auf konjugierte Linolsäureisomere. Der vorliegende Übersichtsartikel bietet einen Überblick über den Stand der bisherigen trans-Fettsäureforschung. Es werden die Struktur und Bildung, die Analytik, das Vorkommen in Lebensmitteln, die Beurteilung der täglichen Aufnahme, die Gehalte in humanen Fettgeweben und Körperflüssigkeiten sowie die physiologischen Eigenschaften von trans-Fettsäuren (TFA) vorgestellt. Besonderer Schwerpunkt wurde auf die konjugierten Linolsäuren (CLA) gelegt, da diese außergewöhnlich positive physiologische Wirkungen zeigen. Der Einfluß von CLA auf die Karzinogenese in *In-vivo*-Versuchen und in Studien mit humanen Krebszellkulturen, auf die Krebsinhibierung über das Immunsystem sowie weitere physiologische Eigenschaften werden vorgestellt. Möglichkeiten, die CLA Gehalte in Lebensmitteln zu beeinflussen, z. B. durch Fütterungseinflüsse oder Prozeßbedingungen, werden ebenfalls diskutiert.

1 Introduction

The scope of the present review is to provide comprehensive information on trans fatty acids (TFA). It covers the usual analytical techniques for identifying and quantifying TFA isomers as well as their occurrence in foods and estimations of the dietary intake. Particular attention is paid to the recognized conjugated linoleic acid (CLA) isomers, which exhibited impressive physiological effects. Animal and cell culture experiments, which revealed the anticancerogenic and antiatherosclerotic properties of CLA are extensively reviewed. The influence of food manufacturing and food processing on TFA and CLA contents is also discussed.

2 Structure and Formation of Trans Fatty Acids

In the diet of most industrial nations, more than one quarter of total daily calories are provided by fatty acids, which contain at least one double bond. The usual configuration of these double bonds is the cis configuration, and the C=C double bonds are typically positioned at the 3rd, 6th or 9th carbon atom from the terminal methyl group, e.g. oleic acid (18:1 c9), or linoleic acid (18:2 c9c12). However, some fatty acids have one or more double bonds in the trans configuration (Fig. 1), the so-called trans fatty acids (TFA).

Both, TFA and CLA arise in the first stomach of ruminants as intermediates of the hydrogenation of dietary unsaturated fatty acids during bacterial fermentation. First step in this biohydrogenation is the isomerization of linoleic acid to mainly 18:2 c9t11 catalyzed by the anaerobic bacterium *Butyrivibrio fibrisolvens*. These intermediates are then hydrogenated to form a mixture of mainly trans vaccenic acid and elaidic acid (Fig. 2) [1]. As a result of this process, the fat in butter, cheese, milk, beef and mutton contains approximately 2–8% TFA by weight [2].

TFAs are also formed in varying amounts during the industrial hydrogenation of vegetable or fish oils. The hydrogenation improves oxidative and thermal stability, in particular for the highly polyunsaturated oils, which contain

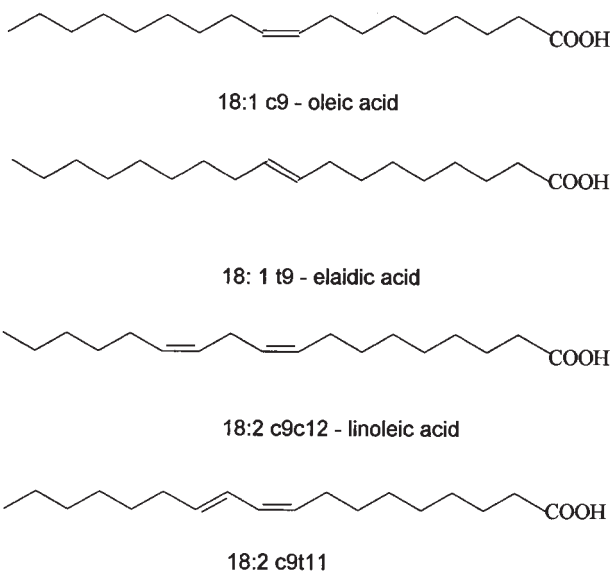

Fig. 1. Structure of oleic acid (18:1 c9), elaidic acid (18:1 t9), linoleic acid (18:2 c9c12), and the main CLA isomer (18:2 c9t11).

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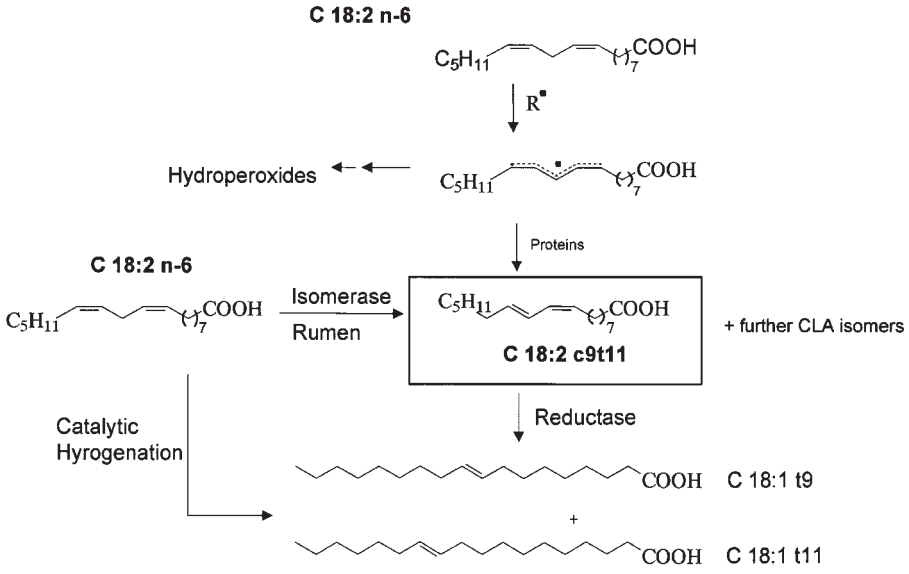

Fig. 2. Formation of trans vaccenic acid, elaidic acid, and CLA.

linolenic acid (18:3 n-3). The extreme case is a fully hydrogenated oil that contains zero percentage TFA. In practice, the TFA content is limited by the thermodynamics of the cis-trans equilibrium to approximately 75% of the total number of double bonds [3, 4].

The predominant 18:1 trans positional isomers in partially hydrogenated vegetable oil form a *Gaussian* distribution that centers around 18:1 t9, 18:1 t10, 18:1 t11, and 18:1 t12 [5]. This isomer distribution is distinct from the pattern for milk fat, which contains trans vaccenic acid (18:1 t11) as the predominant trans isomer (Fig. 3) [6]. In contrast to TFA, CLA are not formed in higher amounts during industrial hydrogenation.

CLA can also be formed through the autoxidation of linoleic acid by free radicals, followed by reprotonation of the pentadienyl radical by proteins, for instance whey proteins (Fig. 2).

Recently, *Chin* et al. [7] investigated the capability of nonruminants (rats) to produce CLA. They supplemented the diet with 5% free linoleic acid or 8.6% corn oil (equivalent to 5% free LA in triglyceride). *Chin* et al. observed higher tissue CLA concentrations in rats fed free LA than in control animals. These investigators concluded that the intestinal bacterial flora of rats is capable of converting free LA (but not LA esterfied in triglyceride) to 18:2 c9t11 and 18:2 t9c11 CLA isomers.

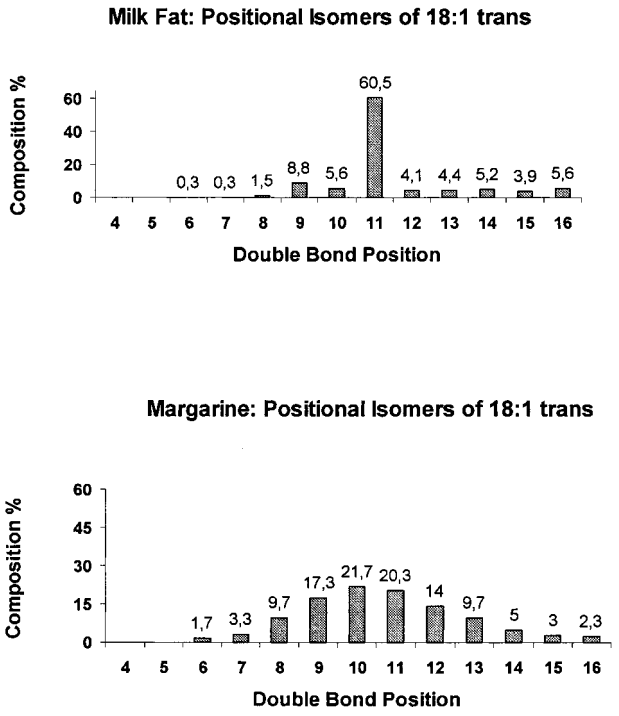

Fig. 3. Trans 18:1 isomer distribution of milk fat and margarine according to [5, 6].

3 Analysis of Trans Fatty Acids

3.1 Infrared spectroscopy (IR)

3.1.1 Principle

Infrared spectroscopy (IR) is the classical method used to determine TFA routinely in foods for the last two decades, as shown in Tab. 1. Isolated trans double bonds show absorption at 966 cm^{-1} (10.3 μm) deriving from the C–H out-of-plane deformation band for trans R_1–HC=CH–R_2 groups accompanied by the CH_3 in-plane rocking band (1121 cm^{-1}) for saturated fatty acid methyl esters (FAMEs). The IR spectrophotometers used are double-beam IR (equipped with reference and sample cell) or *Fourier* Transform IR (FTIR). An IR spectrum (transmission or absorption) is received by scanning from 1050 to 900 cm^{-1}. To obtain true transmission or absorption values the background spectrum is subtracted from the sample spectrum.

Generally IR measurements are carried out using FAMEs. A major problem is that samples analyzed by IR as methyl esters produce trans levels, which are 1.5–3.0% lower for trans values from 1 to 15% [8]. Therefore, correction factors to compensate for the lower absorption of methyl esters were proposed by the Association of Official Analytical Chemists (AOAC Official Method 965.34) [9]. Conjugated trans double bonds also absorb very closely to the isolated trans bond (conjugated trans/trans near 990 cm^{-1} or conjugated cis/trans near 990 and 950 cm^{-1}) and can therefore interfere with the isolated trans measurement [10]. The overlap of the trans absorption by other bands in the spectrum produces a strongly sloping background that converts the trans band into

Tab. 1. Mean trans fatty acid (TFA) and conjugated linoleic acid (CLA) contents of edible oils. Values in brackets are minimum and maximum levels.

food	origin	[reference]	18:1 t [% of total fat]	18:2 c,t/t,c [% of total fat]	total TFA [% of total fat]	CLA [% of total fat]
edible oils						
unrefined oils (n = 8)	Germany	1973/4 [143]			0.1–0.2[b]	
refined oils (n = 7)	UK	1984 [144]			1.6 (0.2–6.7)[b]	
unrefined oils (n = 11)					0.5 (0.2–1.0)[b]	
soybean oil, hydrogenated (n = 3)	USA	1991 [74]				0.3–1.8[a,b]
edible oils (n =6)	Germany	1992 [2]	0.25 (0–1.5)[b]	0.05 (0–0.2)[b]	0.30 (0–1.6)[b]	
coconut oil, olive oil (n = 16)	USA	1992 [75]				0.01–0.07[b]
edible oils, refined (n = 9)	Germany	1997 [69, 70]	< 0.01[b]	< 0.01[b]	< 0.01[b]	< 0.01[b]

[a] Determined by IR.
[b] Determined by GC.

a shoulder at levels below 2% and reduces the accuracy of determination.

Therefore, many procedures have proposed changes to overcome these drawbacks. These include applying arithmetical compensation to eliminate biases, using double-beam differential spectrophotometers to eliminate background interferences [11], applying the internal absorption band rationing procedure [12], eliminating the volatile toxic carbon disulphide solvent by using attenuated total reflection (ATR) [13] or 0.1 mm transmission cells [14, 15], using the advantages offered by FTIR spectroscopy instrumentation [16–18], using regression analysis, using the band height or area [17–20] as the independent variable, modifying the calibration procedure for two-component or multiple-component [13, 14] mixtures that contain a trans reference material instead of calculating the absorptivity of a standard [8, 11], and applying partial least-squares chemometric procedures [15] or post-measurement spectral subtraction manipulations [17].

3.1.2 Attenuated total reflection (ATR)

To eliminate the strongly sloping background of the 966 cm^{-1} trans band, the single-beam (SB) spectrum of the trans containing fat is "ratioed" against that of an unhydrogenated oil or a reference cell containing only cis double bonds. Thus, a symmetric absorption band on a horizontal background is obtained (Fig. 4).

