J. Dairy Sci. 85:295–350
© American Dairy Science Association, 2002.

Invited Review: The Composition of Bovine Milk Lipids: January 1995 to December 2000

Robert G. Jensen
Department of Nutritional Sciences
University of Connecticut, Storrs, CT 06269-4017

ABSTRACT

Data from recent publications on bovine milk lipids are presented and discussed. This includes extraction of lipids, triacylglycerols, phospholipids, other complex lipids, sterols, isoflavones, and fatty acids. Improved gas-liquid and high performance liquid chromatography were used. Data on the *trans* and *cis* isomers of fatty acid and of conjugated linoleic acids are given, and the analyses are described. Papers about the lipids in milks and dairy products from the United States are few; where with the exception of *trans*-fatty acid isomers and conjugated linoleic acids, almost no research has been reported.
(**Key words:** milk lipid, method)

Abbreviation key: **Ag-HPLC** = silver-ion HPLC, **Ag-TLC** = argentation TLC, **Ag-TLC/GLC** = argentation TLC/GLC, **BO** = butter oil, **C** = cholesterol, **CLA** = conjugated linoleic acid, **CN** = carbon number, **CVD** = cardiovascular disease, **DAG** = diacylglycerol, **FA** = fatty acid, **FAIPE** = fatty acid isopropyl ester, **FAME** = fatty acid methyl ester, **GDIa** = disialoganglioside Ia, **GD_{Ib}** = disialoganglioside Ib, **GD_2** = disialoganglioside 2, **GD_3** = disialoganglioside 3, **GL** = glycosphingolipid, **GLC-MS** = GLC mass spectrometry, **GM_1** = monosialoganglioside 1, **GM_2** = monosialoganglioside 2, **GM_3** = monosialoganglioside 3, **GT** = triasialoganglioside, **LDL** = low density lipoprotein, **MF** = milk fat, **MFD** = MF-depressing diet, **ML** = milk lipids, **MLGM** = milk lipid globule membrane, **PL** = phospholipid, **PUFA** = polyunsaturated FA, **RA** = rumenic acid, **RP-HPLC** = reversed-phase HPLC, **RPLC** = reversed-phase liquid chromatography, **SFC** = supercritical fluid chromatography, **TAG** = triacylglycerol, **TG** = triglyceride.

INTRODUCTION

Background

The number of papers published per year that represent the research done on milk lipids (**ML**) has, with some exceptions, remained about the same since my last review (Jensen and Newberg, 1995). This lack of effort is probably due to the belief "we know all that is required." As a result, there are no papers on fatty acids (**FA**) on US milks that were analyzed by the most appropriate methods and contain representative data. The exceptions mentioned are those about about conjugated linoleic (**CLA**) and trans-unsaturated FA. A relatively large number of papers have been published about these FA during the last decade. Only a few have appeared on the other lipids; triacylglycerols (**TAG**), sterols, phospholipids (**PL**), and other complex lipids. Data on these lipids through 1994 are presented in Jensen and Newberg (1995). Other useful reviews are in Christie (1995) and Fox and McSweeney (1998). The trends in research for alternate uses of milk fat (**MF**) are described by Jiminez-Flores (1997). Although I placed a starting date of January 1995 in the title, I included earlier references to ensure continuity.

The Nature of Lipids in Milk

Lipids (3 to 5%) occur as globules emulsified in the aqueous phase (87%) of milk. The globules contain nonpolar or core lipids such as TAG, cholesteryl esters, and retinol esters (Jensen and Newberg, 1995). They are coated with bipolar materials, PL, proteins, cholesterol (**C**), enzymes, etc. into a loose layer called the milk lipid globule membrane (**MLGM**). The MLGM prevents the globules from coalescing and acts as an emulsion stabilizer. The diameter of the globules ranges from <1 to about 10 μm, with most of the globules at 1 μm, but those of 4 μm account for most of the mass. The globules present a large surface area (4.6 m^2/dl) to the lipolytic enzymes encountered during their passage through the digestive tract facilitating lipolysis of milk TAG and absorption of the digestion products. Almost all bovine milk is clarified (centrifugal removal of particulates), pasteurized, and homogenized before consumption. Pasteurization apparently has little effect on the lipid content and composition, although this has not been thoroughly investigated.

Received June 13, 2001.
Accepted October 25, 2001.
E-mail: rjensen@uconnvm.uconn.edu.

Conversely, homogenization, a process that reduces the diameter of the lipid globules from about 3 to 0.8 μm and increases their number at least 100-fold and the surface area about 6 to 10 times, and alters the globule membrane structure and composition. The globule surface is recoated largely, but not completely, with caseins. However, some semblance of the original globule membrane is retained (Keenan and Patton, 1995). Unfortunately, we have very few data on the composition of processed products and virtually none about the digestion of the lipids in the calf or human. However, recent reviews on the cell biology of milk secretion and the origin and secretion of ML are available from Mather and Keenan (1998a, 1998b).

A relatively new technique, forward lobar laser light scattering has been used to determine the size of fat globules in milk and cream. McCrae and Lepoetre (1998) used the method to show that while homogenization effectively disrupts the fat globules, this process was offset by clustering. The method can determine the diameter of smaller globules <1 μm, which cannot be done with precision using the microscope or a photoelectric counter.

A new type of homogenizer, the Microfluidizer, will produce milk-containing globules smaller and in a narrower size range than those produced by the valve homogenizer. The diameter of the droplets is about 100 nm. Dalgleish et al. (1996) compared the process to regular homogenization. After several passes, the microfluidized milks contained very small globules of fat (30 to 50 nm in diameter) coated with protein. The particles existed alone and were also inserted into apparently intact casein micelles. These smaller particles should result in a more stable emulsion, but other effects, e.g., digestion, have apparently not been studied.

EXTRACTION OF LIPIDS

Determination of Content

Instructions to determine content are standardized and available (AOAC, 1995; IDF 350/2000). Information about IDF publications is available in the United States from: United States National Committee (USNAC) of the IDF (c/o Request Ltd. 415 Venture Court POB 930398, Verona, WI, 53593-0398. Email; usnacsec@usnac.org). The amounts will be needed when ML are to be investigated so that the quantities of, for example, bioactive FA, per deciliter of milk can be reported as well as per 100 g of lipid. This amount is usually not given, but must be provided because it is required by the nutritionist and consumer. However, the investigator who wants to study the composition of ML must isolate them from milk by extraction with solvents, as described later. The content can be determined by final evaporation of the extraction solvents in a tared, 100-ml, round flask with a flat bottom on a rotary evaporator. The flask is stored in a vacuum dessicator and weighed. This should be done on all samples unless they were taken from a standardized commercial product, where the actual fat content will always be slightly higher than the legal minimum. For example, in the United States, whole milk must contain at least 3.25% fat. The actual contents are very close, usually 3.34%.

An automatic microgravimetric method for determination of fat in skim milk and cocoa by supercritical fluid extraction and online piezoelectric detection has been described (Manganiello et al., 2000). However, it will not replace the infrared procedure now in use (AOAC, 1995).

Extraction

Lipids are extracted from milk with mixtures of solvents. The procedures are described in our paper (Jensen et al., 1997) on the methods employed to extract ML from human milk for the analysis of environmental contaminants. However, all of these were originally used for bovine milk. I recommend the Mojonnier (AOAC, 1995), Bligh and Dyer (1959), and dry column (Maxwell et al., 1986) extractions. If quantities greater than about 350 mg (10 ml of whole milk) are needed, then the Bligh-Dyer or Mojonnier methods are required. If saving time is important, the dry column procedure is preferable. In addition, a fraction enriched in PL can be obtained, and less solvent is needed. Any surface that will contact extraction solvents must be glass or stainless steel; Teflon stopcocks are required for the funnels. The International Dairy Federation (IDF, 1995), published a modification of the Mojonnier method, and Klump et al. (1982) described an Extrelut column procedure that is rapid, precise, and uses small quantities of solvent. However, PL are not extracted. I mention the method because it was not included in my earlier reviews.

The factors listed in Table 1 influence the lipid contents of milk from individual cows (Palmquist et al., 1993). However, production and processing practices eliminate most of these. The current tendency is to select and breed for low-fat milks, e.g., Holsteins versus Guernseys. Colostral, late, and milks from mastitic or otherwise diseased cows and those treated with antibiotics are excluded and pooling occurs. As mentioned, milk and dairy products have legal minimal lipid contents, and the contents are very close to these standards. The processor can adjust the lipid content

Table 1. Factors associated with variations in the amount and fatty acid composition of bovine milk lipids.[1]

Animal
Genetics
Stage of lactation
Ruminal fermentations[2]
Udder infections[3]
Use of bST[3]
Feed
Grain intake
Amount and composition of dietary fat
Dietary protein intake
Energy intake
Seasonal and regional effects

[1]Adapted from Palmquist et al. (1993).

[2]Added by author.

[3]Kaylegian and Lindsay (1995).

of milk to any amount desired lower than the original quantity by controlled separation (centrifugation).

LIPID CLASSES

The average composition of milk as reported by Bitman and Wood (1990) lipids is given in Table 2. These data, obtained by densitometric analysis of separated ML on TLC plates, were the first reported in many years. Earlier results are compiled in the data from the National Dairy Council (1993). In processed milks, the amounts of lipids will be similar to these data.

Triacylglycerols

The composition of TAG is usually defined in terms of the kinds and amounts of FA present. The FA will be discussed later. However, structure also includes the distribution of FA within the TAG molecule and among the TAG molecules, as well as the identification of the individual molecular species of TAG.

Table 2. Lipids in milk.[1]

Lipid class	Maryland US area	United States
Phospholipid	1.11[2]	0.20–1.00[2]
Cholesterol	0.46	0.419[3]
Triacylglycerol	95.80	97 or 98
1,2-Diacylglycerol	2.25	0.28–0.59
Free fatty acids	0.28	0.10–0.44
Monoacylglycerol	0.08	0.16–0.38
Cholesteryl ester	0.02	. . .
Hydrocarbons[4]	Trace	Trace

[1]Adapted from Jensen and Newberg (1995). Maryland = Bitman and Wood (1990). United States = National Dairy Council (1993).

[2]Includes sphingomyelin.

[3]Does not include cholesteryl esters.

[4]Includes squalene and carotenoids.

Table 3. Composition of the major fatty acids in milk fat.[1]

Fatty acid carbon number	Fatty acid common name	Average range (wt%)
4:0	Butyric	2–5
6:0	Caproic	1–5
8:0	Caprylic	1–3
10:0	Capric	2–4
12:0	Lauric	2–5
14:0	Myristic	8–14
15:0	Pentadecanoic	1–2
16:0	Palmitic	22–35
16:1	Palmitoleic	1–3
17:0	Margaric	0.5–1.5
18:0	Stearic	9–14
18:1[2]	Oleic	20–30
18:2	Linoleic	1–3
18:3	Linolenic	0.5–2

[1]Adapted from Kaylegian and Lindsay (1995).

[2]Also contains about 3% of total as *trans* isomers (Jensen and Newberg, 1995).

The structure of the TAG influences the action of lipolytic enzymes and, therefore, absorption; it also influences the flavor of cheeses (Jensen and Newberg, 1995). The structure of milk TAG is responsible for the melting points, crystallization behavior, and rheological properties of MF as globules. See reviews by Jiminez-Flores (1997), Keenan and Patton (1995), Hawke and Taylor (1995), Walstra (1995), and Walstra et al. (1995), for butter and butter oil (**BO**), see these references and Kaylegian and Lindsay (1995). The FA composition and, hence, bovine milk TAG structure is not greatly directly affected by ordinary changes in diet because of the biohydrogenation of unsaturated FA and production of short-chain FA in the rumen.

Bovine ML contain 12 FA in amounts greater than 1% (Kaylegian and Lindsay, 1995; Table 3). Therefore, it would be theoretically possible to have 12 cubed ($12 \times 12 \times 12$) or 1728 TAG species if all the acids were randomly distributed. The total theoretical possibilities are much greater, since bovine ML contain at least 400 FA. With 400 FA, the theoretical maximum is 400 cubed or 64,000,000 TAG! Because the distribution of FA in milk TAG is not random, the numbers of TAG do not approach this figure, but several thousands are probably present, most in traces. I'll present a brief summary of older data and information about the findings that have been published from 1995 to 2000.

Kaylegian and Lindsay (1995) defined the TAG composition and structure of ML as follows. Composition is expressed as the total carbon number (**CN**; sum of the three FA) or as the TAG class, e.g., trisaturated or SSS. Structure includes the location of the FA in the three positions of the TAG molecule and ultimately, the identity of the major TAG.

Table 4. Average triglyceride composition of milk fat, expressed as total carbon number.[1]

Total fatty acid carbon number	Average range (wt%)
C26	0.1–1.0
C28	0.3–1.3
C30	0.7–1.5
C32	1.8–4.0
C34	4–8
C36	9–14
C38	10–15
C40	9–13
C42	6–7
C44	5–7.5
C46	5–7
C48	7–11
C50	8–12
C52	7–11
C54	1–5

[1]Adapted from Kaylegian and Lindsay (1995). Note lowering effect of 4:0 to 10: on carbon numbers.

The TAG classes are identified by the types and amounts of unsaturated FA in the TAG, e.g., SSS, SSU, SUS, etc. where S is saturated and U, unsaturated. Separation has been done with Ag NO_3-TLC, which resolves six to eight classes or with Ag NO_3-HPLC, which produces many peaks.

Triacylglycerol structure. A list of the major TAG as total carbon numbers of the FA, e.g., tripalmitin is 3×16C or 48, i.e., the CN, is presented in Table 4 (Kaylegian and Lindsay, 1995). Note the relatively large amounts of TAG with low carbon numbers, reflecting the presence of 4:0 to 10:0. Bovine ML is the only major food fat that contains these FA and TAG.

A brief description of the stereospecific position of the major FA in TAG is shown in Table 5 (Kaylegian and Lindsay, 1995; Parodi, 1979). Data on the positions of the FA reported throughout a year are shown in Table 6 (Parodi, 1979). Seasons had almost no effect on the distributions, except for small changes in quantities.

