Occurrence and biochemical characteristics of natural bioactive substances in bovine milk lipids

Joachim Molkentin*

Institute for Chemistry and Physics, Federal Dairy Research Centre, PO Box 6069, D-24121 Kiel, Germany

Bovine milk lipids (BML) contain a number of bioactive substances with positive as well as negative properties, mainly in the class of fatty acids. Besides *trans* fatty acids (TFA), conjugated linoleic acids (CLA) are of particular interest. Apart from ruminant meat products the main source of CLA in food are BML. Although TFA as well as saturated fatty acids (12:0–16:0) are thought to be positively correlated with atherosclerosis and coronary heart disease, CLA are considered antiatherogenic. Further, CLA are reported to reduce adipose fat and to have anticarcinogenic properties. The varying CLA and TFA contents of lipids from milk and dairy products are positively correlated with one another. However, TFA are also negatively correlated with 12:0–16:0 in BML. Anticarcinogenic effects are also ascribed to butyric acid as well as to some phospholipids and ether lipids present in BML. Moreover, the essential fatty acids 18:2*n-6* and 18:3*n-3* are found in BML which are involved in a variety of biochemical processes and thus have numerous functions in human metabolism. Contents of the individual bioactive components of BML are summarised taking into account also seasonal variations. The total content of bioactive substances in BML is approximately 75 % but their overall impact on human health considering benefits and drawbacks is difficult to assess.

Bioactive substances: Bovine milk fat: *Trans* fatty acids: Conjugated linoleic acid

Introduction

In the industrial countries malnutrition is hardly a problem anymore, so that concern increasingly focuses on the quality rather than the quantity of food. Thus, the biological activities of food ingredients are considered to facilitate enhancement of favourable effects or enable avoidance of harmful influences by specific selection of food items. With an average daily per capita consumption of lipids from milk and dairy products of 28·2 g (women) or 33·1 g (men) in Germany (Adolf *et al.* 1994) bovine milk lipids (BML) can make an important contribution to the quality of the total diet. Bovine milk fat comprises several classes of lipids. Besides mono-, di- and triacylglycerols containing a great variety of fatty acids, there are also free fatty acids, phospholipids, glycolipids and steroids (including cholesterol esters) present in milk lipids. Minor constituents include waxes, alcanols, carotinoids, vitamins and lipoproteins. In the following sections classes that are reviewed include substances with known bioactive properties (except for fat-soluble vitamins) comprising positive as well as negative effects. With respect to bovine milk fat, saturated and *trans* fatty acids as well as conjugated linoleic acids have been widely discussed in recent years. In addition to data on the contents of bioactive milk fat components and their seasonal variation, biochemical properties are also summarized here.

Fatty acids

Free fatty acids comprise only approximately 0·1–0·4 wt % of total milk lipids (Frede *et al.* 1990). Fatty acids are mainly bound in triacylglycerols as well as in smaller amounts in diacylglycerols and monoacylglycerols which altogether make up the greatest part of milk lipids. After consumption acylglycerols are lipolized to monoacylglycerols and free fatty acids for absorption, with the rate of lipolysis depending on the position of the fatty acid in the triacylglycerol molecule. Although triacylglycerols are subsequently resynthesized for transport of fatty acids in the body (with the exception of fatty acids up to 10:0), the major bioactive effects are due to the actions of free fatty acids or their metabolites.

The determination of the fatty acid composition of milk fat is usually achieved by base-catalysed *trans*-esterification of the anhydrous fat into fatty acid methyl esters (FAMEs) followed by gas chromatographic analysis of the FAMEs.

Saturated fatty acids

In physiological terms saturated fatty acids can be divided into short-chain (≤4:0), medium-chain (6:0–10:0), and long-chain (≥12:0) fatty acids, although different classifications are used in other contexts. On average, 4:0–10:0

* **Corresponding author:** J. Molkentin, telefax +49 431 6092300, email molkentin@bafm.de

account for 10·6–12·8 wt % of total fatty acids in milk fat (Precht, 1990; Jensen & Newburg, 1995). These short- and medium-chain fatty acids are particularly digestible, as they are hydrolysed preferentially from the triacylglycerols and are transferred directly from the intestine to the portal circulation without resynthesis of triacylglycerols. Thus, there is only a low tendency for adipose formation. Further, short- and medium-chain fatty acids can have the effect of reducing cholesterol levels in serum and liver, as well as triacylglycerols levels (Frede *et al.* 1990). Moreover, butyric acid (4:0) is an antineoplastic agent (Parodi, 1996, 1997). Thus, 4:0 is reported to inhibit proliferation and to induce differentiation (*in vitro*) of a variety of neoplastic cells (Prasad, 1980; Chen & Breitmann, 1994) and to inhibit tumour development in mice (Pouillart *et al.* 1991). It also modulates the expression of oncogenes and suppressor genes (Rabizadeh *et al.* 1993). In milk fat 4:0 accounts for 7·5–13·0 mole % (Parodi, 1970) or 3·42 wt % related to fat (Molkentin & Precht, 1997*a*). There is no obvious relation between the 4:0 content in milk fat and the feeding conditions of cows (Precht *et al.* 1985).