The area under the trans band can then be accurately integrated between the same limits, 990 and 945 cm^{-1}, for all trans levels. To speed up the analysis, an attenuated total reflection (ATR) liquid cell is used, into which oils, melted fats or their methyl esters are poured without weighing or quantitative dilution with carbon disulfide.

The trans levels determined by SB-HATR are closer to those found by gas chromatography (GC) when the hydrogenated fat is measured against an unhydrogenated oil than when it is measured against a cis reference material. Small differences were observable between trans levels in hydrogenated fat test samples and the corresponding methyl ester derivatives (9.3% and 2.2% at about 2% and 41% trans, respectively). The lower limit of identification and quantification is 0.2% and 1%, respectively [22].

3.2 Capillary gas chromatography (GC)

The most important method for investigating fatty acid compositions nowadays is capillary GC. This separation technique requires volatile analytes. Most common is a transesterification to methyl esters, which is the principal chemical reaction performed by lipid analysts [23–25]. The FAMEs are generally detected by a flame ionization detector

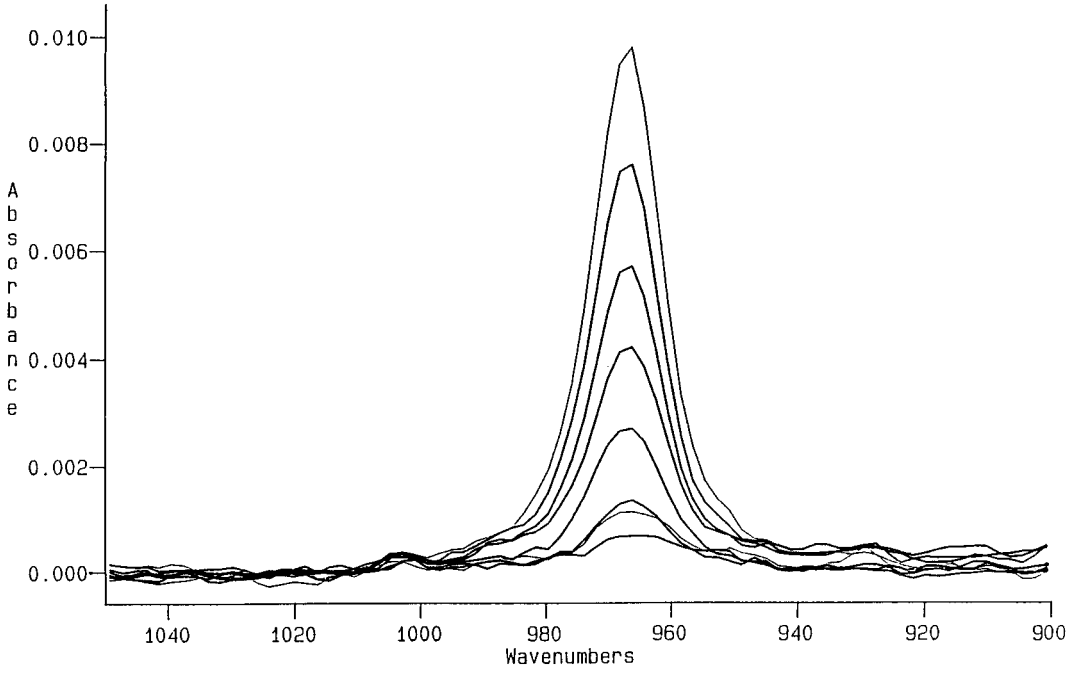

Fig. 4. Single Beam Horizontal Attenuated Total Reflection (SB-HATR)-IR spectra showing the 966 cm^{-1} trans double bond infrared band for different concentrations (0.410, 0.805, 0.998, 2.00, 2.97, 4.12, 5.62, and 7.59% methyl elaidate in methyl oleate used to generate a linear calibration plot) [21].

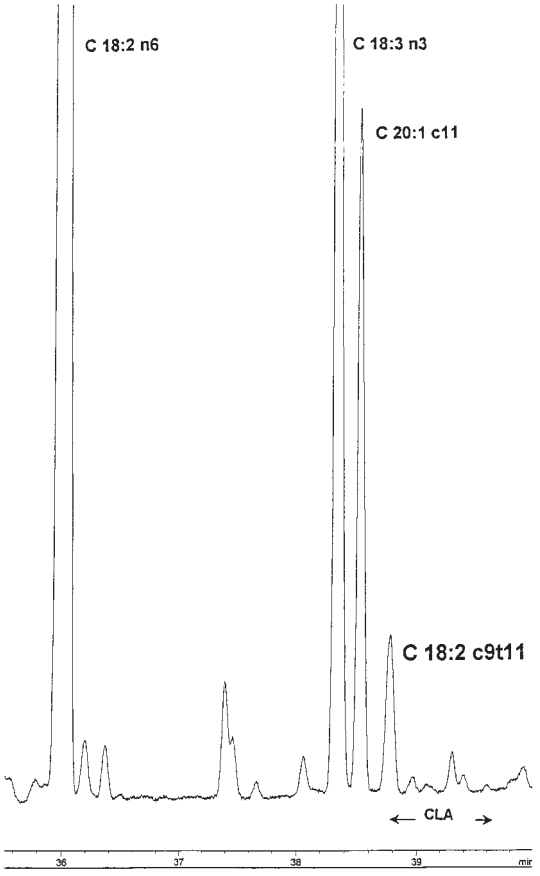

Fig. 5. Capillary GC-FID chromatogram of the CLA region from milk fat recorded as FAMEs on a CP Sil 88 column [70].

(FID). A typical capillary GC-FID chromatogram of the CLA region from a milk fat is shown in Fig. 5.

The current American Oil Chemists' Society (AOCS) Official Method Ce 1c-89 (revised 1990) has been designed to evaluate the general fatty acid composition, including the levels of 18:1 cis and trans isomers, and the total trans unsaturated fatty acid content in hydrogenated and unhydrogenated vegetable oils [26]. For this measurement a direct, one-step capillary GC procedure was developed. The official AOCS method uses a 60 m × 0.25 mm internal diameter fused silica capillary column coated with SP-2340 (biscyanopropylphenylpolysiloxane). This direct GC method without pre-separation by Ag^+ chromatography (thin-layer chromatography, TLC or high-performance liquid chromatography, HPLC) is based on the assumption that 18:1 cis and trans isomers are completely separable on the high polar column. On capillary columns coated with cyanoalkylsiloxane stationary phases (e.g. SP 2560 or CP Sil 88) 18:2 t and 18:3 t are separated as distinct groups without any serious interferences or overlaps (Fig. 6) and levels of these trans polyunsaturates can be obtained directly by GC analysis.

However, because of the multiplicity of positional and geometrical isomers present in partially hydrogenated vegetable oils, a satisfactory separation of 18:1 trans isomers as a group from that of cis isomers is not feasible on cyanoalkylsiloxane phases or any other currently available GC stationary phases [27]. Nevertheless, the separations obtained with the 50-m-column were encouraging, and it seemed possible to improve the separations by increasing the length of the column. Recently, 100-m-capillary columns (SP 2560; CP Sil 88) have been made commercially available, and such columns can improve the resolution of trans-18:1 isomers [28]. For instance, Fig. 7 presents the separation of 18:1 trans positional isomers (Δt8-Δt16) of hydrogenated soybean oil on this extended capillary column [29].

To minimize the underestimation of trans fatty acid levels *Duchateau* et al. [30] developed an optimized method suitable for hydrogenated, as well as for refined, processed oils. The developed method was to comply with the following requirements:

- Maximum resolution between the trans and cis 18:1 isomers, especially the trans-13 18:1 isomer, had to be separated from the cis-9 18:1 isomer;
- 20:1 had to elute with sufficient resolution from linolenic acid isomers, and
- if this was not possible, 20:1 had to elute between the last eluting mono-trans 18:3 isomer (18:3 t9,c12,c15) and 18:3 n-6.

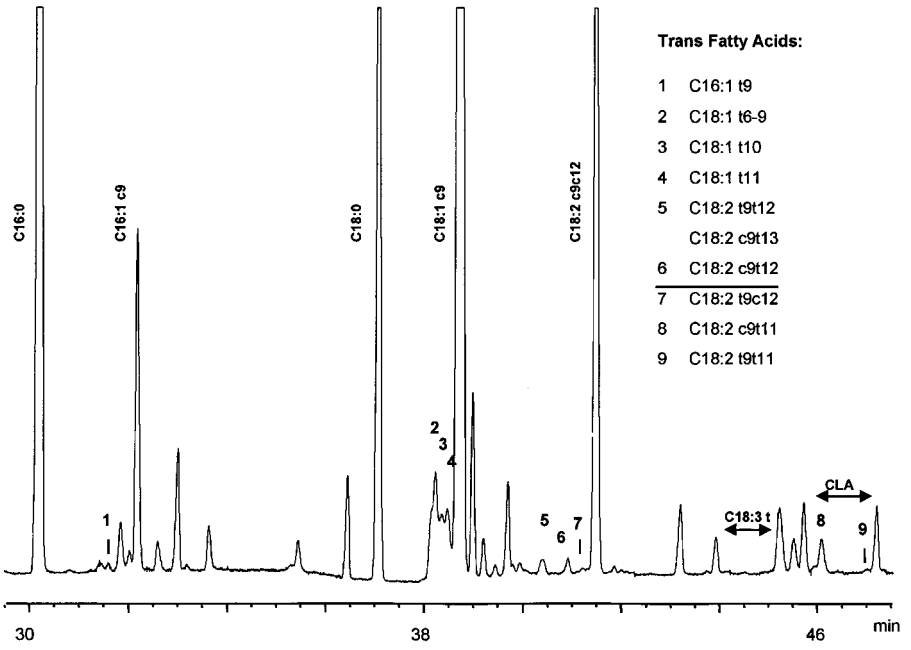

Fig. 6. Capillary gas chromatographic profile for fatty acid methyl esters from human adipose tissue on a 100% coated cyanopropylsiloxane phase [21].

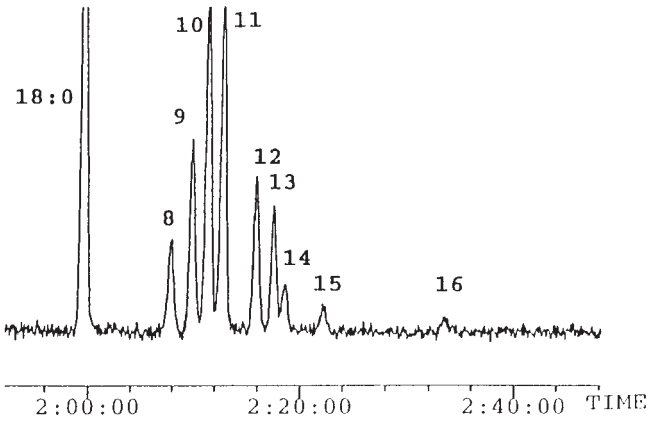

Fig. 7. GC-EIMS chromatogram of 18:1 trans positional isomer DMOX derivatives (Δt8-Δt16) of a complex mixture of partially hydrogenated soybean oil recorded for m/z 113 and m/z 264 on a 100-m-capillary column under 140° C isothermal GC conditions according to *Mossoba* et al. [29] reprinted by permission from AOCS press.

The optimized temperature conditions for CP Sil 88, SP 2340 and BPX 70 GC columns were simulated by computer software. The accuracy of the developed method was checked with cis and trans fatty acid fractions isolated by Ag^+ HPLC.

The trans values obtained with the optimized method were in good agreement with the results obtained for the isolated fractions (deviation from isolated fractions: 0.8%–6.5% (optimized method) in contrast to 21.3%–26.4% (AOCS method) for trans amounts between 12.7% and 43.2% [30]).

3.3 Ag^+ chromatography

Since its introduction by *Morris* in 1963, silver ion (Ag^+) or argentation chromatography has been one of the most important tools available to lipid analysts for the separation of molecular species of lipids [31]. Recently, *Dobson* et al. provided an excellent review of silver ion chromatography of lipids and fatty acids [32]. Furthermore, *Rao* et al. reviewed HPLC techniques for characterization and separation of fatty acids since 1974 [33].

Argentation chromatography can separate fatty acids according to the configuration and the number of their double bonds and also according to the position of the double bonds. The analysis can be carried out either with triacylglycerols or with fatty acids after suitable derivatisation (e.g. FAMEs, phenacyl esters, p-bromophenacyl esters, p-phenylazophenacyl esters) [34–40]. In most of the published work, the Ag^+ chromatography technique has been used in conjunction with TLC with silver nitrate being incorporated into a silica gel layer by various means. Common TLC solvent systems are, for example, hexan:diethyl ether = 90:10 (v/v), benzene for cis/trans-methyl linolenate, and chloroform:acetone:acetic acid = 96:4:0.5 (v/v/v) for cis/trans-methyl arachidonate fractionation. TLC plates are sprayed with 2',7'-dichlorofluorescein solution and dried, and the spots are visualised by UV detection at 254 nm [33]. Using Ag^+ TLC, FAMEs can be separated only by the total number of cis and trans double bonds, and not by the specific location unless extreme conditions are applied (30% silver nitrate in the sorbent layer and –25° C [41]).