Table 5. General description of stereolocation of milk fatty acid in triacylglycerol.[1]

98.1% of 4:0 and 93.2% of 6:0 are located in the *sn*-3 position.
43.5% of 8.0 is esterified to the *sn*-2 position and 52.5% to the *sn*-3 position.
51.4% of 10.0 is located in the *sn*-2 position.
59.8% of 12.0 is found at the *sn*-2 position.
62.2% of 14.0 is esterified to the *sn*-2 position.
44.4% of 16.0 is located at the *sn*-1 position and 43.1% at the *sn*-2 position.
56.2% of 18.0 is acylated at the *sn*-1 position and 27.8% at the *sn*-3 position.
59.3% of 18.1 is found at the *sn*-1 position and 41.3% at the *sn*-3 position.

[1]Adapted from Kaylegian and Lindsay (1995); Parodi (1979). Percentages are mole percents.

Gresti et al. (1993) separated milk TAG into 17 peaks by reversed-phase liquid chromatography. They determined the FA and TAG compositions in each peak by capillary GLC. They calculated the proportions of 223 individual molecular species of individual TAG, accounting for 80% of the total. I have listed the identities of the TAG identified by Gresti et al. (1993) in Table 7. They did not determine the location of the acyl chains within each TAG. I arranged these in Table 7 so that 4:0 and 6:0 are listed at the *sn*-3 positions. All of the TAG with 4:0 and 6.0 at the *sn*-3 position are enantiomers because these FA are not esterified elsewhere. Therefore, an enantiomeric TAG is one that contains different FA at the external or *sn*-1 and *sn*-3 positions. The identity of the *sn*-2 FA does not matter. These occur because the relevant enzymes recognize the *sn*-3 OH. For example, the phosphoryl moiety is attached to the *sn*-3 OH of glycerol in PL. Only one monoacid TAG, 18:1-18:1-18:1 was present in the list, and the distributions were not random. Randomness cannot occur when FA, in this case 4:0, are located in one *sn*- position. I have summarized the data of Gresti et al. in Table 8 (1993). Randomness almost never occurs in nature; Brownian motion is one of the few examples of randomness.

Lipp (1995) and Creamer and MacGibbon (1996) reviewed methods for the analysis of milk TAG, emphasizing the formidable difficulties faced by the analyst and the use of chromatographic methods. They discussed the detection of adulteration of MF with the procedures. Lozada et al. (1995) provided information about the quantitative determination of milk TAG using GLC with capillary columns and a split programmed temperature vaporizer. Molkentin and Precht (1994, 1995a) compared packed and capillary GLC columns for the analysis of milk TAG and described a precise method for their determination. Determination of TAG by GLC was used to detect nondairy fat in cheese (Battelli and Peltegrino, 1994). Precht (1994) also determined seasonal variation of butter fat parameters .

Precht et al. (1998b) collaborated in the certification of an anhydrous butterfat reference material (CRM 519) for certification and C contents. Homogeneity and stability tests were included. The standard is used for the detection of nonmilk fat in butter by calibrating and checking the performance of GLC analysis of milk TAG. The compositions and stability did not change after storage at –18, 4, or 20°C. The samples with added antioxidants were stored in ampoules flushed with nitrogen. A similar standard is not available in

Table 6. Composition and stereospecific distribution of fatty acid (FA) in milk fat triglycerides from bimonthly samples of Maleny butter.[1]

		FA Composition, mole %								
Month	Position	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	18:1
Jan.	TAG	10.1	4.4	2.0	3.4	3.4	10.1	20.9	11.1	19.9
	sn-1	0.1	0.2	0.3	1.3	2.1	7.6	27.8	18.7	23.4
	sn-2	0.1	0.7	3.0	5.4	6.1	18.9	27.0	5.3	11.6
	sn-3	30.2	12.4	2.6	3.7	2.0	3.9	7.8	9.3	24.6
March	TAG	10.2	4.5	2.1	3.5	3.4	9.2	18.4	11.8	21.2
	sn-1	0.2	0.2	1.1	2.1	2.5	7.6	25.8	19.6	24.5
	sn-2	0.0	0.4	3.4	5.9	6.5	18.4	25.0	5.9	14.1
	sn-3	30.2	13.1	2.0	2.5	1.2	1.7	4.4	10.1	25.1
May	TAG	9.8	4.0	1.8	3.1	3.1	9.4	19.9	11.9	22.1
	sn-1	0.1	0.2	0.1	0.5	1.6	7.4	26.5	18.5	25.5
	sn-2	0.0	0.6	2.8	5.0	5.8	18.7	27.6	6.0	14.2
	sn-3	29.4	11.3	2.5	3.7	1.9	2.1	5.7	11.3	26.7
July	TAG	10.6	4.1	1.8	2.9	2.8	8.6	20.0	12.0	23.2
	sn-1	0.1	0.1	0.3	0.9	1.8	6.7	26.8	20.4	26.6
	sn-2	0.1	0.5	3.1	5.1	5.5	17.4	27.3	5.7	14.5
	sn-3	31.7	11.6	2.0	2.6	1.0	1.8	5.9	10.0	28.5
Sept.	TAG	10.8	4.4	1.9	3.2	3.1	9.1	20.0	11.9	22.7
	sn-1	1.0	0.8	1.0	2.0	2.4	7.9	26.2	18.2	25.0
	sn-2	0.1	0.5	3.6	6.0	6.3	18.5	26.8	5.8	14.1
	sn-3	31.4	11.9	1.1	1.7	0.7	0.9	6.9	11.7	29.0
Nov.	TAG	10.2	4.4	2.1	3.6	3.5	10.0	20.3	11.7	21.1
	sn-1	1.6	0.9	0.9	2.2	2.9	9.2	28.3	18.7	20.5
	sn-2	0.1	0.7	3.5	6.3	6.5	18.9	26.5	5.7	14.0
	sn-3	28.8	11.7	1.7	2.4	1.1	1.8	6.2	10.8	28.7
Means[2]	TAG	10.3	4.3	2.0	3.3	3.2	9.4	19.9	11.7	21.7
	sn-1	0.5	4.7	0.6	1.5	2.3	7.7	25.9	19.0	24.3
		(1.6)[3]	(3.1)	(10.3)	(15.2)	(23.7)	(27.3)	(44.1)	(54.0)	(37.3)
	sn-2	0.1	0.5	3.2	5.6	6.1	18.5	26.7	5.7	13.8
		(0.3)	(3.9)	(55.2)	(56.6)	(62.9)	(65.6)	(45.4)	(16.2)	(21.2)
	sn-3	30.1	12.0	2.0	2.8	1.3	2.0	6.2	10.5	27.1
		(48.1)	(93.0)	(34.5)	(28.2)	(13.4)	(7.1)	(10.5)	(29.8)	(41.5)

[1]Adapted from Parodi (1979). TAG = Triacylglycerol.

[2]Means calculated by author.

[3]Percent of fatty acid in *sn* position calculated by author.

Table 7. Amounts of triacylglycerols (TAG) > 1 mol% in bovine milk lipids.[1]

	TAG species[3]		Amounts (mol%)[2]			TAG species[3]		Amounts (mol%)[2]	
			Experimental	Random				Experimental	Random
4:0	14:0	16:0	3.05	1.42	10:0	16:0	18:1	1.60	1.15
6:0	14:0	16:0	1.37	0.72	12:0	16:0	18:1	1.22	1.11
4:0	14:0	18:0	1.35	0.65	14:0	16:0	18:1	2.82	3.39
4:0	16:0	16:0	3.20	1.54	14:0	18:0	18:1	1.45	1.55
6:0	16:0	16:0	1.50	0.78	16:0	16:0	18:1	2.34	3.69
4:0	16:0	18:0	2.47	1.42	16:0	18:0	18:1	2.16	3.39
6:0	16:0	18:0	1.12	0.71	4:0	18:1	18:1	1.48	1.33
4:0	14:0	18:1	1.79	1.31	14:0	18:1	18:1	1.26	1.58
4:0	16:0	18:1	4.17	2.87	16:0	18:1	18:1	2.50	3.43
6:0	16:0	18:1	2.02	1.45	18:0	18:1	18:1	1.21	1.57
4:0	18:0	18:1	1.58	1.31	18:1	18:1	18:1	1.02	1.06
Totals								42.68	37.43

[1]Adapted from Gresti et al. (1993).

[2]Experimental (determined) or random (calculated) mol%.

[3]Position of the acyl chains within each TAG not determined. However, in butyroyl TAG, > 99% 4:0 is at *sn*-3; in hexanoyl TAG, > 93% of 6:0 is at *sn*-3, see Table 4.

Table 8. Summary of triacylglycerol (TAG) in bovine milk as determined by reverse-phase liquid and capillary gas-liquid chromatography.[1]

Quantitated 223 TAG containing even numbered fatty acids (FA) accounting for 80 mole% of the total TAG.
Major TAG were (mole%): 18:1–16:0–4:0, 4:2; 16:0–16:0–4:0; 3.2; and 16:0–14:0–4:0; 3.1. In these and all other butyroyl TAG, 4:0 will be esterified to the *sn*-3 position. These TAG are enantiomers.[2]
Twenty-two TAG (1 > mole%), 42.68 mol% of total, contained at least two of the major FA: 14:0, 16:0, 18:0, and 18:1. In this group there were eight butyroyl diacylglycerols — 19% of the total.
Thirty-six mole% of the TAG contained 4:0 or 6:0 and two long-chain FA. All of the 4:0 and at least 90% of the 6:0 will be at *sn*-3.
In the TAG with 4:0 and 6:0, 14:0, 16:0, and 18:0 were equally distributed among the *sn*-1 and -2 positions.
There was one monoacid TAG > 1 mole %, 18.1 – 18.1 – 18.1 at 1.02%, small quantities of 14.0, 16.0, and 18.0 monoacid TAG, and no monoacid TAG of 4:0 – 12:0.
There were no predominant TAG and the amounts of almost all TAG were nonrandom. These will change as the fatty acid profiles are altered, but the distribution will remain nonrandom.

[1]Adapted from Gresti et al. (1993) and Jensen and Newberg (1995).

[2]Enantiomers are defined as TAG having different FA at the *sn*-1 and *sn*-3 positions.

the United States. Analysis of TAG in goat milk was used by Fontecha et al. (1998) to detect adulteration with foreign fats. Recently, Molkentin and Precht (2000a) determined that packed and capillary columns gave equivalent results for low resolution results in the detection of foreign fats in butter. However, the capillary column is needed to resolve occasional difficulties. The methods developed by Molkentin and Precht (2000b) and Battelli and Pellegrino (1994) were used to detect nondairy fat in several types of cheeses.

Precht (1994) analyzed 250 samples of butter, ascertaining the effects of season on iodine numbers, solid fat contents, penetrometer values, 16:0 and 18:1 contents, 16:0/18:1 ratios, slipping and melting points, refraction indices, C52, C54, C54/C52, and C40/C32 values obtained from the TAG compositions. The effects of season or feed are given. For example, the C54 contents (%) were: wk 3 of year, 4.0, and wk 33, 7.5. The data provide indicators of the spreadability of butter. It is difficult to assess the applicability of their data to the United States. We do not have any study of this type or magnitude. Data on the effects of season on the FA composition exist (discussed later).

Spanos et al. (1995) utilized HPLC with light-scattering detection and desorption chemical-ionization tandem mass spectrometry to characterize milk TAG. They resolved 58 peaks with HPLC, determining the acyl carbon number, the number of double bonds, and the FA in each TAG eluted by HPLC. They listed the TAG structures and identified the most abundant, but did not provide amounts. However, the sequence of FA in the TAG as shown in their Table 1 could not have been determined by the methods employed. They were listed in order of increasing molecular weight. We can deduce the structure from the data presented above. Ruiz Sala et al. (1996) analyzed the triglycerides in milk from ewe, cow, and goat MF with reversed-phase HPLC (**RP-HPLC**) and light scattering detection. They reported the identity of 171 TAG in ewes' milk only. They noted that the amounts (wt%) of short TAG with a CN of ≤34 in the milks were: cow, 10, goat, 15, and ewe, 18.

Manninen et al. (1995) used supercritical fluid chromatography (**SFC**) to separate milk and other oils TAG by CN and degree of unsaturation. However, they did not provide any data except CN peaks about the milk TAG. Later, Kallio et al. (1996) used GC and ^{1}H nuclear magnetic resonance spectroscopy to obtain information about the butyryl groups. They were able to differentiate butyrate at the 1(3)- and 2-positions but again did not give any data. Kemppinen and Kalo (1998) later published information on the butyryl and other short-chained TAG in milk. They identified 42 of the TAG. Their data are shown in Table 9. They employed GLC and silver-ion (**Ag-HPLC**). Essentially all of the butyric (4:0) and caproic (6:0) acids were esterified to the *sn*-3 position. The analyses could not differentiate between the *sn*-1 and -3 positions, but the virtual absence of the acids at *sn*-2 (Table 8) indicated that they were located at *sn*-3 in these TAG. TAG containing 4:0 and 6:0, which would be at the *sn* 3 position are enantiomers. The most abundant TAG in BO (mole%) were: 4:0 – 16:0 – 16:0 + 4:0 – 14:0 – 18:0, 5.59; 4:0 – 14:0 – 16:0 + 4:0 – 12:0 – 18:0, 3.41; 4:0 – 16:0 – 18:0, 2.48; and 6:0 – 16:0 – 16:0 + 6:0 – 14:0 – 18:0, 2.32. The results in this paper agree closely to those of Gresti et al. (1993), see Table 5. Interesterification of the BO randomized the structure of the TAG, as shown by an average ratio of the *sn*-1 (3) to *sn*-2 4:0 and 6:0 of 2.0:1.0.