The average content of the long-chain fatty acids 12:0–16:0 in milk fat is 42·4–44·0 wt % of total fatty acids (Precht, 1990; Jensen & Newburg, 1995). These fatty acids are reported to increase the level of total and LDL cholesterol in plasma (Keys *et al.* 1965; Mattson & Grundy, 1985; Dupont *et al.* 1991; Zöllner & Tatò, 1992; Zock, 1995). An elevated LDL-cholesterol level is considered a risk factor for atherosclerosis (see section on *trans* fatty acids). On the other hand, the long-chain stearic acid (18:0), with an average content of 9·2–12·6 wt % in milk fat (Precht, 1990; Jensen & Newburg, 1995), is reported to be neutral in its effect on lipoprotein cholesterol levels (Hegsted *et al.* 1965; Bonanome & Grundy, 1988; Grundy & Denke, 1990).

Monounsaturated fatty acids

Oleic acid is the predominant monounsaturated fatty acid in milk fat and accounts for 20·1–20·8 wt % of total fatty acids on average (Precht, 1990; Jensen & Newburg, 1995) and for 12·8–27·6 wt % under special conditions. Contents are higher during pasture feeding than during feeding of concentrates. Human diets high in oleic acid are mostly reported to decrease the level of LDL-cholesterol compared to diets containing less oleic acid, whereas HDL-cholesterol levels are not affected significantly (Mattson & Grundy, 1985; Mensink & Katan, 1989; Berry *et al.* 1991; Chan *et al.* 1991; Mata *et al.* 1992; Valsta *et al.* 1992; Katan *et al.* 1994; Zock, 1995). Thus, oleic acid can be regarded as antiatherogenic (see further section on *trans* fatty acids).

Polyunsaturated fatty acids

The polyunsaturated fatty acids (PUFAs) in milk fat mainly comprise linoleic acid (18:2*n-6*) and α-linolenic acid (18:3*n-3*) as well as smaller amounts of their positional and geometric isomers. Conjugated isomers of linoleic acid are dealt with in the next section. On average, 18:2*n-6* and 18:3*n-3* account for 1·2–2·0 wt % and 0·5–0·7 wt % of total fatty acids, respectively (Precht, 1990; Jensen & Newburg, 1995; Precht & Molkentin, 1997*a*). These are essential fatty acids and have many and diverse functions in human metabolism. Their deficiency leads to a variety of physiological aberrations (e.g. Kinsella *et al.* 1990; Horrobin, 1997).

Moreover, 18:2*n-6* is reported to have a LDL-cholesterol-decreasing effect but, according to some studies, may also cause a slight decrease of HDL-cholesterol levels (Mattson & Grundy, 1985; Beynen & Katan, 1989; Mensink & Katan, 1989; Grundy & Denke, 1990; Zöllner & Tatò, 1992; Katan *et al.* 1994). Overall, linoleic acid should promote an antiatherogenic effect (see further section on *trans* fatty acids). However, recent studies have proposed an atherogenic influence of PUFAs present in the triacylglycerol and cholesterolester moieties of LDLs. Due to their ease of oxidation the PUFAs may form cytotoxic compounds in the LDLs (ox-LDLs). These ox-LDLs may then cause atherosclerotic changes in the blood vessels (Witztum & Steinberg, 1991; Holvoet & Collen, 1994; Parthasarathy & Santanam, 1994). However, compared with vegetable fats the PUFA contents in milk fat are relatively low.

The oxidation products of PUFAs are also potentially mutagenic and can lead to the development of malignant tumours (Marnett, 1994). Further, in animal studies the consumption of linoleic acid has been correlated with tumour promotion, whereas some *n*-3 PUFAs have been shown to have protecting properties (Cohen *et al.* 1986; Pariza, 1988; Reddy, 1994). However, this topic remains controversial (Ip, 1997). Finally, PUFAs are known to exhibit immune-modulating activities and properties affecting coagulation of blood (e.g. see references in Pfeuffer, 1997).

Conjugated linoleic acids

Conjugated linoleic acids (CLA) comprise a group of conjugated dienes occurring mainly in milk fat (Chin *et al.* 1992; Precht & Molkentin, 1997*a*). CLA is derived from dietary linoleic acid and, according to a recent hypothesis (Precht & Molkentin, 1998), possibly also from linolenic acid by rumen bacteria. A number of positional and geometric isomers are formed with *cis*-9,*trans*-11-octadecadienoic acid (*cis*-9,*trans*-11-18:2) being the predominant (90 %) isomer (Chin *et al.* 1992). As *cis*-9,*trans*-11-18:2 is derived from linoleic acid and possibly linolenic acid, there is a tendency toward a higher level in milk fat obtained during pasture feeding in summer than in winter when cows are fed more on concentrates (Dhiman *et al.* 1996; Jahreis *et al.* 1996; Precht & Molkentin, 1997*b*; Stanton *et al.* 1997; Kelly *et al.* 1998; Precht & Molkentin, 1998). Therefore, an increase in the content of the favourable *cis*-9,*trans*-11-18:2 can be easily achieved by special feeding conditions. However, this leads to a simultaneous increase in unfavourable *trans*-18:1 acids (see next section) and also to a decrease in saturated fatty acids 12:0–16:0 (Precht & Molkentin, 1997*c*; Precht & Molkentin, 1999). Thus, there is a high correlation between *cis*-9,*trans*-11-18:2 and *trans*-18:1 (total) of $r = 0{\cdot}98$ ($n = 100$) (Precht & Molkentin, 1997*a*) and between *cis*-9,*trans*-11-18:2 and *trans*-11-18:1 of $r = 0{\cdot}78$ ($n = 28$) (Jiang *et al.* 1996) or of $r = 0{\cdot}98$