R_f values of different components are highly variable, being dependent on such factors as the proportion of silver nitrate in the layer, its degree of hydration, atmospheric humidity and temperature and the nature of the mobile phase, factors which are not easily controlled [42].

Recently, a stable silver ion column for HPLC has been developed in which the silver ions are linked via ionic bonds to phenylsulfonic acid moieties, which are bound to a silica matrix [43]. This column has been used extensively for the separation of fatty acid derivatives and triacylglycerols. In particular, excellent separations of positional and geometrical isomers of unsaturated fatty acids have been achieved [44]. In contrast to Ag^+ TLC it is possible to control many of the chromatographic parameters, especially mobile phase composition, flow rate and column temperature, with a high degree of accuracy. Other advantages of Ag^+ HPLC are the reusability of the column, simple and rapid sample separation, short analysis time and recoveries usually above 95% (Ag^+ TLC about 60%) [45]. If the samples are analyzed by GC before and after the separation, the amount of the trans fatty acids in complex mixtures can be determined [46].

The separation of polyunsaturated cis and trans FAMEs is possible with a UV-compatible solvent system (e.g. mobile phase: hexane with 1% acetonitrile) [45]. Baseline separation of the 9-trans and 9-cis 18:1 isomers has been achieved. The elution order when using this mobile phase for the methyl linoleate isomers was [47]:

18:2 t9t12 > t9c12 > c9t12

and differs from that obtained with capillary GC (SP 2330, SP 2340 or SP 2560) in which 18:2 c9t12 elutes before 18:2 t9c12 [48]. The separation of all eight cis-trans isomers of methyl 18:3 was similar to the separation obtained on a 50 m CP Sil 88 capillary GC column. The resolution of 15 of the 16 possible cis/trans isomers of methyl 20:4 farly exceeds the capabilities of HPLC [47].

3.4 Gas chromatography-*Fourier* transform infrared spectrometry (GC-FTIR)

The advantage of coupling IR spectroscopic determination of trans monounsaturated FAMEs with capillary GC is that any interferences, particularly those due to partial overlap of adjacent trans and cis octadecenoic isomer GC peaks will not disturb the accuracy of the measurements [49]. Thus, it is possible to determine the general fatty acid composition of partially hydrogenated vegetable oil by GC/IR.

Three fundamentally different types of interfaces between a GC and a FTIR spectrometer can be distinguished. The most common – light pipe (LP) – interface incorporates a flow-through LP gas cell with vapour-phase spectra, which are measured in real-time at intervals of approximately one second [50]. For systems of this type, the minimum identifiable quantity (MIQ) is rarely less than 5 ng, with the MIQ typically increasing by an order of magnitude for compounds of low absorptivity eluting from the GC column as broad peaks.

Better sensitivity (MIQ in the subnanogram range) is achieved by the use of matrix isolation (MI) or direct deposition (DD) techniques [51]. The MI method involves the adding of argon (1.5% by volume) to the GC carrier gas (helium), and the trapping of the effluent on the outer rim of a slowly rotating gold disk (about 3 mm/min) held at cryogenic temperatures (about 12 K). During a GC run helium is removed by vacuum pumps, and the analyte molecules surrounded by an excess of argon atoms are frozen into a solid matrix. The analytes isolated in the IR-transparent argon matrix are subsequently analyzed by IR spectroscopy. The reflection-absorption spectrum is then measured. The position of each analyte peak on the collection disk is

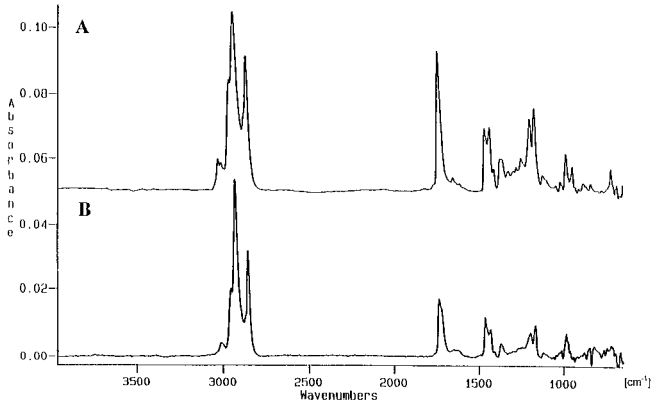

Fig. 8. GC-DD-FTIR spectra observed for the FAME derivatives of A) cis9,trans11 and B) trans9,trans11 18:2 isomers isolated from adipose tissue [55].

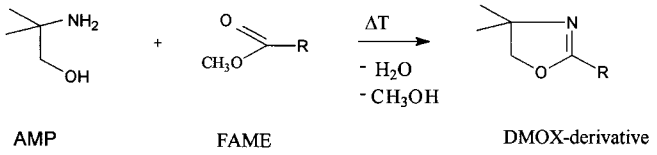

Fig. 9. Condensation of 2-amino-2-methylpropanol (AMP) and fatty acid methyl esters (FAMEs) to the 4,4-dimethyloxazoline (DMOX) derivative.

indexed by its observed GC retention time. By optimizing the performance of the system, including optical alignment and reproducibly locating the peak maximum on the collection disk, the extent of postcolumn peak broadening can be minimized [52].

In the DD interface, GC eluates are condensed on a cooled moving window without dilution in a matrix of any type. The position and movement (e.g. 100 μm during the full-width at half-height (fwhh) of a typical GC peak) of the window are controlled and recorded during a GC run by the spectrometer's software [53]. The width of the sample trace can be reduced to about 100 μm, leading to an approximately 10fold increase in the absorbance per unit weight over a GC/MI-FTIR interface with a 300-μm-wide trace [54].

Another advantage of GC-FTIR is the possibility to confirm double bond configurations even if only nanogram amounts of analytes are available. Fig. 8 shows GC-DD-FTIR spectra observed for the FAME derivatives of cis9,trans11 and trans9,trans11 18:2 isomers isolated from adipose tissue.

Where there are sufficient amounts of minor or unknown fatty acids, nuclear magnetic resonance (NMR) spectroscopy, especially ^{1}H-NMR and ^{13}C-NMR, can be applied to confirm the double bond configuration [56].

3.5 Gas chromatography-mass spectrometry (GC-MS)

To elucidate the structure of fatty acids the use of a mass spectrometer connected to a GC is customary. Recently, *Spitzer* provided a review about structure analysis of fatty acids by GC-MS as their DMOX-derivatives [57].

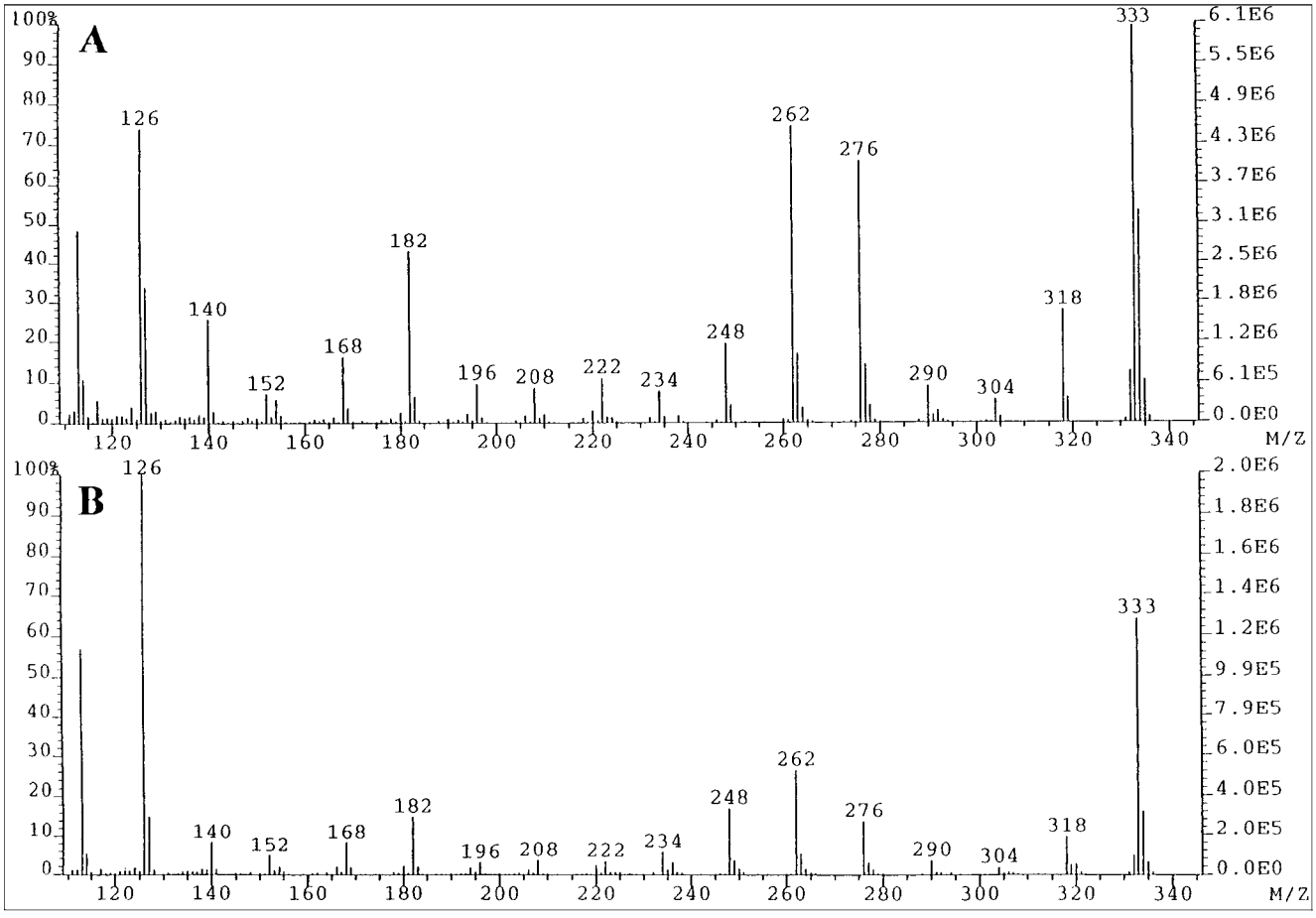

Fig. 10. GC-EIMS spectra observed for the DMOX derivatives of A) cis9,trans11 and B) trans9,trans11 CLA isomers isolated from human adipose tissue. Relatively intense peaks due to allylic cleavage were observed at m/z 182 and 262 [55].

Commonly, saturated fatty acids (SFA) are transferred to methyl or trimethylsilyl esters for GC analysis. Long-chain saturated methyl esters are easily identified but fragmentation patterns of unsaturated FAMEs are not indicative for the position of double bonds [58]. Double bond positions can be determined if unsaturated fatty acids are converted to suitable derivatives. For instance transformations to the pyrrolidine [59], picolinyl, and 4,4-dimethyloxazoline (DMOX) derivatives (so-called "remote-site" derivatives) are proposed for the GC-MS identification of fatty acids with different further functional groups [60, 61]. As a result of these derivatizations unsaturated fatty acids show a reduced tendency to bond migration along the hydrocarbon chain.

The derivatization to DMOX products has proved to be a powerful method in GC-MS fatty acid analysis with a wide range of applicability [61]. Natural and synthetic fatty acids with ethinyl, hydroxy and keto groups as well as methyl branched fatty acids have been analyzed and identified by their low resolution electron impact ionization mass spectra [62]. The preparation of the DMOX derivatives is quite easy to perform and needs only an excess of 2-amino-2-methylpropanol (AMP) in the fatty acids or FAMEs mixture (Fig. 9). The excess of AMP is necessary to avoid the formation of N-O-diacetylated products [57].

Mass spectra of DMOX derivatives of fatty acids give very intense ions at m/z 113 and 126, which are characteristic for DMOX derivatives of fatty acids [63, 64]. Another advantage of DMOX-derivatives is the similar GC behaviour (e.g. corresponding elution order to FAMEs) in contrast to the picolinyl esters and pyrrolidine derivatives, which have disadvantages with regard to volatility and resolution properties. Therefore, elution temperatures are typically only about 10° C higher than those from the corresponding FAMEs, whereas GC analysis of picolinyl esters and pyrrolidine derivatives have been carried out at approximately 50° C higher oven temperatures [60]. Commonly, a wide range of capillary columns has been applied to analyze fatty acids, e.g. SE-54, DB-23, DB-WAX, CP Sil 88, SPB-1, FFAP, HP-5, BPX-70, DB-1, and DB-225 [57].