More information about MF processing and properties of TAG fractionscan be found in Jimenez-Flores (1997) and the book on the subject by Kaylegian and Lindsay (1995). Another useful review has been prepared by German and Dillard (1998). Lin et al. (1996) described the flavor, quality, and texture of a modified FA, high monoene, low saturate butter. Breitschuh and Windhab (1996) discussed several methods for the measurement of thermal fat crystal properties for MF fractionation. They evaluated calorimetry and pulsed nuclear magnetic resonance. With these techniques, they directly determined the solid-fat contents and

Table 9. Proportions (mole%) of *sn*-3 butyryl and caproyl acyl isomers of triacylglycerols (TAG) in butter oils (BO).[1]

Saturates	BO	IBO[2]	Unsaturates	BO	IBO[2]
TAG 32[3]			TAG 36		
4:0–14:0–14:0[4]	1.57	0.79	4:0–14:0–18:1	2.58	1.22
TAG 34			TAG 38		
4:0–14:0–16:0			4:0–16:0–18:1	4.93	1.99
4:0–12:0–18:0	3.41	1.19			
TAG 36			TAG 40		
6:0–14:0–16:0	1.78	0.62	6:0–16:0–18:1	1.75	1.00
4:0–16:0–16:0			16:0–6:0–18:1	1.50	0.88
4:0–14:0–18:0	5.59	1.72			
TAG 38			TAG 42		
6:0–16:0–16:0	2.32	0.66	6:0–18:0–18:1	1.18	0.33
4:0–16:0–18:0	2.41	0.75			
			Diene TAG		
TAG 40			TAG 40		
12:0–12:0–16:0			4:0–18:1–18:1	1.07	0.91
10:0–12:0–18:0			Unsaturated TAG 36-42	14.49	10.45
12:0–14:0–14:0	1.31	1.31	*sn*-2–4:0	1.4	
10:0–12:0–18:0			*sn*-2–6:0	TR	
8:0–16:0–16:0					
8:0–14:0–18:0					
8:0–16:0–16:0	0.92	0.41			
6:0–16:0–18:0	0.94	0.44			
TAG 42					
14:0–14:0–14:0					
12:0–12:0–18:0	2.53	0.83			
10:0–16:0–16:0					
12:0–12:0–18:0	2.53	1.14			
Saturated TAG 27-42	27.43	16.11			

[1]Adapted from Kemppinen and Kalo (1998), 97.6% of 4:0 and 100% of 6:0 are at *sn*-3.

[2]IBO is interesterified butter oil.

[3]TAG 32 is total carbon number of acyl chains.

[4]4:0 and 6:0 are mostly located at the *sn*-3 position of the TAG.

melting characteristics of MF fractions. ten Grotenhuis et al. (1999) studied the crystallization behavior of MF by differential scanning calorimetry and real-time X-ray powder diffraction. They observed the γ, α, and β, but not the β polymorph, which has been previously reported. Transitions between the polymorphs occurred as the MF was stored or heated.

Laakso et al. (1996) gave data on the TAG composition of colostrum. This information should help to elucidate the biosynthesis of milk TAG and provide information on the lipid nutrition in the calf. However, colostrum is excluded from milk that is intended for consumption by humans. Laakso and Manninen (1997) identified these milk TAG by SFC combined with ammonia negative-ion chemical ionization mass spectroscopy. Capillary SFC combined with atmospheric pressure chemical ionization mass spectroscopy was used for ML-TAG at 7 d postpartum. They identified the molecular species and fatty acyl residues in 23 chromatographic peaks, but could not provide TAG structures.

Angers et al. (1998) identified the FA esterified at the *sn*-1, 3 and *sn*-2 positions of milk TAG fractions resolved by HPLC. A summary of their results confirm

and extend earlier observations and are given in Table 10.

Robinson and MacGibbon (1998a) separated New Zealand milk TAG into nine bands based on degree of unsaturation and total length of FA groups by the Ag-TLC method they modified. They determined the mass of material in each band and their FA profiles by GLC. They analyzed samples taken during November 1993, February 1994, and March 1994. Milk flow usually peaks in October/November in New Zealand, with the strong grass growth in the spring. The unsaturated bands, 1, 2, and 5 increased in amount from November to March, and the trisaturate bands, 7 to 9, decreased. Fat hardness gradually decreased with these changes.

Robinson and MacGibbon (1998b) later resolved the milk TAG into 61 peaks with RP-HPLC on the basis of chain length and degree of unsaturation. The bands obtained by their Ag-TLC method were analyzed by HPLC. Data from these analyses are presented in Table 11. By combining their data, they produced the list of proposed identities of TAG in Table 12. In the table, 4:0 is given at the end of each TAG, e.g., 14:0, 8:0, and 4:0. In these TAG, we know from the earlier discussion that 4:0 is esterified almost completely at the *sn*-3 position. All of these TAG are enantiomers. These data are similar to those I presented earlier in this paper. The authors suggest that their procedures can be used to examine seasonal affects on structure to physical properties of butter, such as hardness. They may be somewhat useful in the United States, where, although milk is collected from much larger areas than in New Zealand, season does alter the FA contents.

Lawler and Dimick (1998) discussed the crystallization and polymorphism of fats with a brief section on milk. Van Aken et al. (1999) provided more information. They separated MF into three groups with solvent and dry fractionation. The high melting TAG separated between 25 and 10°C; middle 10 and 5°C, and the low – 13°C and below. The FA composition reflected the melting point (MP) of the fractions, e.g., fewer long-chained and more short-chain melting point acids in the low melting triclycerides LMT group. The stable crystal form was the $\beta($ configuration. Van Aken and Visser (2000) then studied the crystallization and firmness of MF with a Votater, the commercial heat exchanger.

Harmer and Wijesundera (1996) studied the heat stability of MF, a fraction, sunflower, canola, and linola oils. Potato chips were fried in these products, and the effects on them were determined. Heating increased the FFA and polar compound contents, viscosity and dielectric contents of all the fats. Polymers were found in the oils, except for canola, but not in the MF or its fraction. The resistance of MF is due to its relatively low content of polyunsaturated FA (**PUFA**). In an earlier paper, Molkentin and Precht (1993) demonstrated that MF can be protected from autooxidation if stored under nitrogen at –18°C in the dark.

Limb et al. (1999) isolated and characterized monoacetyldiglycerides from the bovine udder for the first time. The acetyl group was located at the primary positions. However the authors did not mention that Parodi (1975) detected the TAG in milk. This would be the natural sequence of their presence in the bovine mammary gland.

Additional information is available on the polymorphism of MF by differential scanning calorimetry (ten Grotenhuis et al., 1999) and the techniques used in ML crystallization studies (Wright et al., 2000). Lenck et al. (1998) described improved recovery of short-chain FA from lipolysates of MF by periodic aqueous extraction. These FA can serve as food flavors.

Significance of TAG structure. I have described the influence of TAG structure in processing parameters. These are particularly applicable to butter, for which spreadability is affected by the TAG or ultimately, the FA composition of ML. The location of the flavor acids (4:0 to 10:0) in the primary positions of TAG makes them accessible to lipases. When free, the acids contribute to the flavor of cheeses. Kaylegian and Lindsay (1995) used differences in structure to prepare potentially useful fractions.

Of equal or possibly more importance are the physiological and nutritional effects of TAG structure. Milk TAG, when consumed by humans, are lipolyzed first in the stomach by gastric lipase. The lipase preferentially hydrolyzes *sn*-3 position FA 4 to 1 compared with *sn*-1 and selectively releases the shorter acids (Jensen and Newberg, 1995). The result is that 4:0 to 10:0 pass through the stomach wall in decreasing quantities as the molecular weight increases, enter the portal vein, and are transported to the liver where they are oxidized. About 25 to 40% of the TAG are digested in the stomach. Milk lipid globules are resistant to pancreatic lipolysis in the small intestine unless they are first exposed to gastric lipolysis. The digestate entering the small intestine will contain bioactive *sn*-1, 2 DAG and very small quantities of 4:0-10:0. These aspects of milk TAG digestion have not been investigated in the human, but have been confirmed in the rat (Lai and Ney, 1998).

We forget that bovine milk is intended for the nutriture of the calf. In my previous reviews, I did not discuss the digestion of milk TAG by the calf. It is similar to the sequence I described above, except that most of the TAG are hydrolyzed by a pregastric ester-

Table 10. Composition of fatty acids (FA; mol%) esterified at the *sn*-1,3 and sn-2 positions of triacylglycerols in the HPLC fractions 5 to 14 of bovine milk fat.[1]

		Fraction/(PN)[2] (mol%)									
FA	Positions	5 (32)	6 (34)	7 (36)	8 (38)	9 (40)	10 (42)	11 (44)	12 (46)	13 (48)	14 (50)
4:0	1,3	40.8 (100)[2]	39.5 (100)	31.4 (100)	13.8 (100)	6.7 (100)	1.3 (100)	–(0)[3]	–(0)	–(0)	–(0)
	2	. . .	. . .	. . .	. . .	. . .	. . .	–(0)	–(0)	–(0)	–(0)
6:0	1,3	6.7 (87)	5.7 (80)	8.3 (92)	18.5 (98)	7.8 (94)	0.9 (86)	–(0)	–(0)	–(0)	–(0)
	2	2.0 (13)	2.8 (20)	1.5 (8)	0.7 (2)	1.0 (6)	0.3 (14)	–(0)	–(0)	–(0)	–(0)
8:0	1,3	2.7 (71)	1.7 (74)	1.8 (78)	5.5 (90)	11.4 (88)	2.6 (76)	0.3 (60)	–(0)	–(0)	–(0)
	2	2.2 (29)	1.2 (26)	1.0 (22)	1.2 (10)	3.1 (12)	1.6 (24)	0.4 (40)	–(0)	–(0)	0.4 (100)
10:0	1,3	4.1 (46)	2.0 (57)	1.9 (63)	4.0 (70)	12.5 (77)	14.1 (75)	4.5 (76)	–(0)	–(0)	–(0)
	2	9.5 (54)	3.0 (43)	2.2 (37)	3.4 (30)	7.4 (23)	9.5 (25)	2.9 (24)	1.8 (100)	–(0)	0.8 (100)
12:0	1,3	6.4 (38)	2.4 (41)	1.5 (53)	2.2 (52)	4.8 (59)	6.8 (60)	11.0 (71)	2.4 (58)	0.6 (100)	–(0)
	2	21.0 (62)	6.8 (59)	2.7 (47)	4.0 (48)	6.7 (41)	9.0 (40)	9.2 (29)	3.5 (42)	–(0)	1.1 (100)
13:0	1,3	–(0)	0.4 (90)	0.2 (80)	–(0)	–(0)	0.2 (80)	0.3 (75)	–(0)	–(0)	–(0)
	2	–(0)	0.1 (11)	0.1 (20)	–(0)	–(0)	0.1 (20)	0.2 (25)	0.3 (100)	–(0)	–(0)
14:0	1,3	8.5 (40)	10.1 (35)	4.3 (33)	3.5 (36)	9.1 (49)	8.9 (48)	15.6 (58)	18.8 (63)	4.3 (42)	0.6 (16)
	2	25.8 (60)	37.2 (65)	17.1 (67)	12.6 (64)	18.9 (51)	19.4 (52)	22.2 (42)	21.7 (37)	11.9 (58)	6.2 (84)
14:1	1,3	0.9 (26)	0.4 (33)	0.4 (62)	0.6 (60)	1.4 (65)	2.4 (67)	3.8 (75)	0.6 (52)	–(0)	–(0)
	2	5.9 (74)	1.6 (67)	0.5 (38)	0.8 (40)	1.5 (35)	2.4 (33)	2.5 (25)	1.1 (48)	–(0)	–(0)
15:0	1,3	1.1 (65)	0.7 (50)	1.2 (53)	0.8 (47)	0.8 (55)	1.0 (57)	1.1 (55)	1.6 (62)	1.0 (49)	0.5 (42)
	2	1.2 (35)	1.4 (50)	2.1 (47)	1.8 (53)	1.3 (45)	1.5 (43)	1.8 (45)	2.0 (38)	2.1 (51)	1.4 (58)
16:0	1,3	14.5 (61)	20.9 (61)	28.6 (54)	22.5 (50)	25.2 (59)	29.4 (65)	27.7 (62)	28.6 (60)	31.9 (54)	22.0 (47)
	2	18.9 (39)	26.6 (39)	48.4 (46)	45.2 (50)	35.4 (41)	31.6 (35)	33.4 (38)	38.9 (40)	54.6 (46)	50.4 (53)
16:1	1,3	1.1 (45)	1.4 (44)	0.5 (42)	0.5 (48)	1.0 (56)	1.2 (55)	2.3 (66)	2.9 (72)	1.1 (100)	0.1 (29)
	2	2.7 (55)	3.6 (56)	1.4 (58)	1.1 (58)	1.6 (44)	2.0 (45)	2.4 (34)	2.2 (28)	–(0)	0.5 (71)
17:0	1,3	–(0)	–(0)	0.4 (73)	0.9 (64)	0.7 (61)	0.6 (75)	0.6 (71)	0.8 (100)	1.4 (100)	0.9 (95)
	2	–(0)	–(0)	0.3 (27)	1.0 (36)	0.9 (39)	0.4 (25)	0.5 (29)	–(0)	–(0)	0.1 (5)
18:0	1,3	3.0 (80)	2.4 (69)	5.2 (79)	15.2 (76)	14.0 (79)	11.8 (79)	9.6 (78)	7.4 (66)	13.8 (76)	45.3 (85)
	2	1.4 (20)	2.2 (31)	2.9 (21)	9.4 (24)	7.7 (21)	6.5 (21)	5.4 (22)	7.7 (34)	8.9 (24)	16.3 (15)
18:1	1,3	6.6 (61)	10.3 (66)	13.6 (59)	10.6 (54)	3.6 (35)	17.4 (71)	21.0 (70)	33.3 (76)	44.6 (80)	30.4 (74)
	2	8.4 (39)	10.7 (34)	18.8 (41)	17.9 (46)	13.3 (65)	14.5 (29)	18.0 (30)	21.0 (24)	22.6 (20)	21.8 (26)
18:2	1,3	2.5 (95)	2.5 (69)	1.0 (67)	1.1 (73)	1.4 (72)	0.9 (64)	1.8 (77)	3.4 (100)	1.1 (100)	0.4 (100)
	2	0.9 (15)	2.2 (31)	1.0 (33)	0.8 (27)	1.1 (28)	1.0 (36)	1.1 (23)	–(0)	–(0)	–(0)
18:3	1,3	2.2 (94)	–(0)	–(0)	–(0)	–(0)	0.3 (100)	0.5 (100)	–(0)	–(0)	–(0)
	2	0.3 (6)	0.2 (100)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)
20:0	1,3	–(0)	–(0)	–(0)	–(0)	–(0)	0.3 (100)	–(0)	–(0)	0.2 (100)	0.8 (100)
	2	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)	–(0)

[1]From Angers et al. (1998). Values are means of duplicate analyses. Note that 4:0–8:0 were found only in partition numbers 32-42.