Table 1. Mean contents of conjugated linoleic acid *cis*-9,*trans*-11-18:2 in milk fat reported from previous literature; *n* = number of samples

Origin (*n*)	Feeding condition	Content	mg/g of	Reference
Homog. milk (3)	–	5.06	F	Chin *et al.* 1992
Butter (4)	–	4.14	F	Chin *et al.* 1992
Butter fat (4)	–	5.43	F	Chin *et al.* 1992
Butter (31)	Average	8.5	F	Henninger & Ulberth, 1994
Milk (4)	–	4.60	F	Lin *et al.* 1995
Milk (22)	50 % concentrate*	5.04	F	Jiang *et al.* 1996
Milk (22)	65 % concentrate*	11.28	F	Jiang *et al.* 1996
Milk (19)	65 % concentrate†	6.56	F	Jiang *et al.* 1996
Butter (2)	–	10.7	FA	Fogerty *et al.* 1988
Milk (12)	Indoor/1 year	3.4	FA	Jahreis *et al.* 1996
Milk (12)	Pasture/1 year	6.1	FA	Jahreis *et al.* 1996
Milk (12)	Ecological/1 year	8.0	FA	Jahreis *et al.* 1996
Butter (927)	Barn	4.5	FA	Precht & Molkentin, 1997*b*
Butter (236)	Transition period	7.6	FA	Precht & Molkentin, 1997*b*
Butter (593)	Pasture	12.0	FA	Precht & Molkentin, 1997*b*
Butter (1756)	Average	7.50	FA	Precht & Molkentin, 1997*b*
Milk (7)	–	11.6	FAME	Fritsche & Steinhart, 1998
Butter (12)	–	9.4	FAME	Fritsche & Steinhart, 1998

F, Fat; FA, fatty acids; FAME, fatty acid methyl esters.
*Restricted amount; †unrestricted amount (ad libitum).

($n = 100$) (Precht & Molkentin, 1997*a*). The contents of *cis*-9,*trans*-11-18:2 found under common feeding conditions are shown in Table 1 and comprise between 4 and 12 mg/g fat or fatty acids. It is important to note that comparisons of CLA contents between individual dairy products can only be made when these have been derived from the same milk source.

The predominant isomer *cis*-9,*trans*-11-18:2 is believed to be the biologically active form of CLA, as a preferred incorporation of this molecule into the phospholipids of cell membranes has been found (Ha *et al.* 1990; Ip *et al.* 1991). In contrast to other *trans* fatty acids, probably this particular CLA isomer exhibits several positive effects. However, some studies on the biological activity of CLA have not only investigated *cis*-9,*trans*-11-18:2 but have also included *trans*-9,*cis*-11-18:2 and further conjugated isomers. Anticarcinogenic properties have been found in animal studies (Ha *et al.* 1987, 1990; Ip *et al.* 1991, 1994) as well as in human cell culture studies (Shultz *et al.* 1992). The anticancer efficacy of CLA is already expressed at concentrations close to human consumption levels (Ip *et al.* 1994). The mode of action is possibly due to antioxidant mechanisms (Ha *et al.* 1990; Ip *et al.* 1991), inhibition of nucleotide synthesis (Shultz *et al.* 1992), reduction of proliferative activity (Ip *et al.* 1994) or other mechanisms (Belury, 1995; Jahreis, 1997; Parodi, 1997). However, a recent study failed to confirm that CLA has an effect as an antioxidant in fats and oils (Chen *et al.* 1997). Moreover, antiatherogenic (Lee *et al.* 1994; Nicolosi *et al.* 1997) and immunomodulating (Michal *et al.* 1992; Cook *et al.* 1993; Miller *et al.* 1994) properties have been reported from animal studies. Further, CLA has been shown to act as a growth factor in rats (Chin *et al.* 1994) and to reduce adipose fat (Dugan *et al.* 1997; Park *et al.* 1997).

Trans fatty acids

Trans fatty acids (TFA) in milk fat result from partial hydrogenation of PUFAs caused by rumen bacteria. Therefore, the TFA content depends among other things on the amount of PUFAs in the fodder. Thus, an extensive supply of linoleic and linolenic acid during pasture feeding in summer leads to higher TFA contents than concentrates given in winter (e.g. Henninger & Ulberth, 1994; Wolff, 1994; Precht, 1995; Precht & Molkentin, 1997*b*). TFAs occurring in milk fat are mostly *trans*-octadecenoic acids (*trans*-18:1). As mentioned above, an increase of *trans*-18:1 is associated strongly with a rise in CLA content (Jiang *et al.* 1996; Precht & Molkentin, 1997*a*, 1999). Moreover, *trans*-18:1 is negatively correlated with the unfavourable saturated fatty acids 12:0–16:0 (Precht & Molkentin, 1997*c*, 1999).