By applying GC-FTIR and GC-MS techniques, complete structure characterization of unknown minor fatty acids is achievable. Recently, *Fritsche* et al. [55] identified minor CLA isomers (18:2 t9c11, 18:2 c9c11, and 18:2 t9t11) in human adipose tissue. As shown in Fig. 10 the mass homologous series m/z 126 + 14 mu is interrupted in the region of the double bond systems. The location of the double bond is indicated by 12 mu gaps.

In comparison with the mass spectra of the pyrrolidine derivatives of conjugated diene C18 fatty acids [65, 66], the fragmentation patterns of the DMOX derivatives are unequivocal in the double bond region and also lead to intensive allylic cleavage peaks, which support the structure assignment.

Another application of structure elucidation using GC-MS (DMOX derivatives), IR, and ^{1}H-, ^{13}C-NMR is given by *Spitzer* et al. [67]. This group could identify, besides some usual fatty acids, two conjugated ene-yne acteylenic fatty acids – trans-10-heptadecen-8-ynoic acid (pyrulic acid) and trans-11-octadecen-9-ynoic acid (ximenynic acid) as well as a novel ene-yne-ene acetylenic fatty acid (cis-7-trans-11-octadecadiene-9-ynoic acid (heisteric acid)), and 9,10-epoxystearic acid in the seed oil of *Heisteria silvanii* (Olacaceae).

Comprehensive information about sophisticated techniques and new applications in lipid analysis have been reported by *McDonald,* and *Mossoba* [68].

4 TFA and CLA Contents in Foods

4.1 Edible oils and margarines

4.1.1 Edible oils

Edible oils such as refined or unrefined walnut, olive, sunflower, safflower, rapeseed, soybean, avocado, cashew, or peanut oil or coconut fat have only negligible amounts of TFA and CLA [69, 70]. As shown in Tab. 1, refined oils contain higher TFA contents than unrefined oils (% of total fat).

The TFA amounts in refined oils are influenced by duration and temperature of refining. Refined and bleached soybean oil treated with temperatures between 160° C and 240° C was found to contain 18:3 trans isomers [71]. Rapeseed oils heated at 275° C for 3 h ("dry heating") contained approximately 4% 18:3 trans isomers [72]. During lower deodorization conditions, e.g. 220° C for 3 h, rapeseed oil contained an approximately 25% lower isomerization rate. One reason for the increased isomerization rate at 275° C might be the more intense oil mixing as a result of the use of steam, which rules out local overheating with its strong influence to trans isomerization [72].

Soybean oil can be partially hydrogenated for use in margarines to prevent flavour reversion [73]. Strong hydrogenation conditions due to detectable CLA concentrations were reported by *Mossoba* et al. [74]. Whereas *Chin* et al. [75] found CLA in coconut and olive oil in an order of about 0.02 g/100 g fat. Another source of CLA was reported by *Spitzer* et al. [76, 77] who identified conjugated linoleic acids in exotic seed oils, e.g. in oil of *Acioa edulis,* which is used for cooking in areas of Brazil. Moreover, *Yurawecz* et al. [78] reported conjugated octadecatrienes (COT) in edible fats and oils ranging from < 0.001 to 0.2%.

4.1.2 Margarines

Regular margarines contain varying contents of partially hydrogenated vegetable oils, and therefore TFA. The main TFAs in margarines are the 18:1 isomers, which have a double bond position from Δ6-Δ16 (Fig. 3). Approximately 85% of total TFA content in vegetable margarines, sunflower margarines, and fat reduced margarines are 18:1 TFAs [79, 80]. The 18:1t, 18:2 c,t, and 18:2 t,c total TFAs, and CLA contents of margarine originating from different countries are presented in Tab. 2.

During recent years, a visible progress in the reduction of TFA contents in European margarines could be observed [79, 81]. This trend is probably attribut to increased public health interests linking TFAs with coronary heart diseases (CHD) and atherosclerosis. Note that the TFA data obtained from IR in the 70s have to be interpreted carefully (probably overestimated) because of error inherent in the older standard IR methods.

Distinct variation of TFA contents in different brands of margarines can be observed. This wide variation can be explained by the different processing parameters, for instance, hydrogenation or/and deodorization conditions. Another reason for reduced TFA content in margarines is probably the changed blend oils in the composition of margarines. Partially hydrogenated oils seem to have been partially replaced by palm, palm kernel or coconut oil to obtain acceptable consistency [82].

With regard to the highest TFA contents in margarines, the sunflower margarines are most prominent [79, 80]. In this case the supplementation of sunflower oil with blend oil is legally restricted in Germany [83]. But the trend to reduced

Tab. 2. Mean trans fatty acid (TFA) and conjugated linoleic acid (CLA) contents of margarines. Values in brackets are minimum and maximum levels.

food	origin	[reference]	18:1 t [% of total fat]	18:2 c,t/t,c [% of total fat]	total TFA [% of total fat]	CLA [% of total fat]
margarines						
margarines (n = 26)	Italy	1967 [145]			22.5 (0.9–34.3)[a]	
margarines (n = 10)	USA	1973 [146]			24.1 (0–36.0)[a]	
margarines (n = 83)	Germany	1973, 1976 [143]	0.1–53.2[b]			
margarines (n = 9)	USA	1977 [147]	18.0 (6.9–31.4)[b]			
margarines (n = 7)	USA	1977 [148]			20.4 (6.3–33.6)[b]	
margarines (n = 9)	Canada	1979 [149]			21.3 (8.7–32.9)[b]	
margarines (n = 40)	USA	1983 [150]			18.4 (6.8–31.0)[b]	
margarines (n = 84)	USA	1985 [151]	19.89 (10.74–30.06)[b]			
margarines (n = 10)	Greece	1991 [152]	5.4–9.5[b]	0–3.65[b]		
margarine	USA	1991 [74]				0.6[a]
margarines (n = 16)	Germany	1992 [2]	7.9 (0.1–23.0)[b]	0.5 (0.1–1.7)[b]	8.5 (0.6– 23.5)[b]	
margarines (n = 9)	Germany	1993 [100]			5.0 (0–10.6)[b]	
margarines (n = 46)	Germany	1994 [153]	9.32 (0.17–25.90)[b]			
margarines	Belgium (n = 9)	1994/5 [154]	5.7 (0.2–16.7)[b]	0.4 (0–0.8)[b]	6.4 (0.5– 17.8)[b]	
	Hungary (n = 7)		13.0 (2.0–22.3)[b]	0.5 (0–0.8)[b]	14.1 (2.0–24.5)[b]	
	GB (n = 3)		9.3 (9.3–10.9)[b]	0.5 (0–1.0)[b]	9.77 (7.7–11.3)[b]	
margarines (n = 17)	Turkey	1994 [85]	0–34.5[b]			0.3–2.0[b]
shortenings (n = 43)	Denmark	1995 [155]	6.8 (0–13.7)[b]	< 0.2[b]		< 0.2[b]
hard margarines (n = 20, > 40% LA)			1.3 (0–5.6)[b]	< 0.2[b]		< 0.2[b]
tub margarines (n = 12)	France	1995 [82]	8.0 (0–17.6)[b]	0.4 (0.06–1.40)[b]		
margarines (n = 24)	Germany	1995 [80]	4.6 (0–20.1)[b]	0.5 (0–0.7)[b]	5.1 (0.4– 21.0)[b]	
margarines (n = 17)	Germany	1997 [69, 70]	1.17 (0.05–4.35)[b]	0.17 (0–0.43)[b]	1.5 (0.15–4.88)[b]	< 0.01[b]
margarines (n = 6)	USA	1997 [156]	0.04–13.29[b]	0–0.26		

[a] Determined by IR.
[b] Determined by GC.

Tab. 3. Mean trans fatty acid (TFA) and conjugated linoleic acid (CLA) contents in milk, and dairy products. Values in brackets are standard deviations or minimum and maximum levels.

food	origin	[reference]	18:1 t [% of total fat]	18:2 c,t/t,c [% of total fat]	total TFA [% of total fat]	CLA [% of total fat]
milk fat						
milk fat (n = 116)	Australia	1971 [157]			6.01 (4.27–7.64)[b]	
milk fat (n = 13)	Canada	1983 [158]			4.0–5.7[a]	
milk fat	Australia	1988 [159]				0.9–1.2[b]
milk fat (n = 13)	Austria	1994 [160]			1.75–5.20 (0.99)[b]	
milk fat (n = 5)	Germany	1992 [2]	1.6–4.0[b]	0.3–0.8[b]	2.4–5.5[b]	
milk fat (n = 2)	USA	1994 [161]				0.45 (0.06)[b]
milk fat (n = 1756)	Germany	1995 [120]			3.62 (1.29–6.75)[b]	
milk fat (n = 132)	Germany	1996 [86]				
		indoor	1.53 (0.11)[b]	0.24 (0.04)[b]	3.32 (0.29)[b]	0.34 (0.04)[b]
		pasture	2.54 (0.46)[b]	0.21 (0.05)[b]	4.73 (0.73)[b]	0.61 (0.08)[b]
		ecological	3.04 (0.68)[b]	0.43 (0.11)[b]	5.78 (1.23)[b]	0.80 (0.21)[b]
milk fat (n = 63)	Sweden	1996 [91]				0.25–1.77[b]
milk fat (n = 7)	Germany	1997 [69, 70]	3.13 (0.24)[b]	0.48 (0.28)[b]	5.12 (1.02)[b]	1.16 (0.10)[b]
dairy products						
cheese (n = 27)	Germany	1992 [2]	1.0–5.1[b]	0.1–1.6[b]	1.6–7.5[b]	
butter (n = 5)	Germany	1992 [2]	1.5–6.3[b]	0.5–0.8[b]	2.5–7.9[b]	
natural cheese (n = 13)	USA	1992 [75]				0.29–0.71[b]
processed cheese (n = 4)						0.45–0.52[b]
homogenized milk (n = 3)						0.55 (0.03)[b]
butter (n = 4)						0.47 (0.04)[b]
cheese (n = 15)	USA	1994 [97]				0.32–0.89[b]
fermented dairy products (n = 3)	USA	1995 [161]				0.38–0.47[b]
fluid milk products (n = 4)						0.40–0.64[b]
cheese (n = 15)						0.36–0.80[b]
cheese (n = 25)	Germany	1997 [69, 70]	2.09 (0.89)[b]	0.51 (0.14)[b]	3.89 (1.92)[b]	0.84 (038)[b]
butter (n = 12)					4.43 (1.99)[b]	0.94 (0.48)[b]

[a] Determined by IR.
[b] Determined by GC.

TFA levels in margarines can also be observed in sunflower margarines, which is documented by studies of *Pfalzgraf*, and *Steinhart* [80].

CLA does not arise in considerable amounts during regular processing conditions [70]. Only a few investigators have reported detectable CLA contents in margarines. These findings reflect different processing conditions such as type and amount of catalyst, hydrogen gas pressure, and temperature [84]. For instance, *Kayahan*, and *Tekin* [85] found CLA in margarines ranging from 0.31 to 2.04% and *Mossoba* et al. [74] reported from 0.2% 18:2 conjugated c,t (and also from 0.2% 18:2 conjugated t,t, and 18:2 c,t, respectively).

4.2 Milk and dairy products

Milk fat generally is a rich dietary source of TFA. The 18:1 t, 18:2 c,t, and 18:2 t,c total TFA, and CLA contents of milk and dairy products are presented in Tab. 3.

The predominant TFA isomers in dairy products are the octadecenoic acid isomers, especially the trans vaccenic acid (18:1 t11). The trans 18:1 isomer distribution of milk fat is shown in Fig. 3. Further TFA isomers such as 14:1 t9, 16:1 t9, 18:1 positional isomers, 18:2 t, and 18:3 t have lower amounts in dairy products [69, 70]. For instance, the contents of palmitelaidic acid (16:1 t9) varied between 0.04% (goat cheese) and 0.21% (*Lightdammer*) [69].

The total TFA content in raw milk ranged from approximately 1.5% to 6.5% [86, 87] and in dairy products from 2.0% to 6.1% [69]. The variations of TFA contents in dairy products can be explained by varying TFA contents of milk fat. The milk fat composition and content are influenced by season and also by different feeding systems of cows [88, 89]. Irish butter (mean 6.5% TFA, data not shown) tends to have higher TFA amounts compared with German butter (mean 3.5% TFA). These different TFA amounts in butter from other countries may reflect different dairy cow breeds.

Another reason for the wide variation of TFA contents in dairy products are processing parameters such as heat treatment during pasteurization. An influence of fermentation on the TFA content in yoghurt or cheese is also possible. For instance, TFA contents in long ripened propionic acid fermented cheeses (e.g. *Old Emmentaler* 5.76%, *Jurassic* 6.06%) or yoghurt with added probiotic cultures seem to be slightly elevated [69].