[2]PN = Partition number, defined as acyl carbon number –2 × DB, where DB is the number of double bonds.

[3]Values in parentheses represent the percentage of fatty acids located between the *sn*-1,3 and *sn*-2 positions in any given fractions.

[4]Fatty acid not detected or value was less than 0.1%.

Table 11. Groups of triacylglycerols (TAG) present in bands from Ag-TLC.[1]

Band from Ag-TLC	TAG Groups present, x, y, z[2]
1	Trienes and higher unsaturates
2	*cis*, *cis* Dienes
3	*cis* Monoenes of type z = $C_{4:0}$, x+y< 36; *cis*, *trans* dienes
4	*cis* Monoenes of type z = $C_{4:0}$ or $C_{6:0}$
5	*cis* Monoenes of type x, y, and z > 4; trace of $C_{6:0}$
6	*cis* Monoenes, regioisomers of band 5
7	Trisaturates of type x and y > 8, z = $C_{4:0}$, x+y > 20; *trans* monoenes
8	Trisaturates of type x and y > 8, z = $C_{6:0}$, x+y > 24
9	Trisaturates of type x, y, and z > 6, CN > 36

[1]Ag-TLC = Argentation TLC; CN = acyl carbon number. Confirmed by reverse-phase. Adapted from Robinson and MacGibbon (1998b).

[2]Designation of TAG groups with different fatty acids.

ase or lipase secreted from the tongue and associated tissues in the calf (Hamosh, 1990). A commercial preparation of the tissues is used in the manufacture of Provolone and Romano cheeses, where it helps produce their characteristic flavors by release of short-chain FA from milk TAG. The lipase preferentially hydrolyzes 4:0 to 12:0 more rapidly than the longer FA. A group of biologically active *sn*-1-2 DG will be produced. The effects of these were unknown until recently. Nagao et al. (2000) observed that diacylglycerol (**DAG**), in contrast to TAG, apparently decreased the accumulation of body fat when fed to men and might reduce the risk of diseases associated with visceral fat disorders. The DAG fed were a mixture that contained about 2 to 1 quantities of 1, 3 to 1, 2-DAG. We do not know if the isomers cause different results, but the *sn* 1,2-DAG would be the isomer produced first. The 1,3-DAG is formed by acid catalyzed acyl migration, but the rate is unknown and passage through the stomach is rapid. More *sn* 1, 2-DAG would be formed from MF than any other fat or oil. About 70% of dietary TAG is hydrolyzed in the abomasum of the calf. Gastric lipase has not been detected in the abomasum. For more information, the reader should consult the review by Hamosh (1990).

Timmen and Patton (1989) observed that the biosynthetic mechanism of TAG in the bovine mammary gland alternatively used 4:0 to 10:0 or 18:1 to maintain liquidity of the MF globules and the intracellular lipid droplets, their precursors. Oleic acid (18:1) is converted from 18:0 as needed by stearoyl Co-A desaturase. The enzyme helps to maintain fluidity of cellular endoplasmic reticulum membrane where TAG biosynthesis occurs. Interestingly, they noted that the small fat globules in the skim milk portion of their samples contained less 4:0 to 10:0 and 18:0 and more 18:1 than the large globules in the cream. Before this, we believed that the FA and TAG in the size range of fat globules did not differ.

Summary of TAG structure. Bovine milk contains a unique assembly of TAG, possibly thousands, and identification of individual TAG is difficult. The distribution is species specific and nonrandom, with most of the 4:0 and 6:0 esterified to the *sn*-3 position. The necessity for liquidity of the MF globule requires that most of the FA be esterified to TAG in combinations that have a melting point at or below 39°C, the body temperature of the cow (Timmen and Patton, 1989). While some do not, e.g., the trisaturated TAG listed in Tables 7, 9, and 12, their quantities are small. These will be codissolved in the liquid TAG. This selectivity of esterification indicates that esterification is directed to produce the required TAG regardless of changes in dietary FA [see Gresti et al. (1993) for more information]. The quantities of TAG will change, but not their structure. Research on milk TAG structure should continue; it may be important to know the location of CLA and the *trans* isomers of 18:1. These acids have beneficial and possibly deleterious effects on the bovine and the human. I will discuss these aspects in the section on FA.

Phospholipids (PL) and Glycosphingolipids (GL)

Data on the contents of these and other lipids are given in Table 2. The amounts of PL, similar to data reported earlier (Jensen and Clark, 1988; Jensen and Newberg, 1995), do not change much during time postpartum in individual cows. These small variations will probably not be observed in pooled, homogenized milks in which the amounts will range from 20 to 30 mg/dl. The few recent publications on PL and GL describe these compounds in whey concentrates and gangliosides in milk. Data are given below.

Composition of PL

The composition of the PL determined by Bitman and Wood (1990) throughout lactation is presented in

Table 12. Summary of proposed triacylglycerols of individual peaks from HPLC.[1]

Peak no.	Typical RT at 20°C (min)	Estimated CN	Estimated PN	Suggested triaclyglycerols
1	5.08	26	28	14:0,8:0,4:0; 12:0,10:0,4:0
2	7.15	28	28	16:0,8:0,4:0; 14:0,10:0,4:0
3	9.84	32	30	10:0,18:1,4:0
4	10.55	30	30	18:0,8:0,4:0; 16:0,10:0,4:0; 14:0,12:0,4:0
5	13.37	36?	32	. . .
6	14.60	34	32	12:0,18:1,4:0
7	15.81	32	32	14:0,14:0,4:0; 16:0,12:0,4:0
8	20.63	38	34	18:2,16:0,4:0?
9	22.22	34 and 36	34	16:0,12:0,6:0; 18:1,14:0,4:0
10	24.24	34	34	16:0,14:0,4:0
11	27.44	42	36	. . .
12	30.33	40	36	18:1,18:1,4:0; 18:1,14:0,6:0
13/14	33.20	38	36	18:1,16:0,4:0; 16:0,14:0,6:0[2]; 18:0,12:0,6:0[2]
15	35.80	36	36	16:0,16:0,4:0; 18:0,14:0,4:0
16	39.95	40	38	18:1,12:0,10:0
17	40.43	40 and 42	38	18:1,18:1,6:0; 18:1,14:0,8:0
18	42.05	38 and 40	38	16:0,14:0,8:0; 16:0,12:0,10:0; 18:1,16:0,6:0
19	44.87	38 and 40	38	18:0,18:1,4:0; 16:0,16:0,6:0; 18:0,14:0,6:0
20	47.93	38	38	18:0,16:0,4:0
21	49.84	44	40	18:1,18:1,8:0
22	51.45	42	40	18:1,14:0,10:0
23	52.33	42?	40	18:1,16:0,8:0
24	53.93	40 and 42	40	18:1,18:0,6:0; 16:0,14:0,10:0
25	55.03	40	40	16:0,16:0,8:0; 18:0,14:0,8:0
26	56.84	40	40	18:0,16:0,6:0
27	59.98	40	40	18:0,18:0,4:0
28	60.15	46	42	18:1,18:1,10:0
29	62.82	44	42	18:1,14:0,12:0; 18:1,16:0,10:0; 18:1,18:0,8:0
30	63.65	44	42	18:1*t*,16:0,10:0
31	65.45	42	42	16:0,16:0,10:0; 18:0,14:0,10:0; 16:0,14:0,12:0
32	66.44	42	42	18:0,16:0,8:0
33	68.23	42	42	18:0,18:0,6:0
34	70.85	48	44	12:0,18:1,18:1
35	71.83	48	44	12:0,18:1,18:1*t*
36	71.37	46	44	16:0,12:0,18:1; 14:0,14:0,18:1; 10:0,18:0,18:1
37	74.58	46	44	16:0,12:0,18:1*t*; 14:0,14:0,18:1*t*; 10:0,18:0,18:1*t*
38	76.08	44	44	16:0,14:0,14:0; 16:0,16:0,12:0; 18:0,16:0,10:0; 18:0, 14:0,12:0
39	78.67	47?	45?	17:0,12:0,18:1?; 15:0,14:0,18:1?
40	80.83	50	46	14:0,18:1,18:1
41	81.88	50	46	14:0,18:1,18:1*t*
42	83.30	48	44 and 46	14:0,16:0,18:1; 16:0,16:0,18:2[3]; 12:0,18:0,18:1
43	84.63	48	46	14:0,16:0,18:1t; 12:0,18:0,18:1*t*
44	86.13	46	46	16:0,16:0,14:0; 12:0,16:0,18:0; 14:0,14:0,18:0
45	88.23	54	48	18:1,18:1,18:1
46	88.43	49?	47?	17:0,14:0,18:1?; 15:0,16:0,18:1
47	90.02	52	48	16:0,18:1,18:1
48	91.23	52	48	16:0,18:1,18:1*t*
49	92.60	50	48	16:0,16:0,18:1; 14:0,18:0,18:1
50	94.03	50	48	16:0,16:0,18:1; 14:0,18:0,18:1*t*
51	95.45	48	48	18:0,16:0,14:0
52	97.08	51?	49?	17:0,16:0,18:1?; 15:0,18:0,18:1
53	98.48	54	50	18:1,18:1,18:0
54	99.88	54	50	18:1,18:1*t*,18:0
55	101.18	52	50	18:1,18:0,16:0
56	102.48	52	50	18:1*t*,18:0,16:0
57	104.18	50	50	16:0,16:0,18:0; 14:0,18:0,18:0
58	108.23	54	52	18:1,18:0,18:0
59	109.17	54	52	18:0,18:0,18:1*t*
50	110.24	52	52	18:0,18:0,16:0
61	113.63	54	54	18:0,18:0,18:0

[1]RT = Retention time; CN = acyl carbon number; PN = partition number defined as acyl carbon number–2× DB, where DB is the number of double bonds. Adapted from Robinson and MacGibbon (1998). 4:0 and 6:0 located mostly in the *sn*-3 position.

[2]Carbon numbers total 36 instead of 38.

[3]Carbon number is 50 instead of 48.

Table 13. Phospholipid composition of cow's milk during lactation.[1]

	Concentration at lactation day (mg/dl)				
Class[2]	3	7	42	180	Pooled SE
SM	5.8[b]	11.9[a]	7.1[b]	3.9[c]	0.5
PC	5.8[c,d]	8.9[a]	6.7[b,c]	4.5[d]	0.6
PS	1.6[b]	3.0[a]	2.1[b]	0.3[b]	0.2
PI	0.8[b]	1.6[a]	1.3[a]	1.5[a]	0.1
PE	6.4[b]	10.0[a]	7.9[b]	2.6[c]	0.8
Total	20.4	35.4	25.1	12.8	2.0
	Percentage of phospholipids				
SM	28.72[b]	34.1[a]	28.7[b]	31.4[a,b]	1.1
PC	28.0[b]	25.1[b]	26.4[b]	35.1[a]	1.1
PS	8.1[d]	8.4[a]	8.5[a]	1.9[b]	0.4
PI	4.1[d]	4.6[c,d]	5.2[b,c]	11.8[a]	0.3
PE	31.0[a]	27.8[a]	31.1[a]	19.8[b]	1.6

[a,b,c,d]Means within a row with different superscripts differ ($P < 0.05$).

[1]Adapted from Bitman and Wood (1990).

[2]SM = Sphingomyelin, PC = phosphatidylcholine, PS = phosphatidylserine, PI = phosphatidyl-inositol, and PE = phosphatidylethanolamine.

Table 13. Sphingomyelin is usually reported as a PL, although it is not a member of this lipid class.

Christie et al. (1987) reported the data from the separation of PL by HPLC (Table 14). They detected almost no PL in samples of commercial powdered whole milk and buttermilk. They attributed the absence to the autooxidation of PUFA in the PL as a result of exposure to heat during the processing. Christie et al. (1987) prepared and analyzed buttermilk, the parent milk, and the resulting skim milk for total PL content. They found (mg/dl): whole milk, 14; buttermilk, 12; and skim milk, 9. More data on the PL and GL contents of milk are given in Table 15 (Christie et al., 1987; Jensen and Newberg, 1995; Rueda et al., 1998). Milk lipids also contain small quantities (0.009%) of glycerol ethers in neutral and PL (Ahrne et al., 1979).

Table 14. Phospholipid classes (PL), wt% of total PL, in various dairy products.

Class	Bovine[1] milk	Lipid globule[2] membrane
Ceramide monohexoside	4.96 ± 0.20	3.5 mmol/mg of protein
Ceramide dihexoside	2.86 ± 0.78	
Phosphatidylethanolamine (PE)	34.17 ± 0.50	27
Phosphatidylinositol	6.23 ± 0.03	11
Phosphatidylserine	2.81 ± 0.32	4
Phosphatidylcholine (PC)	25.41 ± 0.23	36
Sphingomyelin	23.57 ± 0.26	22
Total Pl	22.78 ± 0.66[3]	26–31
Total lipid	3.5–4.0?	0.5–1.1 mg/mg of protein

[1]Christie et al. (1987). Herd milk. Fat content not given; separation by HPLC.

[2]Keenan et al. (1988). Lyso PC and PE also detected.

[3]mg of PL/dl.