Further, smaller amounts of *trans*-octadecadienoic acids (*trans*-18:2), with at least one *trans* double bond, and of *trans*-hexadecenoic acids (*trans*-16:1) are found. To obtain reliable data, quantitation of TFAs should be done by gas chromatography of the *trans*-FAME fraction isolated by Ag-TLC or Ag-HPLC. Moreover, application of 100-m capillary columns then enables separation of almost all positional isomers (Molkentin & Precht, 1995, 1997*b*; Wolff & Bayard, 1995). Table 2 summarizes the typical contents of *trans* fatty acids. Table 3 shows as an example the isomeric distribution of *trans*-18:1 fatty acids typical of German milk fats (Precht & Molkentin, 1996).

In connection with the consumption of *trans* fatty acids, numerous biological effects have been described. Influences on serum cholesterol levels in the different lipoprotein classes have been reported. Thus, *trans*-octadecenoic acids are thought to increase the LDL-cholesterol level unfavourably (Mensink & Katan, 1990; Zock & Katan, 1992; Lichtenstein *et al.* 1993; Siguel & Lerman, 1993; Wood *et al.* 1993; Judd *et al.* 1994; Zock, 1995) as well as to decrease the favourable HDL-cholesterol level (Mensink & Katan, 1990; Siguel & Lerman, 1993; Judd *et al.* 1994; Zock, 1995). Thus, the LDL/HDL ratio is particularly adversely affected. Possibly

Table 2. Contents of *trans* fatty acids (TFA) in milk fat (wt% of fatty acids) mainly obtained by Ag-TLC/GC; *n* = number of samples

Mean	Minimum	Maximum	*n*	Country	TFA	Reference
6·01*	4.27*	7.61*	116	Australia	total	Parodi & Dunstan, 1971
–	4.0*	5.7*	13	Canada	total	DeMan & DeMan, 1983
5·53†	4.52†	7.3†	17	New Zealand	mono‡	Gray, 1973
3·62	1.29	6.75	1756	Germany	18:1	Precht & Molkentin, 1996
3·33	1.75	5.20	31	Austria	18:1	Henninger & Ulberth, 1994
3·80	2.46	5.10	24	France	18:1	Wolff, 1994
0·59§	0.27§	0.90§	31	Austria	18:2‖	Henninger & Ulberth, 1994
0·63	0.11	1.41	1756	Germany	18:2‖	Precht & Molkentin, 1997*b*
0·11	0.04	0.16	24	France	16:1	Wolff, 1994
0·13	0.05	0.25	27	Germany	16:1	Molkentin & Precht, 1997*b*

*obtained by IR; †obtained by Ag-TLC/densitometer; ‡*trans* monoenoic acids; §g/100 g fat; ‖without *cis*-9,*trans*-11-18:2 (CLA).

the increase in LDL-cholesterol could be attributed to a reduction in the activity of lecithin–cholesterol-acyltransferase (Koletzko, 1991) or to an increase in the activity of cholesterol transfer protein (Lagrost, 1992). Moreover, an unfavourable increase of the lipoprotein(a) level has been found (Mensink *et al.* 1992; Nestel *et al.* 1992). These changes caused by 18:1-TFA may contribute to premature atherosclerosis and coronary heart disease where intakes of TFA are particularly high (e.g. see references in Precht & Molkentin, 1995).

Furthermore, TFA have been reported to impair desaturation and chain elongation of the essential fatty acids *cis*-9,*cis*-12-18:2 and *cis*-9,*cis*-12,*cis*-15-18:3 and thus to inhibit prostaglandin synthesis (see references in Koletzko, 1992). These effects were partly related to the consumption of *trans*-9-18:1 and *trans*-9,*trans*-12-18:2. The isomer *trans*-9,*trans*-12-18:2 is also reported to cause a variety of further physiological and toxicological aberrations (Emken, 1984; Hunter & Applewhite, 1986). However, carcinogenic properties of TFA (Enig *et al.* 1978; Ip, 1997) are rather more controversial.

In contrast to *trans*-9,*trans*-12-18:2 the conjugated linoleic acid *cis*-9,*trans*-11-18:2 exclusively exhibits positive biological properties that are dealt with in the preceding section. When interpreting physiological effects, the example of *trans*-18:2 demonstrates that the total amount of TFAs in a fat probably provides less relevant information than the individual isomeric contents. This might also be concluded from epidemiological studies (Willett *et al.* 1993) and may be of particular significance for the positional isomers of *trans*-18:1 (Table 3).

Some studies regard *trans*-16:1 from milk fat as the cause of an elevated incidence of coronary heart disease (CHD). In recent investigations these findings have been questioned because of possibly incorrect gas chromatographic analyses (Molkentin & Precht, 1997*b*). According to this new study bovine milk fats (0·13 wt %) do not contain considerably higher amounts of *trans*-16:1 than partially hydrogenated vegetable fats (0·04 wt %). Moreover, the correlations found in former studies between the content of *trans*-16:1 in human tissues or plasma and the incidence of CHD may be due more to an association of *trans*-16:1 with *trans*-20:1 in partially hydrogenated marine oils, which are the main source of *trans*-16:1 (1·89 wt %) in food. Thus, a negative effect of *trans*-16:1 specifically from milk fat is disputed.