Dairy products are also a major source of CLA (Tab. 3). The CLA content in milk fat ranged from 0.24% to 1.77%. This wide variability is caused by the range of CLA content in the raw material, which is probably influenced by different dairy cow breeds and feeding systems as well as processing parameters. The influence of processing parameters such as heat treatment, composition of starter cultures, storage and ageing on the CLA content in dairy products is discussed in 4.5. However, CLA contents in long ripened propionic acid fermented cheeses or in yoghurt with added probiotic cultures seem to be slightly elevated as has also been seen for TFA contents. Bacterial surface ripened cheeses such as *Münster* and *Tilsiter* did not show different CLA contents to solely lactic acid fermented cheeses [70].

4.3 Meat, meat products and fish

4.3.1 Meat and meat products

The TFA contents in meat are shown in Tab. 4. They ranged from 0.20% in horse up to 10.6% in mutton. Meat from nonruminants such as pork or poultry shows distinctly lower TFA contents than meat from ruminants. The TFA content and isomer distribution in beef is similar to that in milk or other dairy products [2]. The 18:1 trans isomers are the predominant isomers in meat and amount to approximately 80% of total TFA [69].

The TFA content in meat products varied from 0.2% to 3.4% of total fat (Tab. 4). These values assume an intermediate position between meat from ruminants and nonruminants, depending on the raw material used. German sausages contain predominantly pork and therefore reflect comparatively lower TFA amounts.

In addition the CLA content in meat from ruminants, for instance 1.20% in lamb, is higher than in meat from nonruminants (e.g. 0.12% in pork). In the case of nonruminants CLA may occur from dietary sources such as feeding meat meal and tallow [70]. Another explanation may be the formation of CLA by intestinal bacteria, as has been shown for rats [7].

The CLA contents in meat products ranged from 0.27% to 0.44%. The CLA content of the meat products seems to reflect their raw material and has been influenced neither by processing conditions nor by fermentation [70].

4.3.2 Fish

Native fish oil has negligible TFA contents (up to 1.10%, Tab. 5) compared with those of meat or dairy products. The predominant TFA isomers are also the octadecenoic acid isomers (average 60% of total TFA). Carp has the highest TFA amount (1.01%), which might be caused by feeding TFA containing food to the fish in aquaculture [69].

The CLA levels ranged from 0.01% in pike perch to 0.09% in carp. Currently, very few data dealing with CLA content in fish are available. As shown in Tab. 5, sea food has similar contents to fish. Up to now, the maritime formation of CLA remains uncertain.

4.4 Cakes, pastries and other processed foods

The TFA content in chocolates, cakes, and pastries, snack food, and other foods (Tab. 6) ranged from < 0.01% (e.g. plain chocolate, data not shown) to 34.1% (French fries) and originates from dairy fat as well as from partially hydrogenated vegetable oil (e.g. from margarines or frying fat).

These wide variations of TFA could also be observed in the same product category, e.g. potato crisps 1.22% up to 22.01% [69]. A reason for this variability is that products often contain a blend of partially hydrogenated vegetable oils of different sources (e.g. soybean, canola, corn or sunflower oil). The proportions of hydrogenated and unhydrogenated oils in these foods are varied to obtain the desired physical properties, e.g. better mouth feeling [3].

The CLA contents in these food items originate predominantly from dairy fat and reflect its CLA level. In composed foods such as chocolates or nut nougat cremes, which contain a blend of milk and cocoa fat, the percentage of CLA is, therefore, lower than in dairy products. On the other hand, French fries and frying fat, which can contain high amounts of hydrogenated vegetable oil, have negligible contents of CLA (< 0.01%) [70].

4.5 Ways to affect the CLA contents in foods

4.5.1 Influence of feeding conditions

Many successful measures have been taken to reduce TFA contents in foods, which have already been presented in the proceeding chapters. Because of the desirable properties of CLA (7.2, 7.3, and 7.4) attempts have been made to enhance CLA.

To observe the influence of feeding conditions on CLA contents of bovine milk fat investigators have assessed

Tab. 4. Mean trans fatty acid (TFA) and conjugated linoleic acid (CLA) contents in meat, and meat products. Values in brackets are standard deviations.

food	origin	[reference]	18:1 t [% of total fat]	18:2 c,t/t,c [% of total fat]	total TFA [% of total fat]	CLA [% of total fat]
meat						
round beef (n = 4)	USA	1992 [75]				0.29 (0.01)[b]
fresh ground beef (n = 4)						0.43 (0.01)[b]
veal (n = 2)						0.27 (0.02)[b]
lamb (n = 4)						0.56 (0.03)[b]
pork (n = 2)						0.06 (0.01)[b]
chicken (n = 2)						0.09 (0.002)[b]
fresh ground turkey (n = 2)						0.25 (0.004)[b]
calf (n = 3)	Germany	1992 [2]	0.7–1.3[b]	trace – 0.2[b]	0.9–1.7[b]	
beef (n = 5)			1.4–2.4[b]	0–0.3[b]	1.9–3.2[b]	
lamb (n = 3)			5.2–7.0[b]	0.6–0.9[b]	6.6–8.8[b]	
sheep			2.2[b]	0.4[b]	3.2[b]	
mutton (n = 3)			6–8.9[b]	0.9–1.2[b]	8.2–10.6[b]	
pork (n = 3)			0.2–0.4[b]	trace	0.2–0.4[b]	
poultry (n = 5)			0.2–1.2[b]	0–0.2[b]	0.2–1.4[b]	
rabbit			0.3[b]	trace	0.4[b]	
horse (n = 2)			0.2[b]	trance	0.2[b]	
kangaroo (n = 2)			7.9[b]	0.8[b]	9.8[b]	
pork (n = 4)	Germany	1997 [69, 70]	0.22–0.42[b]	0.02–0.03[b]	0.46–0.68[b]	0.12–0.15[b]
lamb (n = 2)			7.53[b]	0.23[b]	9.80[b]	1.20[b]
turkey (n = 2)			0.89[b]	0.08[b]	1.19[b]	0.20[b]
beef steak (n = 2)			2.29[b]	0.19[b]	3.57[b]	0.65[b]
beef liver (n = 2)			2.27[b]	0.03[b]	2.84[b]	0.43[b]
rabbit (n = 2)			0.16[b]	0.03[b]	0.37[b]	0.11[b]
chicken (n = 2)			0.52[b]	0.10[b]	0.88[b]	0.15[b]
meat products						
meat products (n = 25)	Germany	1992 [2]	0.2–2.6[b]	trace – 0.3[b]	0.2–3.4[b]	
meat products (n = 18)	USA	1992 [75]				0.08–0.33[b]
meat products (n = 4)	Germany	1993 [100]			2.6 (2.6)[b]	
meat products (n = 10)	Germany	1997 [69, 70]	0.14–0.53[b]	0.02–0.12[b]	0.48–1.09[b]	0.27–0.44[b]
meat products (n = 3)	USA	1997 [156]			0.20–0.87[b]	

[a] Determined by IR.
[b] Determined by GC.

Tab. 5. Mean trans fatty acid (TFA) and conjugated linoleic acid (CLA) contents in fish. Values in brackets are standard deviations.

food	origin	[reference]	18:1 t [% of total fat]	18:2 c,t/t,c [% of total fat]	total TFA [% of total fat]	CLA [% of total fat]
fish						
salmon (n = 4)	USA	1992 [75]				0.03 (0.005)[b]
lake trout (n = 3)						0.05 (0.005)[b]
sea scallops (n = 2)						0.03 (0.005)[b]
shrimp (n = 2)						0.06 (0.01)[b]
mussels (n = 2)						0.04 (0.004)[b]
herring	Germany	1992 [2]			0.2[b]	
herring filet					0.3[b]	
perch			1.80[b]	0.1[b]	2.3[b]	
rudd			0.1[b]		0.7[b]	
tench			0.3[b]		1.0[b]	
victoria perch	Germany	1997 [69, 70]	0.48[b]	0.12[b]	0.74[b]	0.02[b]
carp			0.50[b]	0.27[b]	1.10[b]	0.09[b]
pike perch			0.56[b]	0.02[b]	0.91[b]	0.01[b]
cod			0.39[b]	0.08[b]	0.83[b]	0.03[b]
ocean perch			0.54[b]	0.04[b]	0.88[b]	0.06[b]
catfish			0.46[b]	0.05[b]	0.66[b]	0.06[b]
salmon			0.33[b]	0.05[b]	0.71[b]	0.07[b]
pollock			0.27[b]	0.05[b]	0.50[b]	0.07[b]
wolf fish			0.36[b]	0.07[b]	0.58[b]	0.05[b]

[a] Determined by IR.
[b] Determined by GC.

the CLA contents in bovine milk fat obtained from different feeding systems (restrictive, intensive). For instance, over a period of a year *Jahreis* et al. [90] collected monthly bulk milk samples from three different types of farms: 1. conventional farming, indoor feeding with silages during the whole year (cereal-rich maize silage rations), 2. conventional farming, grazing during summer season (maize and grass silages), and 3. ecological farming, grazing during summer

Tab. 6. Mean trans fatty acid (TFA) and conjugated linoleic acid (CLA) contents in cakes, pastries, and other foods. Values in brackets are standard deviations.

food	origin	[reference]	18:1 t [% of total fat]	18:2 c,t/t,c [% of total fat]	total TFA [% of total fat]	CLA [% of total fat]
cakes and pastries						
cakes and pastries (n = 13)	Germany	1992 [2]	0–14.3	0–0.8	0–15.5	
cakes and pastries (n = 6)	Germany	1997 [69, 70]	0.41–5.51	0.03–0.34	1.10–5.95[b]	0.02–0.55[b]
processed foods						
chocolates (n = 20)	Germany	1992 [2]	0.2–13.7	trace – 2.7	0.2–15.7[b]	
French fries (n = 5)			5.8–30.9	trace – 1.9	5.8–32.8[b]	
frying fat (n = 5)			0.1–30.2	0–7	0.1–31.8[b]	
crisps (n = 13)			0–14.9	trace – 6.6	trace – 20.2[b]	
instant sauces/soups (n = 5)			2.3–27.7	0.1–0.9	2.9–34.9[b]	
chocolates (n = 9)	Germany	1997 [69, 70]	< 0.01–10.08	< 0.01–0.44	< 0.01– 11.02[b]	< 0.01–0.15[b]
French fries (n = 2)			19.7–29.5	2.70–4.75	26.4–34.1[b]	< 0.01[b]
frying fat (n = 3)			11.37 (10.06)	4.35 (4.14)	15.90 (14.04)[b]	< 0.01[b]

[a] Determined by IR.
[b] Determined by GC.

season, silage feeding in other seasons (clover, alfalfa, grass silages). They reported significant differences between the CLA (18:2 c9t11) content of the indoor group (mean 0.34%) and the ecological group (mean 0.80%) [90].

Jiang et al. [91] reported on a similar study. They fed a control group (n = 11) a diet containing 40–50% silage and 40–55% concentrate (mainly barley, oats and rapeseed expeller), whereas the trial groups were fed a diet with 35:65 forage to concentrate in restricted (8 cows) or unrestricted (9 cows) amounts. Cows fed restricted amounts of the trial diet exhibited the highest concentration (1.13 g/100 g of fat), whereas cows fed *ad libitum* consumption had similar CLA contents to those of the control group (0.66 versus 0.50 g 18:2 c9t11/100 g of fat). These investigators concluded that CLA (mainly 18:2 c9t11) could be increased through suitable dietary regimen.

Shantha et al. [92] assessed the ability of different feeding systems (grass or grain) and the use of a growth promoting hormone implant (zeranol) to affect CLA concentration in beef cattle. They reported that the concentration of CLA (18:2 c9t11) in semimembranosus muscle from cattle on pasture (without added grain, 0.74 g/100 g fat) was significantly higher ($P < 0.05$) than in animals fed grain and grass (0.51 g/100 g fat) or zeranol-implanted animals (0.52 g/100 g fat). *Shantha* et al. [92] also observed significantly lower LA concentrations in the grass-fed group ($P < 0.05$). They concluded that the lower linoleic acid (LA) concentration might result from a conversion of LA to CLA.

4.5.2 Influence of processing conditions

Ageing and heat treatment, e.g. during dairy pasteurization or pan frying of meat, are factors reported to contribute to the formation of CLA in foods.

Therefore, *Shantha* et al. [93] compared the CLA content in unprocessed raw material with the CLA amounts in salted and unsalted butter as well as in nonfat yoghurt [93]. The total CLA concentrations increased 1.32fold in salted and 1.27fold in unsalted butter but did not alter the ratio of 18:2 c9t11 to total CLA. Nonfat yoghurt showed an increase in CLA content with processing. No changes in CLA content were observed in processed dairy products such as lowfat yoghurt, regular yoghurt, low fat and regular ice cream, sour cream or cheese such as *Mozzarella, Gouda,* and *Cheddar. Shantha* et al. [93] concluded that storage (up to six weeks) did not affect CLA concentration in any product and suggested that CLA is a stable food component.