Table 15. Phospholipid and sphingolipid composition of bovine milk.[1]

Phospholipid	Mol%
Phosphatidylcholine	34.5
Phosphatidylethanolamine	31.8
Phosphatidylserine	3.1
Phosphatidylinositol	4.7
Sphingomyelin	25.2
Lysophosphatidylcholine	Trace
Lysophosphatidylethanolamine	Trace
Total choline phospholipids	59.7
Plasmalogens	3
Diphosphatidylglycerol	Trace
Ceramides	Trace
Cerebrosides (neutral glycolipids)	3 mg/L[2]
Gangliosides	1.4 mg/L[3]

[1]Adapted from Jensen and Newberg (1995). Contains about 12 to 35 mg/dL.

[2]Christie et al. (1987).

[3]Rueda et al. (1998).

Some of these sphingolipids are anticarcinogenic. Sphingomyelin inhibited an early stage of colon carcinogenesis in mice (Vesper et al., 1999). The neutral glycosphingolipids, glucosyl-and lactosylceramide inhibited colonic cell formation and aberrant crypt foci formation in CF-1 mice that had been exposed to 1,2-dimethylhydrazine (Schmelz et al., 2000).

The PL and GL contain relatively larger quantities of PUFA than the TAG, but the amounts are so small that they have little nutritional significance. The PL and GL bind cations help to stabilize the emulsion and probably orient enzymes on the globule surface, but their effects in processed milks are unknown.

Glycosphingolipids

Neutral glycolipids. The glycolipid (cerebroside) fraction of bovine milk was reported to be about 20 mg/L, with an ML content of 4% (Hladick and Michaelec, 1966). The principal constituents of this fraction are: glucosylceramide, 35% and lactosylceramide, 65% (Christie et al., 1987). The quantities of these compounds and other glycosphingolipids with references are listed in Table 16. About 70% of the glycolipids are associated with the MLGM.

Gangliosides. Jensen and Newberg (1995) and the recent paper by Rueda et al. (1998) are the basics source of information for this section. Gangliosides are glycosphingolipids composed of a ceramide and an oligosaccharide chain attached to one or more sialic (neuraminic) acids plus several sugars, e.g., glucose and galactose. The gangliosides are named as follows (Bonafede et al., 1989; Puente et al., 1992) $\mathbf{GM_1}$, 2, and 3 are monosialogangliosides with 1, 2, and 3 as

Table 16. Glycosphingolipids of bovine milk.[1]

	mg/L	Reference
Neutral glycolipids or cerebrosides		
Ceramide monohexoside (glucose)	11.3	Christie et al. (1987)
Ceramide monodihexoside (lactose)	6.5	Christie et al. (1987)
Gangliosides. Contain NANA[2]		
Total		
Mature About 8 μg/mg membrane protein		Keenan and Patton (1995)
Mature	11.0	Laegrid et al. (1986)
Colostrum	7.5	Puente et al. (1992)
Mature	1.4	Puente et al. (1992)
Mature	4.0	Pan and Izumi (2000)
Monosialoganglioside 1	0.0012	Laegrid et al. (1986)
	1.2	Laegrid et al. (1986)
Disialoganglioside Ib	0.7	Pan and Izumi (2000)[3]
Monosialoganglioside 2	0.7	Laegrid et al. (1986)
	Trace	Puente et al. (1992)
Disialoganglioside 2	Trace	Bushway and Keenan (1978)
Monosialoganglioside 3	0.3	Laegrid et al. (1986)
20% of total 1.4 mg/L	0.2	Puente et al. (1992)
Disialoganglioside 3	0.1	Pan and Izumi (2000)
Colostrum	92.5	Laegrid et al. (1986)
Mature	8.8	Laegrid et al. (1986)
Mature	2.4	Pan and Izumi (2000)
60–70% of total 1.4 mg/L	0.8–1.0	Puente et al. (1992)
Named $G_{lac}2$ by authors	4.8	Jenneman and Wiegandt (1994)
Triasialoganglioside		
Buttermilk solids, mg/kg	28.0	Takamizawa et al. (1986)
Buttermilk solids, mg/kg	22.4	Ren et al. (1992)
5% of total, 1.4 mg/L	0.07	Puente et al. (1992)
Branched, buttermilk solids, mg/kg	78	Takamizawa et al. (1986)
Branched, buttermilk solids, mg/kg	8.5	Takamizawa et al. (1986) Ren et al. (1992)
9-O-acetyl, GD_3, buttermilk solids, mg/kg	22	Bonafede et al. (1989)
7,9,-di-O-acetyl GT_3, buttermilk solids, mg/kg	24	Bushway and Keenan (1978)
9-O-acetyl, GD_3, buttermilk solids, mg/kg	1.0	Ritter et al. (1990)

[1]Adapted from Jensen and Newberg (1995).

[2]NANA is *N*-acetylneuraminic *N*-glycoyl neuraminic acids or sialic acid.

[3]Pan and Izumi (2000) identified small quantities of disialogangliosade Ia or GD_{1a}.

Table 17. Lipid composition of anhydrous butter fat and dry whey products using amino propyl solid-phase extraction (SPE) compared with those reported in the literature using TLC.

	Total lipids, wt%[1]					Total wt%[2]	
	Anhydrous milk fat						
Lipid classes	SPE	TLC[3]	WPC-75	WPC-34	WP	Commercial	Cheddar
Free fatty acids	0.22 ± 0.03	0.28	2.29 ± 0.10	3.36 ± 0.52	4.95 ± 0.26		
Phospholipids	1.30 ± 0.14	1.11	23.64 ± 1.15	17.53 ± 1.63	18.04 ± 2.55	31.4	12.7
Neutral lipids	98.48 ± 0.21	98.61	74.08 ± 1.45	79.11 ± 1.12	77.01 ± 2.69	65.8	83.6
Cholesterol esters	0.02[4]	0.02	1.52 ± 0.11	1.7 ± 0.37	1.84 ± 0.57		
Triacylglycerols	95.44 ± 0.22	95.80	61.12 ± 2.44	64.10 ± 3.86	60.82 ± 2.74		
Cholesterol	0.46[4]	0.46	2.41 ± 0.20	2.54 ± 0.30	3.21 ± 0.53		
Diacylglycerols	2.17 ± 0.06	2.25	5.46 ± 0.16	4.87 ± 0.49	5.32 ± 0.38		
Monoacylglycerols	0.07 ± 0.01	0.08	3.30 ± 0.04	5.70 ± 0.05	5.83 ± 0.10		
Total lipids, % recovery	99.64 ± 1.22	. . .	99.74 ± 1.42	99.80 ± 0.97	99.01 ± 1.51		
Total lipids, %			. . .	7.87 ± 0.25	. . .	3.9 ± 0.2	17.2 ± 0.2
Total protein, %			75.0	34.0	12.0	18.3 ± 0.0	23.9 ± 0.2

[1]Adapted from Vaghela and Kilara (1995a). WP-75, WP-34, and WP were commercial products from New Zealand. Commercial was a dried whey and Cheddar, liquid whey from the United States. Procedure is SPE unless indicated.

[2]Adapted from Boyd et al. (1999). Commercial was a dried whey and Cheddar, a liquid whey from the United States.

[3]From Bitman and Wood (1990).

[4]The amounts given in the paper are incorrect. They were cholesterol esters and cholesterol, 0.22. They are changed to 0.02% and 0.46% from Bitman and Wood (1990); although butterol was used for SPE and extracted fat for TLC. These will differ.

Table 18. Composition of phospholipids from dry whey products.

Classes of phospholipid	wt% (% of the Total phospholipids)[1]			w% of Total	
	WPC-75	WPC-34	Whey power	Commercial[2]	Cheddar[2]
Cerebrosides	3.57 ± 0.13	2.87 ± 0.35	3.97 ± 0.41	5.0 ± 0.5	0.9 ± 0.4
Phosphatidylinositol	18.13 ± 1.23	21.95 ± 2.51	18.41 ± 1.85	8.5 ± 1.5	4.1 ± 1.0
Phosphatidylethanolamine	4.45 ± 0.21	2.21 ± 0.18	1.94 ± 0.17	34.2 ± 0.5	60.1 ± 3.2
Phosphatidylserine	7.48 ± 0.58	9.56 ± 1.66	8.35 ± 0.64	Combined with phosphatidylinositol	
Phosphatidylcholine	30.54 ± 1.84	31.59 ± 1.15	33.34 ± 1.11	48.6 ± 1.3	33.3 ± 4.1
Sphingomyelin	35.82 ± 1.16	31.83 ± 1.98	33.99 ± 2.01	3.7 ± 0.3	1.7 ± 0.3
Lysophosphatidylcholine	ND[3]	ND	ND	ND	ND

[1]Adapted from Vaghela and Kilara (1996b). WP-75, WO-34, and WP were commercial products from New Zealand. Commercial was a dried whey, and Cheddar the whey from the preparation of the cheese (a liquid whey from the United States).

[2]Adapted from Boyd et al. (1999).

[3]ND = Not detected.

subscripts; (**GD**) is disialo, and (**GT**) trisialo. The additional sugars are attached to the oliogsaccharide chain in specific sequences. The formulas for the gangliosides in milk are presented in Jensen and Newberg (1995; Table 16).

Keenan et al. (1988) reported that the MLGM has the same ganglioside profile as the apical plasma membranes of the secretory cells in mammary gland, that 90% of the gangliosides are found in the MLGM, and the principal gangliosides are GM_3, GM_2, and GD_3. Several others, GD_2 and GD_{1b} were present in smaller quantities. Keenan and his colleagues (Keenan and Patton,1995; Keenan et al., 1988) have done much of the basic research on the MLGM, providing information about the organization of the other components, such as proteins.

The gangliosides mediate several important metabolic and cellular events. Earlier information on gangliosides is available in Pan and Izumi (2000) and Dirk and Reimerdes (1988). The monosialoganglioside GM_1 inhibited the enterotoxins of *Escherichia coli* and *Vibrio cholera* (Newberg and Chaturvedi, 1992). Although human milk contains 10 times more GM_1 than bovine milk, human milk is not converted to butter. Many thousands of pounds of spray-dried buttermilk are available as a source of GM_1 and other gangliosides. Bonafede et al. (1989) isolated about 1.2 mg of 9-*O*-acetyl-GD_3 from 230 g of spray-dried buttermilk by solvent extraction and ion-exchange and silica gel column chromatographic procedures. Ren et al. (1992) suggested this ganglioside might be important in murine development at the two-cell stage and in some differentiated tissue. However, their purification procedure was prolonged and complex, and yields were low. Moving from bench to large-scale operations is difficult, but the availability of this and other gangliosides would provide sufficient material for use in research.

Ren et al. (1992) observed 9-*O*-acetyl-GD_3 in hamster melanomas. They postulated that the ganglioside might modulate critical events in cell metastasis and growth in melanoma cells. They obtained *O*-acetylated gangliosides from 1.7 kg of spray-dried buttermilk, as follows (mg): 7,9-GD_3, 2; 7- or 9-GD_3; 37; and *O*-acetyl-GT_3, 40. These yields are better and could provide sufficient material for tests with animals and possibly humans. GD_3, GM_3, and sphingomyelin purified from buttermilk are available from Matreya, Inc. (Pleasant Gap, PA). It is possible that the gangliosides would be designated as generally recognized as safe (GRAS). Ritter et al. (1990) isolated 0.15 mg of 9-*O*-acetyl-GD_3 from 150 g of buttermilk. Their yields were much smaller than those obtained by Ren et al. (1992), but the acetyl group was attached solely to the sialic acid moiety. This compound induced an immune response against human melanoma cell surface antigens in the mouse.

The gangliosides are present in dried buttermilk, and laboratory procedures are available for their isolation. It remains to be seen if these methods can be expanded. This includes the recovery of MLGM material in the pilot plant because basic data are lacking. I was unable to find any papers that provided data obtained by modern methods on the volumes of product, amounts of lipids, PL, and complex lipids and their FA and base contents in the processing of 1) milk to skim milk and cream, 2) conversion of cream to butter and buttermilk, and 3), conversion of butter to BO and serum. This area of considerable potential benefit has been ignored. Maxwell et al. (1986) found that the PL content of buttermilk lipid was 7.2% and that of skim milk from the same whole milk, 2.2%.

Vaghela and Kilara (1995a) developed methods for extraction of total lipids and separation of their classes with solid-phase extraction from whey protein concentrates. Their results are shown in Table 17. The re-

Table 19. The cholesterol contents of various dairy products.[1]

Identity of product	Fat (%)	Cholesterol (mg/100 g)
Skim milk	0.25	2
Whole milk	3.34	14
Half and half	11.50	37
Light cream	19.31	66
Medium cream	25.00	88
Nonfat dry milk	0.77	20
Cottage cheese, creamed	4.51	15
Cream cheese	34.87	110
Ice cream, vanilla	11.01	44
Blue	28.74	75
Brie	27.68	100
Cheddar	33.14	105
Mozzarella, whole milk	21.60	78
Neufchatel	23.43	76
Swiss	27.45	92
Butter	81.11	219
Sherbert orange	2.00	6

[1]From Jensen and Newberg (1995); USDA Nutrient Data Base (1999).

sults of Boyd et al. (1999) are included. Their procedure is easy, quantitative, and can be performed on a quantitative scale on concentrates containing large mounts of protein. It should be useful for the analysis of dried buttermilk. Vaghela and Kilara (1996a) evaluated the foaming and emulsifying properties of the concentrates and determined the lipid and PL composition of several laboratory and commercial concentrates (Vaghela and Kilara, 1995b, 1996b). The PL compositions of several laboratory and one commercial whey concentrates are given in Table 18 (Vaghela and Kilara, 1995b). The results of Boyd et al. (1999) are also presented.

Sterols

Milk contains 10 to 20 mg/dl of C or 308 to 606 mg/100 g of fat in whole milk containing 3.3% fat (Jensen and Newberg, 1995; Table 2). The amount is positively correlated with the fat content of the dairy product as shown in Table 19. (USDA Nutrient Database, 1999). Cholesterol is the major sterol, 95% of total, and is located mostly in the MLGM (Jensen and Newberg, 1995). Some of the C is bound to β-lactoglobulin (Wang et al., 1997). About 10% of the C is esterified. Small quantities of other sterols have been detected, e.g., 7-dehydrocholesterol and small amounts of phytosterols (IDF, 1992). Because C accounts for 95% of the total sterols, nonspecific methods have been employed. I have listed these in Table 20 and will comment on them below. The manner in which milk is obtained, elimination of colostrums, and pooling reduces the effects of time postpartum and diurnal rhythm seen in individual samples. Products produced by oxidation of C in dried milk products have been investigated (results discussed later).