Table 3. Mean isomeric distribution of *trans*-18:1 fatty acids (% of total *trans*-18:1) derived from 1756 German milk fats (Precht & Molkentin, 1996)

Isomer	Δ4	Δ5	Δ6–8	Δ9	Δ10	Δ11	Δ12	Δ13/14	Δ15	Δ16
Proportion	1·6	1·5	4·7	6·9	4·7	43·2	6·3	14·2	7·9	9·8

Phospholipids

Phospholipids are associated with the milk fat globule membrane and account for 0·2–1·0 wt % of total milk lipids (Christie *et al.* 1987). Some phospholipids exhibit antioxidative properties in milk/milk fat products with low water content (Frede *et al.* 1990; Saito & Ishihara, 1997). Further, sphingomyelin and its metabolites ceramide and sphingosine are reported to have tumour-suppressing properties (Parodi, 1996, 1997) by influencing cell proliferation. Sphingomyelin accounts for 20–35 wt % of total milk phospholipids (Christie *et al.* 1987; Frede *et al.* 1990).

Ether lipids

Ether lipids present in milk fat include alkyldiacylglycerols and alkylacylphospholipids. Milk neutral lipids are reported to contain 0·01 wt % of 1-*O*-alkyldiacylglycerols and milk phospholipids to contain 0·16 wt% 1-*O*-alkylacylphospholipids (Hallgren *et al.* 1974). Ether lipids and their derivatives have antineoplastic effects (Parodi, 1996, 1997). The biological activities exerted on cancer cells comprise inhibition of growth, antimetastatic activity and induction of differentiation and apoptosis (Berdel, 1991; Diomede *et al.* 1993). It is believed that ether lipids are incorporated and accumulated in cell membranes and thereby influence biochemical and biophysical processes.

Steroids

Cholesterol is the major sterol in milk comprising 0·25–0·45 wt % of total lipids. Its content depends among other things on the method of fat extraction and is believed to be near 0·4 wt % in most cases (Frede *et al.* 1990). Further, milk fat contains small amounts of lanosterol, dihydrolanosterol and 7-dehydrocholesterol (provitamin D_3).

Cholesterol is important for the resorption of fats and is a precursor in the synthesis of steroid hormones. Negative effects result from elevated plasma cholesterol levels in particular in combination with certain fatty acids with regard to atherosclerosis and coronary heart diseases (see section on fatty acids).

Conclusion

Bovine milk lipids contain a variety of bioactive substances, exhibiting positive as well as negative physiological effects. The assessment of the overall impact on human health considering benefits and drawbacks is difficult. Current efforts to increase CLA in BML should not ignore their positive correlation with TFA. However, an independent raising of CLA could further improve the physiological properties of milk fat.

References

Adolf T, Eberhardt W, Heseker H, Hartmann S, Herwig A, Matiaske B, Moch KJ, Schneider R & Kübler W (1994) Lebensmittel- und Nährstoffaufnahme in der Bundesrepublik Deutschland. In *VERA-Schriftenreihe, Band XII* [W Kübler, HJ Anders and W Heeschen, editors]. Niederkleen: Wissenschaftlicher Fachverlag Dr. Fleck.

Belury MA (1995) Conjugated dienoic linoleate: a polyunsaturated fatty acid with unique chemopreventive properties. *Nutrition Reviews* **53**, 83–89.

Berdel WE (1991) Membrane-interactive lipids as experimental anticancer drugs. *British Journal of Cancer* **64**, 208–211.

Berry EM, Eisenberg S, Haratz D, Friedlander Y, Norman Y, Kaufmann NA & Stein Y (1991) Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins – The Jerusalem Nutrition Study: high MUFAs vs high PUFAs. *American Journal of Clinical Nutrition* **53**, 899–907.

Beynen AC & Katan MB (1989) Impact of dietary cholesterol and fatty acids on serum lipids and lipoproteins in man. In *Role of Fats in Human Nutrition*, pp. 237–286 [AJ Vergroesen and M Crawford, editors]. London: Academic Press.

Bonanome A & Grundy SM (1988) Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. *New England Journal of Medicine* **318**, 1244–1248.

Chan JK, Bruce VM & McDonald BE (1991) Dietary ω3-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. *American Journal of Clinical Nutrition* **53**, 1230–1234.

Chen ZX & Breitman TR (1994) Tributyrin: a prodrug of butyric acid for potential clinical application in differentiation therapy. *Cancer Research* **54**, 3494–3499.

Chen ZY, Chan PT, Kwan KY & Zhang A (1997) Reassessment of the antioxidant activity of conjugated linoleic acids. *Journal of the American Oil Chemists' Society* **74**, 749–753.

Chin SF, Liu W, Storkson JM, Ha YL & Pariza MW (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *Journal of Food Composition and Analysis* **5**, 185–197.

Chin SF, Storkson JM, Albright KJ, Cook ME & Pariza MW (1994) Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *Journal of Nutrition* **124**, 2344–2349.

Christie WW, Noble RC & Davies G (1987) Phospholipids in milk and dairy products. *Journal of the Society of Dairy Technology* **40**, 10–12.

Cohen LA, Thompson DO, Maeura Y, Choi K, Blank ME & Rose DP (1986) Dietary fat and mammary cancer. I. Promoting effects of different dietary fats on *N*-nitrosomethylurea-induced rat mammary tumorigenesis. *Journal of the National Cancer Institute* **77**, 33–42.

Cook MC, Miller CC, Park Y & Pariza M (1993) Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poultry Science* **72**, 1301–1305.

DeMan L & DeMan JM (1983) *Trans* fatty acids in milk fat. *Journal of the American Oil Chemists' Society* **60**, 1095–1098.