Aneja and *Murthi* [94] studied the effect of different processing methods for ghee (anhydrous milk fat from cows and buffaloes) on the total CLA contents. The CLA contents of cows' and buffaloes' milk fat were 0.6% and 0.5%, respectively. Natural microbial fermentation during curd formation increased the CLA contents to 1.0% and 0.9%, resp. for cow and buffalo. The CLA contents were further increased substantially during clarifying at a temperature of 120° C.

The influence of different cooking conditions (frying, baking, boiling, and microwaving process) on the CLA content in beef (ribeye, round, T-bone, and sirloin) was examined by *Shantha* et al. [95]. These investigators concluded when CLA concentrations were compared on a milligram of CLA per gram fat basis, there were no significant differences in CLA between either cooking methods or degree of doneness. Furthermore, they observed no substantial decrease of CLA content during a seven-day- storage.

4.5.3 Influence of fermentation conditions and food additives

Other causes for the wide variation of the CLA content in dairy products might be different fermentation conditions (e.g. starter cultures, ageing). The interaction between CLA and food additives, e.g. natural or synthetic antioxidants like ascorbate or butylated hydroxytoluene (BHT) are also discussed as affecting the CLA contents in dairy products.

Werner et al. [96] quantified the CLA contents in three *Cheddar*-type cheeses (*Cougar Gold, Cheddar,* and *Viking* cheeses) relative to the effects of different cheese cultures, processing conditions, and ageing periods. *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* were included in the starter cultures of all these cheeses. *Lactobacillus delbrueckii* ssp. *bulgaricus* was added additionally to both the *Cougar Gold* and *Viking* cheese, and *Streptococcus salivarius* ssp. *thermophilus* was included in the starter culture for the *Viking* cheese. Starter cultures, processing conditions (*Cougar Gold* cheeses and *Cheddar* cheeses were cooked from 31.1° C to 36.7° C over a period of 30 min and held at 36.7° C for additional 45 min. *Viking* cheese was cooked from 31.1° C to 37.8° C over a period of 50 min with no hold time), and ageing periods (three 13-month aged cheeses and one unaged cheese) had negligible effects on the CLA content in the three *Cheddar*-type cheeses but did influence the CLA isomer distribution [96].

Shantha et al. [97] reported the effects of various hydrogen donors such as BHT, propyl gallate (PG), cysteine and ascorbic acid on CLA formation in processed cheese. Dairy additives such as sodium caseinate, nonfat dry milk and sweet whey powder have also been assessed for their ability to increase CLA concentration. Furthermore, the ability of the oxidation system Fe^{2+}/Fe^{3+}-ascorbate to increase CLA concentration has been checked. PG was the most effective hydrogen donor tested, increasing the total CLA and 18:2 c9t11 contents 1.42fold and 1.59fold, respectively. Sodium caseinate at concentrations of 6% was the most effective dairy additive, increasing the total CLA concentration 1.65fold compared to processed cheese with no additive (3.30 ± 0.19 mg/g fat 18:2 c9t11; 4.14 ± 0.24 mg/g fat total CLA, n = 6). Nonfat dry milk and whey powder produced 1.57fold and 1.56fold increase in CLA concentration, respectively. At 500 mg/kg Fe^{2+} the increase in total CLA was 1.56fold and 1.44fold for Fe^{3+}-ascorbate. The different CLA forming activity between PG and BHT may be due to different solubility characteristics. The ability of ascorbic acid and cysteine to increase CLA formation could be envisioned in two ways. First, ascorbate and cysteine could act as hydrogen donor, or, alternatively, they could react with the iron originally present in cheese, serving as a radical initiating system to promote the formation of linoleic acid radicals [98].

However, a considerable amount of the original LA (14–18 mg/g fat) is not converted to CLA during processing. This suggests that other limiting factors are involved in the formation of CLA. One possible factor is the surface area of the fat in the cheese. Since the formation of CLA would require interaction between the fat and additives, increasing the surface area could result in an increase in CLA formation [97].

5 Estimation and Evaluation of Daily TFA and CLA Intake

The dietary TFA has been estimated from dietary questionnaires or recall data, analysis of adipose tissue or milk fat data, or food content analysis data. Commonly, the basis of TFA intake calculation varies resulting in varying intake levels in literature. Another reason for the vacillation are the differing eating habits of individuals as well as of cultures. Different analytical techniques such as GC determination and IR absorption measurements are also responsible for widely varying intake levels. Tab. 7 presents an overview of the daily TFA and CLA intake in different countries.

The main dietary TFAs are the trans octadecenoic acids, which contribute to approximately 80–90% of total TFA content in foods. As can be seen from Tab. 7 individuals from Mediterranean countries such as Spain or Italy seem to have a lower TFA intake compared to individuals from other European countries. *Pfalzgraf* and *Steinhart* [99] estimated the daily TFA intake in Germany in 1992 to be 4.1 g/d (men). In view of decreased TFA contents in German margarines and lower meat consumption since 1992 the estimation of the daily TFA intake in Germany was up-dated in 1997 to 2.3 g/d [69]. These results show a decrease of TFA intake since 1992 by approximately 40%. The reported TFA intake by *Fritsche* and *Steinhart* [69] is in a similar range to the estimation by *Demmelmair* et al. [100]. *Wolff* reported elevated 18:1 TFA intake levels for Germany (3.7 g/d) in the order of the values five years ago [101]. This higher 18:1 TFA intake for Germany is probably caused by overestimated TFA amounts for German margarines.

Tab. 7. Estimation of daily TFA and CLA intake in different countries (g/d).

Country	[reference]	[g TFA/day]	[g CLA/day]	basis
TFA				
USA	1986 [104]	7.6		market size and share data combined with TFA product data
GB	1984 [162]	7		
USA	1989 [105]	8.1		market size and share data combined with TFA product data
USA	1990 [163]	12.8		availability data
Germany	1992 [99]	3.4 (women) 4.1 (men)		German Nutrition Study and TFA survey-data
Spain	1994 [164]	2		
France	1995 [101]	2.8		availability data from ruminant sources and margarines
Italy		1.7		
Germany		3.7		
Netherlands		4.7		
Belgium-Luxembourg		4.9		
Denmark		5.8		
Spain		1.5		
Portugal		2.1		
Greece		2.2		
Ireland		2.6		
GB		3.1		
Germany	1996 [100]	1.5–3.1 (children)		dietary plans
Germany	1997 [69]	1.9 (women) 2.3 (men)		German Nutrition Study and TFA survey-data (1996)
GB	1997 [102]	1.7 (women) 2.0 (men)		analysis data from frying oils and fried foods
CLA				
Australia	1994 [159]		0.5–1.5	nutrition data and CLA content of milk fat and CLA content of ruminant depot fat
Germany	1997 [105]		0.31	German Nutrition Study and CLA data
Germany	1997 [70]		0.35 (women) 0.43 (men)	German Nutrition Study and CLA survey-data

Recently, the British Ministry of Agriculture, Fisheries and Food published average TFA intake data. IR and GC were used to determine the TFA levels from frying oils and foods cooked in frying oils. The average intake was estimated to be 2.0 g and 1.7 g/d for men and women, respectively [102].

The TFA intake in the USA seems to be elevated compared to the intake in European countries, probably due to higher total fat intake. Since 1984 the per capita availability of TFAs from household salad, cooking oils, margarines, and spreads has decreased, however [103]. This decreased TFA intake in the case of household salad and cooking oil is probably due to the switch of most manufacturers to unhydrogenated oil for this product category. The per capita TFA consumption from household shortenings decreased in a similar manner (approximately 0.55 g/person and day in 1984 [104] to 0.31 g/person and day in 1989), probably caused by a decline in market size during the 1980s. Although the margarine and spread production has increased in the USA since 1984, the per capita availability of TFAs from margarines and spreads has decreased slightly during this period, caused by the continuing popularity of tub margarines, which have lower TFA content than stick margarines [103]. Per capita TFA consumption from meat and dairy products has remained relatively constant and was estimated to 1.34 g/person and day in 1989 for the USA [103] (without edible tallow).

Another trend of the US food industry should be mentioned here. Since 1989 many restaurants have substituted edible tallow by partially hydrogenated vegetable oils (approximately 30% TFA), because of lowering SFA levels in the products. Many of the fast-food chains now consider switching from the use of solid partially hydrogenated vegetable oil to liquid oils, which have lower amounts of both SFA and TFA (about 15%). Therefore, the contribution of TFA from fast-food service will return to a similar range as that reported before 1989. Over all, *Hunter* and *Applewhite* [103] assumed that the total TFA content in the US diet has been relatively constant since the 1980s and will be in the same range in the near future.

Currently, only a few data concerning the CLA intake are available. The daily CLA intake in Germany has been estimated to be 0.36 g/d for women and 0.44 g/d for men [70]. These data are in a similar range to results from *Jahreis* [105], who estimated a slightly lower daily CLA intake in Germany (Tab. 7). Compared with the estimated daily TFA intake, the CLA intake is approximately one fifth. Therefore, human nutrition in Germany could provide about one fifth of the 0.1% beneficial level of CLA in the diet (based on the relative contents of the 18:2 c9t11 isomer and total food intake of 2434 g/d for men and 2000 g/d for women [106]. This level has proven to be able to reduce the incidence of rat mammary carcinogenesis (section 7).

6 TFA Contents in Human Adipose Tissue and Fluids

6.1 Adipose tissue and plasma

Fig. 11 shows the mean 18:1 TFA contents in human tissue and plasma from patients without CHD and with habitual diet. The 18:2 trans isomers account for only approximately 20% of total TFA.

High-fat foods, which contain partially hydrogenated vegetable oil and dairy products, are the main source of the 18:1 positional isomers in adipose tissue triacylglycerols. The 18:1 isomer distribution patterns for the adipose tissue clearly resemble more closely the pattern for partially hydrogenated vegetable oil than they do for milk fat.

The probable source of 16:1 trans isomers, mainly 16:1 t9, is dairy fat, because only dairy fat contains 16:1 t9 in higher amounts. Another potential source of 16:1 t9 is the β-oxidation of the trans vaccenic acid (18:1 t11) which occurs in both, butter and partially hydrogenated vegetable oil. However, studies with deuterium-labelled 18:1 positional isomers indicate that only trace amounts of the 16:1 isomers are

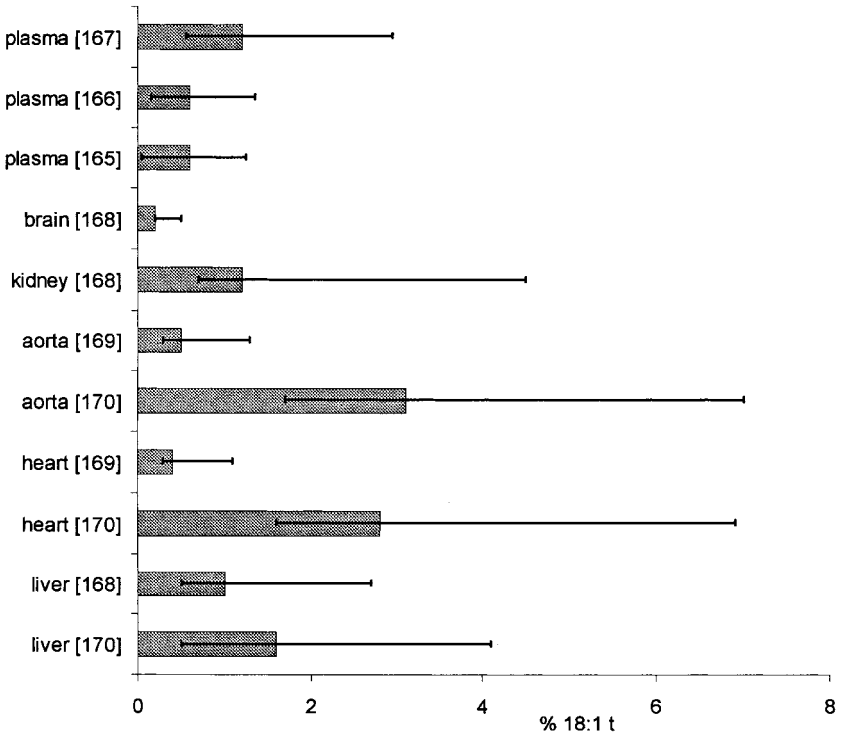

Fig. 11. Mean 18:1 trans content in tissue and plasma from patients without CHD (habitual diet).

released from the β-oxidation pathway [107]. Furthermore, the pattern of the 16:1 trans positional isomers in adipose tissue does not resemble the 18:1 trans positional isomer pattern, because only the latter shows a *Gaussian* isomer distribution from 18:1 t6–t16. These findings suggest that partial β-oxidation of 18:1 trans isomers is not a significant source of the 16:1 trans isomers in human adipose tissue.