The least expensive, easiest, and most widely applicable of the methods for the determination of C is the colorimetric method of Bachman et al. (1976). Because C is the major sterol in ML, 95% of the total, we need not be concerned that the procedure is not specific. The C in 1 ml of milk, 144 μg, can be readily detected. Also, the amounts of C in dairy foods will usually be about the same as those available from the USDA web site, www.nal.usda.gov/fnic (USDA Nutrient Database, 1999), and the minimum fat contents are fixed by statute, so these data can be employed. Unless the researcher is developing a new product, investigating the metabolism of C, or identifying vitamin D metabolites, there is little need to analyze C. Exceptions are the analyses of mixtures of vegetable oils and MF (IDF, 1992; Alonso, et al., 1997). A standard BO is available for checking the methods (Precht et al., 1998b). How to determine C oxides will be discussed later.

The procedure in method 7 (Fletouris et al., 1996; Table 20, for the preparation of milk samples before analysis of C by GLC), e.g., saponification in a capped tube followed by extraction with hexane is identical to that used in method 1 (Bachman et al., 1976) and is similar to the AOAC method 10 (Klatt et al., 1995). However, the earlier paper was not cited by Fletouris et al., 1998). I will use this as an excuse to give an anecdote to remind readers that the usefulness of a paper does not vanish because it was published more than 10 yr in the past. In 1984, I attended a meeting on methods of analyses for the components in human milk. Many of these had been developed for use by the dairy industry. The investigators interested in proteins argued for 3 d about the best method for determining the protein content of human milk. They finally selected the Kjeldahl method. The description of this procedure was published in 1883! Foote (1999) recently commented on the need to be a student of history.

Because consumption of C has been related to the incidence and severity of cardiovascular incidents, and milk products have been designated as major contributors to dietary C, removal of the sterol from milk has been discussed and investigated. Cholesterol can be removed from dairy products by several methods. For example, mixing β-cyclodextrin with homogenized milk removed 92.2 to 93.5% of the C (Lee et al., 1999). The justification for removal was discussed and rejected by Sieber (1994). The contribution of dietary C to increased plasma C does not justify the cost.

Oxidized sterols. These are found in dairy products that have been heated, dried, and stored, have been

Table 20. Methods for the determination of cholesterol in milk lipids.

	Sample preparation	Results	References
1. Colorimetric, *o*-phthaldehyde derivatives	1 to 10 ml of milk. Saponification, extraction with hexane	Milk: 3.4% fat; 14.0 mg/100 g Skim milk: 0.13% fat 2.8 mg/100 g	Bachman et al. (1976)
2. GLC by capillary columns. Internal standard	About 2 g. Saponification, extraction with Et_2O, TLC, silylation of sterols.	300 mg/100 g fat, milk; 3.9% fat, 11.7 mg/100 g. 7-dehydrocholesterol, 0.7 to 4% of total sterols	IDF (1992)
3. GLC, as above, semicapillary	Solvent extraction	239 mg/100 g fat	Kisza et al. (1994)
4. GLC, as above, capillary. Transesterification.	Alkaline transesterification. Injection of hexane extract.	Detect 5% vegetable oils added to milk fat	Alonso et al. (1997)
5. GLC, capillary column, programmed-temperature vaporizer injector. Internal standard	Direct injection	270 mg ± 1.7 SD/100 mg of fat	Alonso et al. (1995)
6. GLC, packed column, cholesteryl butyrate standard	Extraction with $CHCl_3$-MeOH, saponification	. . .	Sheppard (1992)
7. GLC, capillary column	Same as in method 1	Milk, 12.2 mg ± SD 0.18/100 g. Butter, 228.1 ± SD 4.36/100 g	Fletouris et al. (1998)
8. HPLC, internal standard	Saponification, extraction with hexane-Et_2O (1:1 vol/vol)	Butter oil, 296.5 mg/100 g. Cholesterol and phytosterols not separable	Casirighi et al. (1998)
9. Supercritical fluid chromatography (SFC), internal standard	Saponification, extraction with hexane	CRM sample 164, 273 mg/100 g of fat[1] SF3 270 mg GLC 302 mg	Huber et al. (1995)
10. Capillary GLC	Saponification		AOAC method Klatt et al. (1995)

[1]CRM 519 = Anhydrous butter oil obtained from the Community Bureau of Standards, Brussels, Belgium (Precht et al., 1998b).

implicated in the initiation of the atherosclerotic process (Appelqvist, 1996). However, the oxides are found in other foods treated similarly. Analyses for the oxides is difficult and "true" values have not been established, but 2 ppm or 2 mg/kg of C oxides in reference skim milk powder lipids was selected as the correct value for the product. The quantity for fresh skim milk powder was 0.7 ppm. Nielsen et al. (1996a) found 4 mg/kg of oxysterol in butter stored at –4°C, and 7-dehydrocholesterol was the major oxysterol. Nielsen et al. (1996b) also analyzed Feta cheese produced from high bleached and nonbleached BO and stored for 5 mo. They employed solid-phase extraction, preparative HPLC, and separation and quantification of the oxysterols by GLC mass spectrometry (**GLC-MS**). In the reference Feta cheese, the concentration of 9-keto-C was 1.2 mg/kg of lipid, while in the bleached cheese, it ranged from 10.4 to 13.1 mg/kg of lipid. 7-keto C made up about 55% of the total oxysterols. Nielsen et al. (1996a) also found 4 mg/kg of oxysterol in butter stored at –4°C, 7-dehydrocholesterol was the major oxysterol. The reliability of these data has been questioned (Jimenez-Flores, 1997), since saponification, even at 20°C can create oxidation products. The factors causing oxidation were eliminated to the extent possible by Przygonski et al. (2000) in their analyses of milk powder samples and infant formulas for oxysterols. They found 5.21to 6.351 μg/g of lipid extract in three milk powder samples. The compounds present in largest quantities were (μg/g of lipid): α-epoxy C, 1.56 to 2.43 and 7-keto-C, 1.76 to 2.08.

Are the oxysterols in relevant dairy products hazardous? The answer is possibly, according to Zhou et al. (2000), who observed that excess concentrations of oxysterols were cytotoxic to cultural human endothelial cells. This group (Kummerow et al., 2000), also found a significant correlation between the concentrations of oxidation products rather than C in the plasma in catheterized patients with identified cardiac stenosis. These results support the belief that oxidized low-density lipoprotein (**LDL**) initiates the pathological events leading to atherosclerosis. Are dietary oxysterols involved?

While this question cannot be answered now, it appears unlikely because of the following observations. Lyons et al. (1999) found that dietary 7-keto C, the major oxysterol, is rapidly metabolized and excreted by the rat's liver. Enterohepatic recycling of the sterol was negligible. Their results indicate that the diet may not be a major source of oxysterols in atherosclerotic plaques, and that dietary oxysterols probably make little or no contribution to atherogenesis. With these

results in mind, we should be aware of, but not overconcerned about, the oxysterols found in milk and dairy products.

Isoflavanoids. Evidence suggess that isoflavonoids may provide some benefits if consumed. However, because they have estrogenic properties based on animal studies, their consumption by infants and young children might be undesirable. Because various feed materials consumed by dairy cattle contain isoflavonoids and there are few data about their contents in milk, King et al. (1998) analyzed the amounts in Australian milks. They determined the daidzein and genistein contents by HPLC in 148 samples from 76 farms and a factory from September 1995 throughout January 1996. The mean daizeden content was <5 ng/ml. The mean genistein contents ranged from about 2 ng/ml in the summer samples to 20 to 30 ng/ml in spring samples, when clover was the major pasture forage. The contents were 45 ng/ml in the summer milks and 293 ng/ml in the spring samples. Pasteurization had no effect on the contents. The authors concluded that the low contents were not likely to have pronounced biological effects if consumed by humans. They also noted that soy foods are the major sources of isoflavonoids in foods for humans. The effects of feeding soy products to dairy cattle upon the contents of isoflavonoids in their milk has not been reported.

FATTY ACIDS

The abbreviations for identification of FA are as described by Lobb and Chow (1999). This terminology is used in the American Journal of Clinical Nutrition (McGlade et al., 1996) and recently, in Lipids. For example, oleic acid is 9*c*-18:1; linoleic acid, 9*c*, 12*c*-18:2, and CLA is 9*c*, 11*t*-18:2. The prefix C is not needed nor used. Neither is the subscript 18:1. While there is no official abbreviated nomenclature, this is, in my opinion, the easiest to use and understand. Classes of unsaturated FA are named as n-6 or n-3, which identifies the position of the first double bond from the terminal methyl group.

Recent publications on milk FA in the United States have focused on the effects of various feeding regimens, on CLA, *trans* isomers, and the FA profiles. The complete FA profiles have not been reported. Only one of the analyses was done with the best analytical method available, the argentation-TLC/GLC (**Ag-TLC/GLC**) procedure (Wolff et al.,1998a; Wollf, 1999). With this method, quantification of the *trans* and *cis* isomers of 18:1 and other unsaturated FA can be attained. If this method is not used, the quantities of 9*c*-18:1 will be overestimated, and *trans*-18:1 underestimated. I will present examples later. Briefly, the method requires 1) analysis of the FA as methyl (**FAME**) or isopropyl esters (**FAIPE**) with a 100-m capillary column coated with a polar material and 2) Ag-TLC to resolve the *trans* and *cis* ester bands, followed by analysis of the esters in the bands with the GLC column used in 1). Details will be given later.

Analysis

Sampling. If a commercial product is to be analyzed, the fat content is known and need not be determined (discussed earlier), but it must be reported. When milk from individual cows is being tested, the fat content must be determined (discussed earlier), and determination with infrared analyzers can be used. Because the total lipids must be extracted, gravimetric determination is easily done. An alternative is to use an external standard during the GLC analysis. This will be required anyway.

Preparation of esters. Methyl, butyl, and isopropyl esters have been used-butyl and isopropyl esters because they are less volatile than the FAME and reduce the inevitable loss of methyl butyrate that occurs during analytical processing. Nevertheless, FAME can be employed with proper care. Sodium methoxide catalyzed transesterification is recommended because acid catalysts, HCl, H_2SO4, acetyl chloride, and BF_3, decrease the major CLA, 9*c*, 11*t*-18:2, increase 9t, 11*t*-18:2, and produce allylic methoxy artifacts (Kramer et al., 1997). Conversely, Yamasaki et al. (1999) found that CLA were not affected by acid catalysis if proper amounts (0.5 or 1.0 ml) of dimethylsulfoxide or dimethylformamide were added to the reaction mixture. Acid catalysts will be required if substantial amounts of free FA are present in the product being analyzed. Their presence can be detected by TLC. Yurawecz et al. (1999) reviewed the methylation procedures for CLA and describe the methods for many matrices. Analysts should read this paper for comprehensive instructions. Information about base and acid catalyzed methods is available from the IDF (1999a).

Ulberth et al. (1999) tested the response of a flame-ionization detector to methyl, ethyl, propyl, and butyl esters of 4:0 to 18:0 and 18:1. The esters were prepared by transesterification of a mixture of monoacid TAG resembling the FA in ML. They compared the theoretical response and empirical response factors [(weight%)/(area%)]. The authors preferred using butyl esters, although when MF was analyzed, butyl and FAME profiles were in close agreement. However, analysts should check the validity of their procedures by routine use of a TAG mixture with a FA profile similar to that of MF.

Wolff (1994) determined the theoretical conversion factors relative to 18:0 required to convert peak area percentages to FA weight percentages. He checked C1-C5 alcohols versus the chain length of the FA and found that the conversion factors for butyrate were: methyl, 1.39; isopropyl, 1.03, and butyl, 0.8. Use of FAIPE eliminated the need to convert peak area percentages into FA weight percentages using conversion factors.

Wolff et al. (1995) described a procedure for the direct transfer of the FAIPE esterification mixture to the GLC instrument without intermediate evaporation. Otherwise, the volatile FAME of short-chain esters will be partly or completely lost by evaporation. Although it is unlikely that labels listing the contents of classes of FA, e.g., saturates will be required on dairy products, they are, or will be on foods to which fats and oils have been added. The FDA will require that the amounts be given as TAG equivalents or the amounts of FA plus the portion of glycerol added to it. The importance is that glycerol contributes to caloric intake, which has been ignored. The factors required to convert the amount of an FA to its TAG equivalent are listed by Sheppard (1992) and can be calculated as described by Jensen (1999). The factors range from 1.14 for 4:0 to 1.05 for 16:0.

Gas-liquid chromatography. A 100-m capillary column coated with a cyanopropyl polysiloxane stationary phase (CP-Sil 88, SP-2560, or BPX-70) is required (Wolff et al., 1998a). According to Precht (personal communication, 2000), the initial temperature of the oven should be 50°C, 1 min isothermal, then 5°C/min to 225°C, held at this temperature for 15 min and finally raised by 5°C/min to 260C. Wolff (1994) employed a similar program. An internal standard must be used (Wolff et al., 1998a) and the detector response should be checked regularly with a known mixture of TAG with a FA content similar to MF. Information about the use of GLC to determine the FA composition of MF is available from the IDF (1999b). However, the method above will provide more information.

European reference standard, COM 164, anhydrous MF is available as BCR-16.4 from R.T.C. (Laramie, WY). However, the FA composition determined by the Ag-TLC/GLC method is not available.

Fatty Acids

I will discuss FA data on ML from three areas based on the GLC method employed. These are: short, packed (old), capillary columns alone (cap), and AgCl-TLC/GLC methods (new). I will present new data first, since they must be used to illustrate deficiencies in the cap and old data. I have prepared a brief review on milk FA that includes some of the information above Jensen (2000). The data presented on old data are limited to those required to illustrate the differences in amounts of 18:1, 18:2, and their *trans* and *cis* isomers. More cap data will be given because it was used in most recent studies.