Dhiman TR, Anand GR, Satter LD & Pariza M (1996) Conjugated linoleic acid content of milk from cows fed different diets. *Journal of Dairy Science* **79S1**, 35.

Diomede L, Colotta F, Piovani B, Re F, Modest EJ & Salmona M (1993) Induction of apoptosis in human leukemic cells by the ether lipid 1-octadecyl-2-methyl-*rac*-glycero-3-phosphocholine. A possible basis for its selective action. *International Journal of Cancer* **53**, 124–130.

Dugan MER, Aalhus JL, Schaefer AL & Kramer JKG (1997) The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. *Canadian Journal of Animal Science* **77**, 723–725.

Dupont J, White PJ & Feldman EB (1991) Saturated and hydrogenated fats in food in relation to health. *Journal of the American College of Nutrition* **10**, 577–592.

Emken EA (1984) Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. *Annual Review of Nutrition* **4**, 339–376.

Enig MG, Munn RJ & Keeney M (1978) Dietary fat and cancer trends – a critique. *Federal Proceedings* **37**, 2215–2220.

Fogerty AC, Ford GL & Svoronos D (1988) Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk. *Nutrition Reports International* **38**, 937–944.

Frede E, Precht D & Timmen H (1990) Lipide: Fettsäuren, Fette und Fettbegleitstoffe einschließlich fettlöslicher Vitamine. In *Kompendium zur milchwirtschaftlichen Chemie*, pp. 57–78 [E Schlimme, editors]. München: Volkswirtschaftlicher Verlag.

Fritsche J & Steinhart H (1998) Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A* **206**, 77–82.

Gray IK (1973) Seasonal variations in the composition and thermal properties of New Zealand milk fat. *Journal of Dairy Research* **40**, 207–214.

Grundy SM & Denke MA (1990) Dietary influences on serum lipids and lipoproteins. *Journal of Lipid Research* **31**, 1149–1172.

Ha YL, Grimm NK & Pariza MW (1987) Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* **8**, 1881–1887.

Ha YL, Storkson JM & Pariza MW (1990) Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Research* **50**, 1097–1101.

Hallgren B, Niklasson A, Stallberg G & Thorin H (1974) On the occurrence of 1-*O*-alkylglycerols and 1-*O*(2-methoxyalkyl)glycerols in human colostrum, human milk, cow's milk, sheep's milk, human red bone marrow, red cells, blood plasma and a uterine carcinoma. *Acta Chemica Scandinavica* B**28**, 1029–1034.

Hegsted DM, McGandy RB & Myers ML (1965) Quantitative effects of dietary fat on serum cholesterol in man. *American Journal of Clinical Nutrition* **17**, 281–295.

Henninger M & Ulberth F (1994) *Trans* fatty acid content of bovine milk fat. *Milchwissenschaft* **49**, 555–558.

Holvoet P & Collen D (1994) Oxidized lipoproteins in atherosclerosis and thrombosis. *FASEB Journal* **8**, 1279–1284.

Horrobin DF (1997) Essentiality of oils and fats for human nutrition and disease. In *Modern Developments in Food Lipids*, pp. 171–192 [VKS Shukla and SP Kochhar, editors]. Aarhus/Danmark: International Food Science Center (ISBN 87-894166-3-4).

Hunter JE & Applewhite TH (1986) Isomeric fatty acids in the US diet: levels and health perspectives. *American Journal of Clinical Nutrition* **44**, 707–717.

Ip C (1997) Review of the effects of *trans* fatty acids, oleic acid, *n*-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. *American Journal of Clinical Nutrition* **66**, (Suppl), 1523S–1529S.

Ip C, Chin SF, Scimeca JA & Pariza MW (1991) Mammary cancer prevention by conjugated linoleic acid. *Cancer Research* **51**, 6118–6124.

Ip C, Singh M, Thompson HJ & Scimeca JA (1994) Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. *Cancer Research* **54**, 1212–1215.

Jahreis G (1997) Krebshemmende Fettsäuren in Milch und Rindfleisch. *Ernährungs-Umschau* **44**, 168–172.

Jahreis G, Fritsche J & Steinhart H (1996) Monthly variation of milk composition with special regard to fatty acids depending on season and farm management sytems – conventional versus ecological. *Fett/Lipid* **98**, 356–359.

Jensen RG & Newburg DC (1995) Bovine milk lipids. In *Handbook of Milk Composition*, pp. 543–575 [RG Jensen, editors]. San Diego/CF: Academic Press.

Jiang J, Bjoerk L, Fonden R & Emanuelson M (1996) Occurrence of conjugated *cis*-9,*trans*-11-octadecadienoic acid in bovine milk: effects of feed and dietary regimen. *Journal of Dairy Science* **79**, 438–445.

Judd JT, Clevidence BA, Muesing RA, Wittes J, Sunkin ME & Podczasy JJ (1994) Dietary *trans* fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. *American Journal of Clinical Nutrition* **59**, 861–868.

Katan MB, Zock PL & Mensink RP (1994) Effects of fat and fatty acids on blood lipids in humans: an overview. *American Journal of Clinical Nutrition* **60**, (Suppl), 1017S–1022S.

Kelly ML, Kolver ES, Bauman DE, Van Amburgh ME & Muller LD (1998) Effect of intake of pasture on concentration of conjugated linoleic acid in milk of lactating cows. *Journal of Dairy Science* **81**, 1630–1636.