Numerous linoleic and linolenic isomers also exist in human adipose tissue (Fig. 6). Both, dairy products and partially hydrogenated vegetable oils, are dietary sources for these geometrical isomers. The c,t and t,c-18:2 isomers occur in approximately equal amounts in adipose tissue. Partially hydrogenated vegetable oil is probably a source of the traces of 18:3 n-3 isomers in human adipose tissue.

CLA, predominantly 18:2 c9t11, is also present in human adipose tissue [108]. Recently, *Fritsche* et al. [55] have identified three minor CLA-isomers – 18:2 t9t11, 18:2 c9c11, and 18:2 t9c11 – in human adipose tissue (Fig. 10). In general, the small amounts of CLA isomers in biological samples are difficult to detect by routine GC methods. For ultimate identification of unknown CLA minor isomers sophisticated techniques such as GC-DD-FTIR and GC-MS are necessary to determine the position and configuration of the double bond.

The occurrence of the 18:1 trans isomers in human plasma lipid fractions such as cholesteryl esters, triacylglycerols, and phospholipids has been reported by several investigators [109, 110].

6.2 Milk fat

The contents of total TFA, 18:1 trans, and 18:2 trans in human milk from different countries are presented in Tab. 8.

These data indicate the wide variation of TFA content of milk lipid caused by the different nutrition habits of individuals and countries. The monounsaturated isomers, mainly 18:1 isomers, contribute to approximately 70–80% of the total TFAs in human milk, similar to the percentage in partially hydrogenated vegetable oil and adipose tissue. Normally, the TFA content of human milk reflects the TFA level of the previous day's diet [111]. This fact is derived from stable-isotope-tracer data that show an approximately lag time of 10 h for incorporation of dietary fats into milk triacylglycerols [112]. Because milk fat (short-term) and adipose tissue (long-term) have different TFA storage properties, the range in the TFA content for milk fat is expected to be larger than the range for adipose tissue data.

The influence of dietary TFA content on the milk fat TFA level can be shown in results from Canadian studies, where women were fed a 35% TFA diet [113]. The TFA level increased up to approximately 6%. In the same Canadian study a 2.5% TFA milk fat level was reported when a 13% TFA diet was fed [113]. These data suggested, together with tissue lipid fatty acid composition data, that incorporation of TFA appears to be restricted to some extent when the diet contains high amounts of TFA. On the other hand, the turnover of TFA is rapid. Most of the TFA are believed to be provided by mobilisation of TFA stored in adipose tissue triacylglycerol. Therefore, the TFA content of human milk lipids is higher and more variable than that for other tissue lipids because dietary fat composition has a large direct influence on the triacylglycerol fatty acid composition of milk lipid [112].

Tab. 8. Content of total TFA, 18:1 trans, and 18:2 trans in human milk from different countries. Values in brackets are minimum and maximum levels.

reference	total TFA	18:1 trans	18:2 trans
Chen et al. [48]	7.2 (4.17–10.23)	5.9 (3.38–8.42)	0.94
Boatella et al. [165]	1.3	1.0 (0.5–1.5)	0.29 (0.23–0.35)
Koletzko et al. [166]	4.4 (2.2–1.6)	3.1 (1.5–4.4)	0.20 (0.1–0.4)
Wolff [101]		1.99 (1.2–3.17)	

7 Physiological Properties

7.1 Physiological properties of TFAs

In the 1990s there has been increasing public health concern about epidemiological studies, which have suggested that TFAs increase the risk of coronary heart diseases (CHD) [114]. By contrast, analysis of human adipose tissue TFAs indicated no significant correlation between dietary TFA intake and acute myocardial infarction [115], cardiovascular risk factors [116], and sudden cardiac death [117].

Furthermore, clinical studies discovered that high intakes of TFA raise total cholesterol and low-density lipoprotein (LDL) cholesterol and lower high-density lipoprotein (HDL) cholesterol. An effect of TFA on the lipoprotein-A-level, which may be an independent risk factor for CHD, is also discussed.

Exhaustive information about the effect of TFA on human plasma cholesterol, lipoprotein, and triglyceride levels and their correlations with atherosclerosis and CHD has also been published elsewhere [118–121]. Therefore, the present review focuses on the physiological properties of the particular group of conjugated linoleic acids (CLA).

7.2 Physiological properties of CLA

7.2.1 Effect on carcinogenesis *in vivo* animal studies

CLA revealed impressive protective effects against tumorgenesis in animal studies. Because of the unique chemoprotective properties of CLA numerous studies were carried out to investigate the background of CLA. An excellent review about the possible modes of action of CLA was given by *Belury* [122]. In the following a brief overview of the chemoprotective properties of CLA is reported. The discussed physiological properties of CLA are summarized in Fig. 12.

To test the anti-initiation activity of CLA *Ha* et al. [123] used synthetically prepared CLA in the two-stage mouse epidermal carcinogenesis system. Mice were treated with 7,12-dimethylbenz(a)anthracene (DMBA) to induce cancer and after that with 12-O-tetradecanoylphorbol-13-acetate (TPA) to effect tumor promotion. Mice which were treated with CLA prior to DMBA gavage developed about half as many papillomas and exhibited a lower tumor incidence compared with the control mice.

In 1990 *Ha* et al. [124] investigated the inhibition of benzo-a-pyrene-induced mouse forestomach neoplasia by CLA in a similar experiment reported to that above. Mice treated with CLA developed only about half as many neoplasias/animal as mice in control groups ($P < 0.025$), in two of the experiments tumor incidence was also reduced ($P < 0.05$). Furthermore, CLA was more potent as an antioxidant than α-tocopherol and almost as effective as BHT. *Ha* et al. concluded that CLA might serve as an *in situ* defense mechanism against membrane attack by free radi-

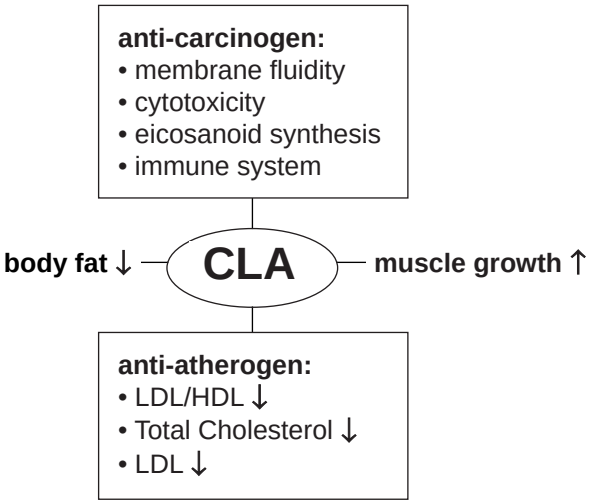

Fig. 12. Physiological effects of conjugated linoleic acids (CLA).

cals, which may explain the anticarcinogenic properties of CLA [124].

Ip et al. [125] studied the effect of CLA on the inhibition of development of mammary tumors induced by DMBA. Rats were fed either CLA free diet or diet supplemented with CLA (0.5, 1.0 or 1.5% by weight). The total number of mammary adenocarcinomas in CLA groups was reduced dose dependent by 32, 56, and 60%, respectively. Chronic feeding of up to 1.5% CLA produced no adverse consequences in the rats. The feeding of CLA for either one or six months resulted in a decrease in the extent of lipid peroxidation in the mammary gland, but not in the liver. *Ip* et al. found maximal antioxidant efficacy of CLA with only 0.25% CLA in the diet, whereas maximal tumor inhibition was achieved at about 1% CLA. This discrepancy between the antioxidant efficacy and anticarcinogenic potency led to the assumption that other mechanisms have to be involved in cancer protection [125].

The magnitude of tumor inhibition by 1% CLA was not influenced by the level or type of fat in the diet [126]. Fatty acid analysis showed that CLA was incorporated predominantly in mammary tissue neutral lipids, while the increase of CLA in mammary tissue phospholipids was minimal. Analysis of neutral lipids and phospholipids indicated that

- the accumulation of CLA in mammary tissue was dose dependent from 0.5% to 2.0%,
- CLA concentrations were ten times higher in neutral lipids than in phospholipids,
- the incorporation of CLA in either fraction was not affected by the availability of linoleic acid, and
- CLA did not appear to displace linoleic acid or arachidonic acid in the mammary tissue [127].

Ip et al. concluded that cancer preventive activity of CLA is unlikely to be mediated by interference with the metabolic cascade involved in converting linoleic acid to eicosanoids [126]. They also checked the antioxidative properties of CLA in mammary tissue. Treatment with CLA resulted in lower levels of mammary tissue malonaldehyde but failed to change the levels of 8-hydroxydeoxyguanosine, a known marker of oxidatively damaged desoxyribonucleic acid (DNA). Therefore, *Ip* et al. suggested that the proposed anticarcinogenic activity cannot be accounted for by protecting the target cell DNA against oxidative damage [126].

In further studies *Ip* et al. [128] investigated how the timing and duration of CLA feeding affect the development of mammary carcinogenesis in the methylnitrosourea (MNU) model in rats. They found that exposure to 1% CLA during the early postweaning and pubertal period was sufficient to reduce subsequent carcinogenesis. A continuous intake of CLA was required for maximal inhibition of tumorgenesis, however, when CLA feeding was started after MNU administration, it suggested that some active metabolites of CLA might be involved in suppressing the process of neoplastic progression. *Ip* et al. [125] also verified that the anticancer activities of free CLA and triglyceride CLA are essentially identical. These findings are important for further CLA study designs because free CLA isomers are more available.

In a continued study CLA was given to rats 1% by weight in the diet for either 4 weeks, 8 weeks or continuously following carcinogen administration [129]. Tumor inhibition was only significant in rats that were given CLA for the entire duration of the experiment (20 weeks). The rate of disappearance of neutral lipid CLA (rather than phospholipid CLA) subsequent to CLA withdrawal paralleled more closely the rate of occurrence of new tumors in the target tissue. *Ip* et al. proposed that neutral lipid CLA possibly are a more sensitive marker of tumor protection than phospholipid CLA [129].

Belury et al. [130] determined the role of increasing levels of dietary CLA in mouse skin tumor promotion elicited by TPA. Mice were fed no CLA diet during initiation, then the feed switched to diets containing 0.5, 1.0, or 1.5% by weight CLA during skin tumor promotion by TPA. Body weights of mice fed 0.5% up to 1.5% CLA were similar to each other but were significantly lower ($P < 0.05$) than weights of mice fed no CLA throughout promotion. A reduction in papilloma incidence was observed in mice fed 1.5% CLA from week 8 to 24 compared with mice fed diets containing 0–1.0% CLA ($P < 0.05$). 24 weeks after tumor promotion began, diets containing 1.0% and 1.5% CLA inhibited tumor yield compared with diets without CLA or 0.5% CLA. *Belury* et al. concluded that CLA inhibits tumor promotion in a manner that is independent of its anti-initiator activity [130].

Liew et al. [131] administered CLA to male rats on alternating days in week 1–4, while 2-amino-3-methylimidazo[4,5-f]-quinoline (IQ) was given every other day in week 3 and 4. A significant decrease in colonic aberrant crypt foci (ACF) of CLA-treated rats could be observed ($P < 0.05$). Furthermore, rats given CLA also had significantly lower IQ-DNA adducts in the colon as determined by ^{32}P-postlabeling analysis. In contrast to mechanism studies, which had indicated that CLA and other fatty acids interact with certain heterocyclic amines in a manner consistent with substrate-ligand binding, no such interaction occurred with IQ. CLA also failed to inhibit significantly the mutagenicity of N-hydroxy-IQ in the *Salmonella* assay. *Liew* et al. concluded that these results support a mechanism involving inhibition of carcinogen activation by CLA, as opposed to direct interaction with the procarcinogen or selective induction of phase I detoxification pathways [131].

7.2.2 Effect on human cancer cell cultures

During the last five years the effect of CLA on various human cancer cell cultures have been examined by numerous investigators. *Shultz* et al. [132] assessed the effect of CLA and β-carotene on human cancer cells (M21-HPB, malignant melanoma; HT-29, colorectal; MCF-7, breast). The incubation of cancer cells with CLA showed significant reductions of proliferation compared to control cultures (18–100%). M21-HPB and MCF-7 cell mortality was dose-

and time-dependent. MCF-7 cells supplemented with CLA incorporated significantly less [^{3}H]-leucine, [^{3}H]-uridine, and [^{3}H]-thymidine than control cultures. *Shultz* et al. deduced that growth inhibition may be due to an ability of CLA to inhibit protein and nucleotide biosynthesis [132]. These *in vitro* results suggested that CLA may be cytotoxic to human cancer cells *in vivo*.

Schønberg and *Krokan* examined the effect of linoleic acid and CLA on the growth of three different lung adenocarcinoma cell lines (A-427, SK-LU-1, A-549) and one human glioblastoma cell line (A-172) [133]. They found that CLA inhibited proliferation of the three human lung carcinoma cell lines in a dose and time dependent manner, but had no effect on proliferation of the human glioblastoma cell line. In contrast to CLA, linoleic acid exhibited no inhibitory effect. A significant increase of lipid peroxidation, measured as formation of malondialdehyde (MDA), was observed after treatment of the lung cell lines with 40 μm CLA.