Determination of FA by the Ag-TLC/GLC method. The method was described by Ratnayake et al. (1990) and used to obtain some data on the *trans* isomers in butter (Ackman and Macpherson, 1994). Chen et al. (1995) gave data on the contents of *cis* and *trans* isomers in human MF, but they were in bar graphs and small quantities are difficult to estimate. Strocchi and Holman (1971) and Smith et al. (1978) earlier analyzed the various bands separated by Ag-TLC, but the columns they employed could not resolve the *cis* and *trans* isomers of 18:1. The information about *trans* FA has been reviewed by Fritsche et al. (1999a).

Data obtained with the method by Wolff et al. (1995) and Precht et al. (1999) are presented in Tables 21 to 24. Earlier information on the procedure (Precht and Molkentin, 1996) and the effects of feeding (Precht and Molkentin, 1997b) are available. More complete coverage on the influence of season is given in this reference. Additional information about the isomers of 18:2 is presented in Precht and Molkentin (1997a). They reported the following information on the CLA contents of 238 German milk samples (wt%): mean, 0.81; SD, 0.392, and range, 0.25 to 1.95.

Zegarska et al. (1996) analyzed Polish milk FA with the Ag-TLC/GLC method during the barn and pasture feeding periods. They reported for barn and pasture feeding, respectively, these amounts of trans isomers (wt%): total *trans*-18:1; barn, 1.83; pasture, 5.73; 10+11 *trans*-18:1, 0.91 and 3.69. Precht et al. (2001) determined the amounts of positional individual isomeric *trans* and *cis*-18:1 acids in the milks of cows, goats, and ewes. The authors presented more data on the effects of season on the *trans* isomer contents in milks from the three species. They confirmed that vaccenic acid, 11*t*-18:1, is the major FA in all the milks. This information is useful because the isomer can be desaturated to 9c, 11*t*-18:2, a bioactive CLA, in the human (Adlof et al., 2000). This isomer requires additional techniques for identification (Yurawecz et al., 1998). I will discuss CLA later in a separate section.

Destaillats et al. (2000) identified the individual *trans* and *cis* isomers of 16:1 in cow, goat, and blue cheese fats. They found that the major *trans* isomer was 9*t*. Unless the Ag-TLC/GLC method is used, branched 17:0 and 16:1 *cis* isomers overlap with the *trans* 16:1 peak during GLC analysis, resulting in erroneously high val-

Table 21. Fatty acid composition (wt%) of French butters collected at different periods of the year.[1]

Fatty acid	January[2]	March	May-June	July-August	October-November
4:0	3.95 ± 0.21	4.40 ± 0.25	3.83 ± 0.13	4.21 ± 0.27	4.29 ± 0.24
5:0	0.03 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
6:0	2.53 ± 0.09	2.67 ± 0.09	2.38 ± 0.08	2.52 ± 0.11	2.55 ± 0.11
7:0	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
8:0	1.52 ± 0.05	1.56 ± 0.04	1.42 ± 0.07	1.46 ± 0.09	1.52 ± 0.08
9:0	0.04 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
10:0	3.45 ± 0.11	3.46 ± 0.11	3.14 ± 0.19	3.13 ± 0.26	3.28 ± 0.20
10:1	0.34 ± 0.02	0.34 ± 0.01	0.30 ± 0.01	0.34 ± 0.02	0.35 ± 0.02
11:0	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
12:0	3.99 ± 0.11	3.95 ± 0.16	3.57 ± 0.26	3.50 ± 0.30	3.75 ± 0.27
13:0	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.10 ± 0.01
Iso-14:0	0.12 ± 0.02	0.12 ± 0.03	0.13 ± 0.01	0.13 ± 0.02	0.12 ± 0.02
14:0	12.08 ± 0.21	11.93 ± 0.38	11.03 ± 0.36	11.16 ± 0.41	11.32 ± 0.33
Iso-15:0	0.23 ± 0.04	0.31 ± 0.07	0.34 ± 0.04	0.36 ± 0.05	0.27 ± 0.02
Anteiso-iso-15:0	0.37 ± 0.11	0.49 ± 0.09	0.58 ± 0.04	0.59 ± 0.08	0.67 ± 0.10
14:1	1.12 ± 0.05	0.95 ± 0.07	0.91 ± 0.07	1.05 ± 0.14	0.88 ± 0.07
15:0	1.16 ± 0.09	1.20 ± 0.10	1.15 ± 0.05	1.19 ± 0.09	1.13 ± 0.08
Iso-16:0	0.28 ± 0.05	0.27 ± 0.04	0.27 ± 0.02	0.29 ± 0.07	0.24 ± 0.03
16:0	33.34 ± 0.74	32.52 ± 1.06	27.05 ± 1.37	28.27 ± 2.20	29.27 ± 1.91
16:1	0.27 ± 0.04	0.20 ± 0.06	0.35 ± 0.04	0.37 ± 0.07	0.27 ± 0.06
Iso-17:0	0.30 ± 0.05	0.29 ± 0.14	0.42 ± 0.02	0.43 ± 0.8	0.34 ± 0.02
16:19	1.54 ± 0.14	1.57 ± 0.10	1.43 ± 0.08	1.46 ± 0.12	1.67 ± 0.16
Anteiso-iso-17:0	0.52 ± 0.06	0.56 ± 0.05	0.1 ± 0.02	0.53 ± 0.06	0.45 ± 0.03
17:0	0.51 ± 0.04	0.54 ± 0.02	0.70 ± 0.04	0.57 ± 0.05	0.65 ± 0.03
17:1	0.24 ± 0.02	0.25 ± 0.02	0.38 ± 0.03	0.33 ± 0.04	0.35 ± 0.04
18:0	9.01 ± 0.54	8.95 ± 0.55	10.96 ± 0.69	10.54 ± 0.91	9.61 ± 0.54
18:1[3]	19.16 ± 0.54	19.67 ± 0.89	24.00 ± 1.03	22.93 ± 1.50	22.62 ± 1.58
18:2 isomers	0.41 ± 0.12	0.41 ± 0.07	0.93 ± 0.08	0.48 ± 0.10	0.68 ± 0.12
18:2n-6	1.37 ± 0.14	1.49 ± 0.27	1.17 ± 0.15	1.26 ± 0.15	1.35 ± 0.15
20:0	0.12 ± 0.01	0.10 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.13 ± 0.01
18:3n-3	0.25 ± 0.05	0.25 ± 0.05	0.60 ± 0.07	0.53 ± 0.10	0.46 ± 0.09
20:1	0.12 ± 0.01	0.12 ± 0.02	0.10 ± 0.02	0.11 ± 0.02	0.12 ± 0.01
18:2 conjugated	0.38 ± 0.06	0.46 ± 0.05	0.74 ± 0.16	0.65 ± 0.17	0.48 ± 0.10
Others	1.04 ± 0.17	0.78 ± 0.33	1.21 ± 0.31	1.24 ± 0.38	0.91 ± 0.36

[1]Adapted from Wolff et al. (1995).
[2]Mean + SD for 12 samples of butter at each period.
[3]*cis* and *trans* isomers.

ues for *trans* 16:1. The authors believe that this decrease in real contents of *trans* 16:1 invalidates the allegation that the acid is involved in the etiology of ischemic heart disease (Thomas et al., 1987). They found 4-14*t*- and 7,9,10-13*c* isomers of 16:1. They reported, for the first time, the presence of 3*t*-16:1.

Determination of FA with capillary columns. Almost all of the recent data on milk FA in North America were captured with capillary columns alone. Information about milks from the United States and butter from Canada (Jensen and Newberg, 1995) is given in Table 25. Table XV in Jensen and Newberg

Table 22. Content and distribution profile of *trans*-18:1 isomers in French butters as a function of the period of collection.[1]

Collection Period	Total *trans*-18:1 acids[3]	Individual *trans*-18:1 isomers[2]					
		6-9	10+11	12	13+14	15	16
January	2.37 ± 0.27	10.10 ± 1.20	48.40 ± 3.00	8.90 ± 0.70	17.00 ± 0.90	6.60 ± 0.60	9.00 ± 1.10
March	2.37 ± 0.23	6.70 ± 1.30	47.80 ± 1.90	10.20 ± 0.70	19.40 ± 1.10	7.40 ± 0.50	8.40 ± 0.70
May-June	4.28 ± 0.47	7.20 ± 0.80	58.20 ± 3.80	6.20 ± 0.70	15.40 ± 1.70	5.70 ± 0.60	7.30 ± 0.90
July-August	3.68 ± 0.68	8.00 ± 1.20	56.50 ± 3.80	7.30 ± 2.40	14.90 ± 1.40	5.70 ± 0.60	7.60 ± 1.10
Oct-Nov	3.22 ± 0.44	9.60 ± 1.30	50.40 ± 3.90	7.20 ± 0.70	17.20 ± 1.60	6.60 ± 1.60	9.00 ± 1.20

[1]Adapted from Wolff et al. (1995). N = 2 for each period.
[2]As weight percent of total *trans* 18:1 isomers.
[3]As weight percent of total fatty acids.

Table 23. Fatty acid contents of German milk lipids.[1]

Summer milk lipids			Winter milk lipids		
Fatty acid		wt%	Fatty acid		wt%
4:0		3.79	4:0		3.85
5:0		0.02	5:0		0.02
6:0		2.10	6:0		2.37
7:0		0.02	7:0		0.03
8:0		1.19	8:0		1.39
9:0		0.02	9:0		0.04
10:0		2.44	10:0		3.03
10:1		0.27	10:1		0.27
11:0		0.04	11:0		0.06
12:0		2.98	12:0		3.57
12:1		0.08	12:1		0.09
13 iso		0.14	13 iso		0.13
13 aiso		0.02	13 aiso		. . .
13:0		0.08	13:0		0.10
14 iso		0.16	14 iso		0.10
14:0		9.75	14:0		11.11
14:1		1.08	14:1		1.07
15 iso		0.43	15 iso		0.29
15 aiso		0.74	15 aiso		0.50
15:0		1.35	15:0		1.17
16 iso		0.30	16 iso		0.22
16:0		23.46	16:0		30.30
16:1		2.00	16:1		2.03
17 iso		0.65	17 iso		0.55
17 aiso		0.55	17 aiso		0.52
17:0		0.72	17:0		0.64
17:1		0.39	17:1		0.36
18 iso		0.05	18 iso		0.08
18:0		10.58	18:0		9.42
18:1	*trans* 4	0.02	18:1	*trans* 4	0.01
	trans 5	0.02		*trans* 5	0.01
	trans 6/7/8	0.26		*trans* 6/7/8	0.21
	trans 9	0.27		*trans* 9	0.17
	trans 10	0.29		*trans* 10	0.25
	trans 11	3.82		*trans* 11	1.11
	trans 12	0.33		*trans* 12	0.27
	trans 13/14	0.34		*trans* 13/14	0.24
	trans 15	0.51		*trans* 15	0.38
	trans 16	0.26		*trans* 16	0.19
	trans (total)	6.12		*trans* (total)	2.84
	cis 9 (Oleic acid)	19.44		*cis* 9 (Oleic acid)	17.28
	cis 11	0.55		*cis* 11	0.53
	cis 12	0.10		*cis* 12	0.14
	cis 13	0.06		*cis* 13	0.08
	cis 15	0.02		*cis* 15	0.03
	cis (total) other than 9	0.73		*cis* (total) other than 9	0.78
18:2 (total)		2.17	18:2 (total)		2.18
18:3	*cis* 9/12/15	0.61	18:3	*cis* 9/12/15	0.42
19:0		0.09	19:0		0.05
20:0		0.16	20:0		0.15
20:1		0.31	20:1		0.22
20:4		0.09	20:4		. . .
22:0		0.07	22:0		0.06
22:1		0.03	22:1		. . .
24:0		0.04	24:0		0.04

[1]Adapted from Precht at al. (1999).

(1995) has some errors, as follows: the ai for anteiso (where applicable) and i for iso were omitted from 13:0, 14:0, 15:0,16:0, 17:0, and 18:0. The amount 2.9 given in the second column for 18:3 n6 should be deleted and replaced with a dash. The sample did not contain this FA.

With long polar columns, the resolution of the *trans* isomers of 18:1 is incomplete. The *trans*-18:1 contents are underestimated by a general factor of 1.58% in German milks, because the 12 to 15 *trans* isomers coeluted with the 9*c*-18:1 peak. As a result, the 18:1 peak is overestimated. Information on this is shown

Table 24. Contents of $C_{18:2}$ and $C_{18:3}$ isomers in German milk lipids.[1]

Fatty acid	wt%
Summer milk lipids	
trans, *trans* NMID[2]	0.11
cis-9, *trans*-13+*trans*-9, *trans*-12	0.22
trans-8, *cis*-13	0.06
cis-9, *trans*-12	0.10
trans-9, *cis*-12	0.07
trans-11, *cis*-15	0.54
total *trans*-18:2	1.10
cis-9, *trans*-11 (CLA[3])	1.71
cis-9, *cis*-12 (linoleic acid)	1.05
cis-9, *cis*-15	0.02
total *cis*-18:2	1.07
cis-9, *cis*-12, *cis*-15 (α linolenic acid)	0.61
Other components	
Cholesterol, mg/100 g	310
α-tocopherol mg/kg	29.6
Winter milk lipids	
trans, *trans* NMID[2]	0.04
cis-9, *trans*-13+*trans*-9, *trans*-12	0.20
trans-8, *cis*-13	0.09
cis-9, *trans*-12	0.06
trans-9, *cis*-12	0.03
trans-11, *cis*-15	0.17
total *trans*-18:2	0.59
cis-9, *trans*-11 (CLA)	0.50
cis-9, *cis*-12 (linoleic acid)	1.55
cis-9, *cis*-15	0.04
total *cis*-18:2	1.57
cis-9, *cis*-12, *cis*-15 (α linolenic acid)	0.42
Other components	
Cholesterol, mg/100 g	240
α-tocopherol mg/kg	17.3

[1]Adapted from Precht et al. (1999). Ag-TLC/GLC method and 20°C data used.