Keys A, Anderson JT & Grande F (1965) Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism* **14**, 776–787.

Kinsella JE, Broughton KS & Whelan JW (1990) Dietary unsaturated fatty acids: Interaction and possible needs in relation to eicosanoid synthesis. *Journal of Nutritional Biochemistry* **1**, 123–141.

Koletzko B (1991) Zufuhr, Stoffwechsel und biologische Wirkungen *trans*-isomerer Fettsäuren bei Säuglingen. *Nahrung* **35**, 229–283.

Koletzko B (1992) *Trans* fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. *Acta Pædiatrica* **81**, 302–306.

Lagrost L (1992) Differential effects of *cis* and *trans* fatty acid isomers, oleic and elaidic acid, on the cholesteryl ester transfer protein activity. *Biochimica et Biophysica Acta* **1124**, 159–162.

Lee KN, Kritchewsky D & Pariza MW (1994) Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* **108**, 19–25.

Lichtenstein AH, Ausman LM, Carrasco W, Jenner JL, Ordovas JM & Schaefer EJ (1993) Hydrogenation impairs the hypolipidemic effect of corn oil in humans. *Arteriosclerosis and Thrombosis* **13**, 154–161.

Lin H, Boylston TD, Chang MJ, Luedecke LO & Shultz TD (1995) Survey of the conjugated linoleic acid contents of dairy products. *Journal of Dairy Science* **78**, 2358–2365.

Marnett LJ (1994) Generation of mutagens during arachidonic acid metabolism. *Cancer Metastasis Reviews* **13**, 303–308.

Mata P, Garrido JA, Ordovas JM, Blazquez E, Alvarez-Sala LA, Rubio MJ, Alonso R & de Oya M (1992) Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women. *American Journal of Clinical Nutrition* **56**, 77–83.

Mattson FH & Grundy SM (1985) Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *Journal of Lipid Research* **26**, 194–202.

Mensink RP & Katan MB (1989) Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. *New England Journal of Medicine* **321**, 436–441.

Mensink RP & Katan MB (1990) Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *New England Journal of Medicine* **323**, 439–445.

Mensink RP, Zock PL, Katan MB & Hornstra G (1992) Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein(a) levels in humans. *Journal of Lipid Research* **33**, 1493–1501.

Michal JJ, Chew BP, Shultz TD, Wong TS & Magnuson NS (1992) Interaction of conjugated dienoic derivates of linoleic acid with β-carotene on cellular host defence. *FASEB Journal* **6**, A1102.

Miller CC, Park Y, Pariza MW & Cook ME (1994) Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochemical and Biophysical Research Communications* **198**, 1107–1112.

Molkentin J & Precht D (1995) Optimized analysis of *trans*-octadecenoic acids in edible fats. *Chromatographia* **41**, 267–272.

Molkentin J & Precht D (1997*a*) Representative determination of the butyric acid content in European milk fats. *Milchwissenschaft* **52**, 82–85.

Molkentin J & Precht D (1997*b*) Occurrence of *trans*-C16:1 acids in bovine milkfat and partially hydrogenated edible fats. *Milchwissenschaft* **52**, 380–385.

Nestel P, Noakes M, Belling B, McArthur R, Clifton P, Janus E & Abbey M (1992) Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. *Journal of Lipid Research* **33**, 1029–1036.

Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA & Huth PJ (1997) Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. *Artery* **22**, 266–277.

Pariza MW (1988) Dietary fat and cancer risk: evidence and research needs. *Annual Review of Nutrition* **8**, 167–183.

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME & Pariza MW (1997) Effect of conjugated linoleic acid on body composition in mice. *Lipids* **32**, 853–858.

Parodi PW (1970) Fatty acid composition of Australian butter and milk fat. *Australian Journal of Dairy Technology* **25**, 200–205.

Parodi PW (1996) Milk fat components: possible chemopreventive agents for cancer and other diseases. *Australian Journal of Dairy Technology* **51**, 24–32.

Parodi PW (1997) Cow's milk fat components as potential anticarcinogenic agents. *Journal of Nutrition* **127**, 1055–1060.

Parodi PW & Dunstan RJ (1971) The *trans* unsaturated content of Queensland milk fats. *Australian Journal of Dairy Technology* **26**, 60–62.