Cunningham et al. [134] investigated the potential mechanism for the stimulation or inhibition of cell growth by LA and CLA by using eicosanoid synthesis inhibitors. Normal human mammary epithelial cells (HMEC) and MCF-7 breast cancer cells were incubated in a serum-free medium supplement with LA or CLA and cyclooxygenase (indomethacin, INDO, inhibiting prostaglandin synthesis) or lipoxygenase (nordihydroguaiaretic acid, NDGA, inhibiting leukotriene synthesis) inhibitors. LA stimulated the growth and [^{3}H]-thymidine incorporation of normal HMEC and MCF-7 cancer cells, while CLA was inhibitory. Supplementation with linoleic acid increased intracellular lipid peroxide concentrations in normal HMEC and MCF-7 cancer cells, whereas CLA did not affect lipid peroxide formation. The supplementation of HMEC and MCF-7 cells with linoleic acid and INDO or NDGA resulted in growth inhibition. The treatment of normal HMEC with CLA and NDGA or INDO stimulated cell growth, which seems to be paradoxical. However, the addition of CLA and NDGA (but not INDO) to MCF-7 cells resulted in synergistic growth suppression, suggesting that CLA effects were mediated through inhibition of leukotriene synthesis.

Durgam and *Fernandes* [135] studied whether the inhibitor action of CLA on MCF-7 human breast cancer cells is related to the estrogen responsiveness of MCF-7 cells. They showed that CLA selectively inhibits proliferation of estrogen receptor positive MCF-7 cells as compared with estrogen receptor negative MDA-MB-231 cells. Cell cycle studies indicated that a higher percentage of CLA treated MCF-7 cells remained in the G_0/G_1 phase as compared to control and those treated with LA. CLA also inhibited expression of protooncogene c-myc in MCF-7 cells. *Durgam* and *Fernandes* concluded that CLA may inhibit MCF-7 cell growth by interfering with the hormone regulated mitogenic pathway [135].

7.2.3 Effect on cancer inhibition via the immune system

Chew et al. [136] studied the *in vitro* effects of CLA isomers in combination with β-carotene on the immune system, which plays a central role in cancer defense. Porcine blood lymphocytes and murine peritoneal macrophages were incubated with CLA and β-carotene. CLA alone stimulated mitogen-induced lymphocyte proliferation, lymphocyte cytotoxic activity, and macrophage bactericidal activity, but CLA inhibited interleukin-2 production by lymphocytes and suppressed the phagocytic activity of macrophages. When present together, CLA and β-carotene interacted in an additive manner to further enhance lymphocyte cytotoxicity and spontaneous lymphocyte proliferation. Therefore, CLA and β-carotene, alone and in combination, act to modulate different aspects of cellular host defense [136].

Contrary results concerning lymphocyte cytotoxicity and interleukin-2 production by lymphophages were reported by *Wong* et al. [137], who studied the effect of CLA on lymphocyte function and growth of a transplantable murine mammary tumor. Female *Balbic* mice were fed 0.1, 0.3 or 0.9% CLA for 3 or 6 weeks. Lymphocyte proliferation in mice fed 0.3% and 0.9% CLA was enhanced in phytohemagglutinin-induced but not in concanavalin A or lipopolysaccharide-stimulated cultures. Production of interleukin-2 was also stimulated by CLA. In contrast, CLA had no effect on lymphocyte cytotoxicity.

In another experiment mice were fed the same diets for 2 weeks before being infused with 1×10^6 WAZ-2T metastatic mammary tumor cells into the right inguinal mammary gland. Tumor volume and latency were recorded for 45 days. Dietary CLA did not affect mammary tumor growth. Tumor latency, tumor incidence and tumor lipid peroxidation activity also were unaffected by CLA. Body weights and feed intake were similar among treatments. Therefore, *Wong* et al. suggested that dietary CLA modulated certain aspects of the immune defence but had no obvious effect on the growth of an established, aggressive mammary tumor [137]. The discrepancy between the results of *Chew* et al. [136] and results of *Wong* et al. [137] is probably due to differences between the *in vitro* and *in vivo* systems.

Visonneau et al. [138] examined the effect of dietary CLA mixture on the growth of human breast adenocarcinoma cells in serve combined immunodeficient (SCID) mice. Mice were fed 1% CLA for two weeks prior to subcutaneous inoculation of 10^7 MDA-MB 468 cells and throughout the study. Dietary CLA inhibited local tumor growth by 73% and 30% at 9 and 14 weeks post-inoculation, respectively. Moreover, CLA completely abrogated the spread of breast cancer cells to lungs, peripheral blood, and bone marrow. *Visonneau* et al. suggested, that these results indicate the ability of dietary CLA to block both the local growth and systemic spread of human breast cancer via mechanisms independent of the host immune system [138].

7.2.4 Further physiological properties

In addition to the chemoprotective properties of CLAs mentioned above CLAs have also been linked to an influence on growth and development of rats. A beneficial influence on the development of atherosclerosis has also been associated with CLA.

Belury and *Kempa-Steczko* [139] further examined the role of CLA in lipid metabolism. Mice were fed with 0, 0.5, 1.0, and 1.5% by weight CLA for six weeks. Mice fed 0.5% up to 1.5% CLA exhibited lower body weights compared with mice fed diets without CLA. In the liver, dietary CLA was incorporated into neutral and phospholipids at the expense of LA in diets. Oleate increased and arachidonate decreased in neutral lipids of CLA diet groups. In addition, increasing dietary CLA was associated with reduced LA in hepatic phospholipids. In an *in vitro* assay, CLA was desaturated to an unidentified 18:3 product to a similar extent as LA conversion to γ-linolenate. *Belury* and *Kempa-Steczko* suggested that CLA may affect metabolic interconversion of fatty acids in liver, which may ultimately result in a modified fatty acid composition and arachidonate-derived eicosanoid production in extrahepatic tissue [139].

Similar findings were reported by *Sébédio* et al. [140]. These authors studied the metabolism of CLA in rats by feeding high quantities of CLA (180 mg/day) for six days to animals, which had been reared on a fat-free diet for two weeks [140]. HPLC analysis revealed the presence of conjugated polyunsaturated fatty acids (PUFAs) in the total liver lipids. Three fatty acid metabolites were identified by GC-MS as being 20:3 Δ8,12,14, 20:4 Δ5,8,12,14, and 20:4 Δ5,8,11,13. A higher quantity of 20:4 Δ5,8,12,14 compared to 20:4 Δ5,8,11,13 was observed. These findings suggested that the formation of the observed conjugated PUFAs could be concluded from the elongation and desaturation of 18:2 Δ10,12, and 18:2 Δ9,11, respectively, and confirmed the results of *Belury* and *Kempa-Steczko* [139].

Miller et al. [141] examined the ability of CLA to prevent endotoxin-induced growth suppression. Mice fed a basal diet (containing 2.5% by weight LA) or diet with 0.5% by weight fish oil (contained mainly n-3 fatty acids) lost twice as much body weight after endotoxin injection (lipopolysaccharide from *E. coli* 055:B5) than mice fed CLA. By 72 h post-injection, mice fed CLA had body weights similar to vehicle injected controls. However, body weights of basal and fish oil fed mice injected with endotoxin were reduced.

Chin et al. [7] studied the effect of CLA on rat growth and development. Rats were fed control or CLA-supplemented (0.25% or 0.5% by weight CLA) diets during gestation and/or lactation. CLA was incorporated into milk fat and tissue lipids proportional to the level of CLA and the duration of CLA feeding. CLA was incorporated into fetal and neonatal tissues and did not affect litter size or induce apparent abnormalities. In contrast, feeding CLA to the dams during gestation and lactation improved the postnatal body weight gain of pups, measured for 10 days of lactation. Pups that continued to receive the CLA-supplemented diet after weaning had significantly greater body weight gain and improved feed efficiency relative to control animals ($P < 0.05$).

Currently, only a few data about the effect of CLA on atherosclerosis are available. *Lee* et al. [142] assessed the effect of CLA on atherosclerosis in rabbits. They fed 12 rabbits with a semi-synthetic diet (14% fat and 0.1% cholesterol) for 22 weeks. For 6 rabbits the diet was supplemented with 0.5 g CLA/rabbit per day. By 12 weeks total and LDL-cholesterol and triglycerides were markedly lower in the CLA-fed group. Additionally, the LDL-cholesterol to HDL-cholesterol ratio was significantly reduced in the CLA-fed group ($P < 0.05$). Examination of the aortas of CLA-fed rabbits showed less atherosclerosis.

8 Conclusion

Partially hydrogenated vegetable oils, dietary products and meat from ruminants are the major sources of TFA in the diet. During the past five years the TFA content in most European margarines has decreased significantly. Therefore, the total daily per capita availability of TFAs has decreased in a same manner. At the same time the consumption of beef products has also decreased due to the well-reported bovine spongiform encephalopathy (BSE). It has to be taken into consideration, however, that this might only be a temporary trend towards decreased beef consumption.

In numerous foods, particularly in dairy foods, CLA isomers have been observed. In contrast to the conventional TFAs, CLAs are linked with unique chemoprotective anticarcinogenic properties. Diverse investigators have reported that CLA exerts anticarcinogenic activity in different kinds of tumors and different stages of tumor development. In addition, CLA has been reported to modulate immune-induced growth suppression, to overcome catabolic responses due to injection of endotoxin, to reduce atherosclerosis and total cholesterol and to alter the LDL/HDL ratio advantageously in rabbits. More recently, CLA has been reported to inhibit the growth and metastases of human breast adenocarcinoma cells implanted in mice. Currently, the mechanisms by which CLA inhibits carcinogenesis at different stages remain uncertain. Because CLA is incorporated into cell membrane phospolipids it may inhibit carcinogenesis by modulating several cellular events that are mediated in part of the plasma membrane. The replacement of other PUFAs with CLAs in the plasma membrane may affect events such as oxidative stress, eicosanoid synthesis, and signal transduction. Each of these events may, in turn, modulate carcinogenesis.

To date, all reported physiological studies of CLA have been carried out with mixtures of CLA isomers (mainly 18:2 c9t11). Therefore, the biologically active isomer(s) remain(s) unknown. In a number of foods, particularly dairy products, and in human adipose tissue some minor CLA isomers have been detected. To evaluate their biological significance, their structure and double bond configuration have to be elucidated unequivocally. Pure individual reference CLA isomers will have to be synthesized in larger amounts. The properties of these isomers should be investigated in further studies. It may also be possible that CLA metabolites, e.g. oxidation products (furan fatty acids), are responsible for the biological effects.

Although it is controversial to directly extrapolate from animal studies or cell cultures studies to human beings, CLA may be able to render lasting protection against subsequent cancer risk. Adverse sideeffects of CLAs have not been observed as yet. Thus, it might be desirable to enhance the CLA concentrations in foods to obtain beneficial level. A supplementation of CLAs should therefore be considered.

Abbreviations

ACF, aberrant crypt foci; AMP, 2-amino-2-methylpropanole; AOAC, Association of Official Analytical Chemists; AOCS, American Oil Chemists' Society; ATR, attenuated total reflection; BHT, butylated hydroxytoluene; BSE, bovine spongiform encephalopathy; CHD, coronary heart disease; CLA, conjugated linoleic acids; COT, conjugated octadecatrienes; DD, direct deposition; DMBA, 7,12-dimethylbenz(a)anthracene; DMOX, 4,4-dimethyl oxazoline; DNA, desoxyribonucleic acid; EIMS, electron impact ionization mass spectrometry; FAMEs, fatty acid methyl esters; FID, flame ionization detector; FTIR, *Fourier* transform infrared spectrometry; fwhh, full width half height; GC, gas chromatography; HATR, horizontal attenuated total reflection; HDL, high-density lipoprotein; HMEC, human mammary epithelia cell; HPLC, high-performance liquid chromatography; INDO, indomethacin; IQ, 2-amino-3-methyl imidazo[4,5-f]-quinoline; IR, infrared; K, degree Kelvin; LA, linoleic acid; LDL, low-density lipoprotein; LP, light pipe; MDA, malondialdehyde; MI, matrix isolation; MIQ, minimum identifiable quantity; MNU, methyl nitrosourea; MS, mass spectrometry; NDGA, nordihydroguaiaretic acid; NMR, nuclear magnetic resonance; P, level of probability; PG, propyl gallate, PUFA, polyunsaturated fatty acid; resp, respectively; R_f retention factor; SB, single beam; SCID, severe combined immunodeficient; SFA, saturated fatty acids; TBARS, thiobarbituric reactive substances; TFA, trans fatty acids; TLC, thin-layer chromatography; TPA, 12-O-tetradecanoylphorbol-13-acetate; UV, ultraviolet.

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