Parthasarathy S & Santanam N (1994) Mechanisms of oxidation,

antioxidants, and atherosclerosis. *Current Opinion in Lipidology* **5**, 371–375.
Pfeuffer M (1997) Bedeutung mehrfach ungesättigter Fettsäuren in der Ernährung. In *Fette in der Ernährung, Schriftenreihe des BML, Reihe A: Angewandte Wissenschaft, Heft 464*, pp. 35–67 [S Warwel and N Weber, editors]. Bonn: Köllen-Verlag.
Pouillart P, Cerutti I, Ronco G, Villa P & Chany C (1991) Butyric monosaccharide ester-induced cell differentiation and antitumor activity in mice. Importance of their prolonged biological effect for clinical applications in cancer therapy. *International Journal of Cancer* **49**, 89–95.
Prasad KN (1980) Butyric acid: a small fatty acid with diverse biological functions. *Life Science* **27**, 1351–1358.
Precht D (1990) Quantitativer Nachweis von Milchfett in Schokoladenmischungen I: Bestimmung von Milchfettanteilen in Kakaobutter. *Fat Science and Technology* **92**, 153–161.
Precht D (1995) Variation of *trans* fatty acids in milk fats. *Zeitschrift für Ernährungswissenschaft* **34**, 27–29.
Precht D, Frede E, Hagemeister H & Timmen H (1985) Zur fütterungsbedingten Variation des Milchfettes der Kuh unter besonderer Berücksichtigung von Regulationsmechanismen beim Fettstoffwechsel. *Fette Seifen Anstrichmittel* **87**, 117–126.
Precht D & Molkentin J (1995) *Trans* fatty acids: Implications for health, analytical methods, incidence in edible fats and intake. *Nahrung* **39**, 343–374.
Precht D & Molkentin J (1996) Rapid analysis of the isomers of *trans*-octadecenoic acid in milk fat. *International Dairy Journal* **6**, 791–809.
Precht D & Molkentin J (1997*a*) *Trans*-geometrical and positional isomers of linoleic acid including conjugated linoleic acid (CLA) in German milk and vegetable fats. *Fett/Lipid* **99**, 319–326.
Precht D & Molkentin J (1997*b*) Effect of feeding on conjugated *cis*Δ9,*trans*Δ11-octadecadienoic acid and other isomers of linoleic acid in bovine milk fats. *Nahrung* **41**, 330–335.
Precht D & Molkentin J (1997*c*) Vergleich der Fettsäuren und der Isomerenverteilung der *trans*-C18:1-Fettsäuren von Milchfett, Margarine, Back-, Brat- und Diätfetten. *Kieler Milchwirtschaftliche Forschungsberichte* **49**, 17–34.
Precht D & Molkentin J (1998) Correlations of anticarcinogenic conjugated linoleic acid with other C18 fatty acids in German bovine milk fat. In *European Research Towards Safer and Better Food. Proceedings 3rd Karlsruhe Nutrition Symposium 1998. Part 2*, pp. 150–158 [V Gaukel and WEL Spieß, editors]. Karlsruhe: Bundesanstalt für Ernährung.
Precht D & Molkentin J (1999) Analysis and seasonal variation of conjugated linoleic acid and further *cis*-/*trans*-isomers of C18:1 and C18:2 in bovine milk fat. *Kieler Milchwirtschaftliche Forschungsberichte* **51**, 63–78.
Rabizadeh E, Shaklai M, Nudelman A, Eisenbach L & Rephaeli A (1993) Rapid alteration of c-*myc* and c-*jun* expression in leukemic cells induced to differentiate by a butyric acid prodrug. *FEBS Letters* **328**, 225–229.
Reddy BS (1994) Chemoprevention of colon cancer by dietary fatty acids. *Cancer Metastasis Reviews* **13**, 285–302.
Saito H & Ishihara K (1997) Antioxidant activity and active sites of phospholipids as antioxidants. *Journal of the American Oil Chemists' Society* **74**, 1531–1536.
Shultz TD, Chew BP, Seaman WR & Luedecke LO (1992) Inhibitory effects of conjugated dienoic derivatives of linoleic acid and beta-carotene on the *in vitro* growth of human cancer cells. *Cancer Letters* **63**, 125–133.
Siguel EN & Lerman RH (1993) *Trans* fatty acid patterns in patients with angiographically documented coronary artery disease. *American Journal of Cardiology* **71**, 916–920.
Stanton C, Lawless F, Kjellmer G, Harrington D, Devery R, Connolly JF & Murphy J (1997) Dietary influences on bovine milk *cis*-9,*trans*-11-conjugated linoleic acid content. *Journal of Food Science* **62**, 1083–1086.
Valsta LM, Jauhiainen M, Aro A, Katan MB & Mutanen M (1992) Effects of a monounsaturated rapeseed oil and a polyunsaturated sunflower oil diet on lipoprotein levels in humans. *Arteriosclerosis and Thrombosis* **12**, 50–57.
Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA, Sampson LA & Hennekens CH (1993) Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet* **341**, 581–585.
Witztum JL & Steinberg D (1991) Role of oxidized low density lipoprotein in atherogenesis. *Journal of Clinical Investigation* **88**, 1785–1792.
Wolff RL (1994) Contribution of *trans*-18:1 acids from dairy fat to European diets. *Journal of the American Oil Chemists' Society* **71**, 277–283.
Wolff RL & Bayard CC (1995) Improvement in the resolution of individual *trans*-18:1 isomers by capillary gas-liquid chromatography: use of a 100-m CP-Sil 88 column. *Journal of the American Oil Chemists' Society* **72**, 1197–1201.
Wood R, Kubena K, O'Brien B, Tseng S & Martin G (1993) Effect of butter, mono- and polyunsaturated fatty acid-enriched butter, *trans* fatty acid margarine, and zero *trans* fatty acid margarine on serum lipids and lipoproteins in healthy men. *Journal of Lipid Research* **34**, 1–11.
Zock PL (1995) Dietary fatty acids and risk factors for coronary heart disease: controlled studies in healthy volunteers. PhD Thesis, Universtity of Wageningen.
Zock PL & Katan MB (1992) Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. *Journal of Lipid Research* **33**, 399–410.
Zöllner N & Tatò F (1992) Fatty acid composition of the diet: impact on serum lipids and atherosclerosis. *Clinical Investigation* **70**, 968–1009.