[2]NMID = Nonmethylene interrupted dienes.

[3]Conjugated linoleic acid.

in Table 26 (Wolff et al., 1998a). The factors can be applied to US milks, depending on the season.

Capillary columns of various lengths and containing a variety of polar coatings were used beginning in 1959. The early methods are described by Firestone and Sheppard (1992). For example, Lanza and Slover (1981) used 60- and 100-m glass capillary columns coated with SP-2340 to determine the total *trans*-FA contents in many fast foods, some of which contained dairy products. Sampugna et al. (1982) employed a 15-m glass capillary column coated with SP-2340 and calculated factors to estimate the total *trans*-FA contents in 220 foods (Enig et al., 1983). Analyses required only 15 to 30 min. The *trans*-FA content of three samples of butter was 3.37%. Later, they derived the correction factor for the *cis*-18:1 FA (Atal et al., 1994). Nevertheless, the contents of *trans*-FA determined by this method was similar to the amounts found by Ag-TLC/GLC method (Wolff et al., 1998a).

I have calculated the amounts of 18:1s and 18:2s from the data in Table 23 that would be found when capillary (cap) and old (packed) columns were used. The results in Table 27 show the overestimation of 18:1 and 18:2 and underestimation of *trans*-18:1. The data were determined with a short packed (old) or a 100-m cap column. I have provided correction factors to convert data in old and cap papers to new and reliable quantities. The year's average factors can be employed when seasonal data are not available. The factors are reasonably reliable because the amounts of FA involved will remain stable unless an exotic ration is fed. Many papers describe the effects of different feeding regimens on milk FA profiles and the changes in contents of *trans*-FA and CLA. I will discuss these but will now give information on the contents of butyric acid, the FA and long-chain bases in sphingomyelin and hydroxy FA.

Molkentin and Precht (1997b) analyzed 130 samples of European milk for their contents of butyric acid and obtained these results (wt%): mean, 3.42; median, 3.44; range 3.07 to 3.75, and standard deviation, 0.144. They employed a 25-m capillary column and methyl valerate as an external standard. They later validated the method for the determination of MF contents in mixed fats (Molkentin and Precht, 2000b).

Sphingomyelin composition. Before presenting data on milk sphingomyelins, I will describe their structure. They are composed of a polar head group (a phosphorylated component), an *N*-acetylated FA, and a long-chain base, also named a sphingoid base (Ollson et al., 1997). The long-chain bases are usually dihydroxy analogs, saturated (sphinganine) or unsaturated (sphingosine), which are identified as d 18:0 and d 18:1, respectively. Milk sphingolipids were investigated because of the potential use in cosmetics and as pharmacological agents. (See the section on PL composition.)

Nyberg (1995) identified the long-chain bases and FA in a commercial sphingomyelin. Her results and those of Olsson et al. (1997) and Valeur et al. (1994) are shown in Table 28. Ollson et al. (1997) and Karlsson et al. (1998) provided the data in Table 29. Olsson et al. (1997) identified the derivatized long-chain bases in milk sphingomyelin with GLC-MS, given in Table 30. They, in contrast to Valeur et al. (1994) and Karlsson et al. (1997, 1998), found several with branched chains. Valeur et al. (1994) and Karlsson et al. (1997, 1998) employed HPLC-MS for separation and identification. The data of Valeur et al. (1994) on actual amounts and the relative quantities found by Karlsson et al. (1998) are also shown in Table 28. Information on the quantities of long-chain bases and FA each are presented in Table 29 and of their totals plus data on glucosyl-and lactosyl glycosides in Table 30 (Karlsson et al., 1997). Information about the preparation of the

Table 25. Fatty acids (FA) in US milks[1] and Canadian butter[2] determined by GLC with capillary columns.

Fatty acid	Milk[1] (wt%)	Butter[2] (wt% ± SD)	FA	Milk (wt%)	Butter (wt% ± SD)
Saturated fatty acids					
4:0	4.5	5.31 ± 0.30	16:0 i[3]	. . .	0.29 ± 0.01
6:0	2.3	2.81 ± 0.09	16:0	28.2	28.13 ± 0.37
8:0	1.3	1.56 ± 0.08	17:0 i	0.7	0.52 ± 0.01
10:0	2.7	3.14 ± 0.06	17:0 ai	. . .	0.50 ± 0.01
11:0	0.3	. . .			
12:0	3.0	3.39 ± 0.06	17:0	0.6	0.57 ± 0.01
13:0 i[3]	. . .	0.13 ± 0.01	18:0 i	. . .	0.09 ± 0.01
13:0	0.2	0.11 ± 0.00	18:0	12.6	10.62 ± 0.11
14:0 i	0.1	0.15 ± 0.00	19:0	. . .	0.14 ± 0.03
14:0	10.6	10.78 ± 0.17	20:0	0.2	0.20 ± 0.03
15:0 i	0.7	0.30 ± 0.01			
15:0 ai[3]	. . .	0.49 ± 0.01			
15:0	1.0	1.03 ± 0.01			
Monounsaturated fatty acids					
10:1	. . .	0.31 ± 0.01	18:1 t	1.7	
12:1	. . .	0.09 ± 0.01	18:1n9	21.4	20.84 ± 0.79
14:1n5	0.9	0.90 ± 0.02	18:1n7	. . .	0.15 ± 0.02
15:1	0.3	. . .			
16:1t	. . .	0.27 ± 0.02	20:1 n9	0.6	0.29 ± 0.06
16:1n7	1.8	1.38 ± 0.03	22:1	. . .	0.09 ± 0.05
17:1	0.4	0.28 ± 0.04			
Polyunsaturated fatty acids					
18:2t	0.4	0.47 ± 0.04	20:2n6	. . .	0.03 ± 0.02
18:2n7	. . .	0.15 ± 0.02	20:3	. . .	0.10 ± 0.01
18:2n6	2.9	2.01 ± 0.14	20:4n6	0.2	0.14 ± 0.01
18:2n4	. . .	0.09 ± 0.03	20:4n4	. . .	0.11 ± 0.05
18:3n6	. . .	0.08 ± 0.02	20:5n3	. . .	0.08 ± 0.04
18:3n3	0.3	0.48 ± 0.05	22:6n3	. . .	0.09 ± 0.05
18:4n3	. . .	0.27 ± 0.04	Unknown	. . .	0.23 ± 0.26

Personal communication, J. Sampugna, University of Maryland (1993). Analyses done with butyl esters of 4:0–14:0 and methyl esters of 14:0 and up and temperature programming. Capillary GLC columns, 12.5 m crosslinked dimethyl silicone, and 30 m SP-2380 coatings, used.

[2]Personal communication, S. J. Iverson and R. G. Ackman, Technical University of Nova Scotia (1993). Analyses done with butyl esters and temperature programming. Capillary column, 30 m with J&W DB 23 coating, used.

sources of the sphingomyelins was not given in any of these papers.

Nyberg (1995) also gave data on the FA in phosphatidylethanolamine and choline. I present them here in Table 31 because they are, insofar as I know, the only data on these milk PL that have been recently published. None of these investigators presented data on *trans*-FA, probably because they did not use appropriate methods. *Trans*-FA were detected much earlier in milk sphingomyelins, as described by Karlsson et al. (1997).

Hydroxy FA. Chance et al. (1998) utilized GLC-MS to identify the hydroxy FA in skim milk. Some of the longer C2 and C3 FA may have been derived from sphingomyelins. Their results are presented in Table 32. They could not identify the C4- and 5-hydroxy FA because they spontaneously converted to the gamma and delta lactones. These FA and lactones have been investigated because some of the latter are flavor compounds believed to be produced by heating of MF (Kurtz et al., 1974). If we assume that the gamma and delta lactones found in butter (Kurtz et al., 1974) were all derived from 6-19 and 4-OH FA, then 24 hydroxy FA, in addition to those in Table 32, have been identified in ML. The presence of unsaturated and branched-chain lactones has been reported, but identification should be done with modern methods (Chance et al., 1998). Lehman et al. (1985) identified the stereoisomers of the C8 to C16 delta and gamma lactones in dairy products. The quantities in heated products, e.g., butter, were much larger than in pasteurized whole milk. Lactones in dairy products were mostly 70 to 80% the R configuration. This stereoconfiguration suggests a natural origin for the precursor hydroxy FA. The delta isomer was found in larger quantities than the gamma form in all products analyzed. The amounts of the C10 lactone in country butter were (μg/kg) delta, 3859, and gamma, 46.

I have included a paper by Tuomala and Kailio (1996) here because they identified the FFA and some other volatiles in Swiss cheese with a unique method. The frozen ground cheese powder was placed in a car-

Table 26. Quantities of *trans*-18:1 in butter and milk fat samples determined by GLC with capillary columns and corrected by analyses with the argentation (Ag)-TLC/GLC method.[1]

Source of lipids	n	Method of analysis		Correction factors	Reference
		Capillary only[2]	Ag-TLC/GLC[3]		
France[4]	60	2.00	3.30	1.65	Wolff et al. (1995a)
France[5]	24	1.82	3.22	1.77	Wolff (1994)
France[6]	24	2.78	4.28	1.54	Wolff (1994)
Germany[7]	1756	2.47	3.83	1.55	Precht and Molkentin (1996)
Germany[8]	927	1.53	2.65	1.73	Precht and Molkentin (1997b)
Germany[9]	236	2.42	3.80	1.57	Precht and Molkentin (1997b)
Germany[10]	593	3.53	5.08	1.44	Precht and Molkentin (1997b)
Germany[11]	58	2.92	4.35	1.49	Precht and Molkentin (1997b)

[1]Adapted from Wolff et al. (1998a) portion of table.

[2]*Trans* 5 to 11 isomers visible on GLC chart. These are capillary (cap) data obtained with 30 to 60 m columns.

[3]Corrected to include masked *trans* 12 to 16 18:1 isomer that were determined by Ag-TLC/GLC method. These are the new and "real" data.

[4]Butter samples obtained throughout year (quantities are from papers).

[5]Autumn butter.

[6]Spring butter.

[7]Milk from different seasons.

[8]Milk from barn fed cows (winter).

[9]Milk from winter to summer.

[10]Milk from pasture fed cows (summer).

[11]Slight energy deficit.

tridge. The material in the cartridge was extracted with supercritical CO_2, which swept on-line into a GLC-MS. They identified 30 compounds, mostly FFA which did not require derivatization.

Oxo FA. Saturated and unsaturated oxo FA were identified in milk by Brechany and Christie (1992, 1994). Twenty-one of the 36 saturated FA identified in Table 33 had been found before by Weihrauch (1974), but the remaining 15 are new. Brechany and Christie (1994) also found 15 unsaturated oxo FA (Table 34), and only five had been previously identified. The authors suggested that the precursors were hydroperoxides of PUFA in the diets of the cows that were altered by ruminal biohydrogenation.

Furan FA. Furan FA were observed in milk by Guth and Grosch (1992). They identified nine of the FA in butter and BO in amounts of 116 to 476 mg/kg. The acids disappeared when butter was irradiated with fluorescent light. Some of them are apparently precursors of 3-methyl-2,4-nonanedione, which is primarily responsible for the light-induced off-flavor in butter and BO. Furan acids are produced by the autoxidation of CLA (Yurawecz et al., 1995).

Other. Weidong et al. (2000) analyzed the volatile compounds in milk as related to the stages of the estrus cycle using a 30-m capillary column and GLC-MS. They identified 23 of the 59 peaks observed finding esters, FA, aldehydes, ketones, alcohols, and lactones. Absolute quantities of components were not given. Processing of milk would reduce the amounts or eliminate some completely.

Jensen and Nielsen (1996) investigated some of the lipids in the MLGM and fat globule core from cream in cows' milk. They injected two of three cows with emulsified DL-α-tocopherol to determine the extent of incorporation of tocopherols, retinol, and β-carotene into the MLGM. Because the injection of tocopherol apparently did not alter the composition of FA in the core and MLGM membrane, I have provided data from a cow that was not injected and one that did receive the tocopherol in Table 35. There were significant differences in the distributions of the FA between the two compartments, with more unsaturated FA in the MLGM. This would be expected because almost all of the PL, which contain these acids, are located in the MLGM. Only 2.7% of the FA were found in this compartment. The α-tocopherol was almost equally divided between the globule core and MLGM in the control cow. Gamma tocopherol, retinol, and β-carotene were not detected in the MLGM. There was more tocopherol in the MLGM than in the core from the injected cow. Again, the other vitamins were not detected. The authors discuss the potential value of α-tocopherol as an antioxidant in the MLGM.

A cap survey of the FA in Australian milks is available (Thomas and Rowney, 1996). The study covered January through December in 1994. Analyses were done with a 25-m capillary column, but the data are

Table 27. Amounts (wt%) of 9*c*–18:1 and 9*c*,12*c*–18:2 as determined by short-packed columns (old) and capillary (cap) columns and the argentation (Ag)-TLC/GLC method.[1]

18:1			18:2		
Isomer	Summer	Winter	Isomer	Summer	Winter
1. 9*c*[1]	19.44	17.28	1. 9*c*, 12*c*[1]	1.05	1.55
2. 4 - 11*t*[1]	4.68	1.76	2. total trans[1]	1.10	0.59
3. 12 - 15*t*[1]	1.18	0.89	3. 16*t* - 18:1[1]	0.26	0.19
4. total trans[1]	6.12	2.84	4. 12-15*c*-18:1[1]	0.18	0.25
5. *cis* less 9[7]	0.79	0.78			
Old					
18:1. 1-4[2]	26.29	20.72	18:2. 1-4[2]	2.59	2.58
trans	. . .	. . .		. . .	. . .
Cap					
18:1. 1+3[3]	20.62	18.17	18:2	1.05	1.55
trans. 2[4]	4.68	1.95	*trans.* 2[4]	1.10	0.59
New					
18:1.1	19.44	17.28	18:2	1.05	1.55
trans 2+3+16*t*	6.12	2.84	*trans.* 2.	1.10	0.59
Correction factors, %[5]					
Old to new	73.9	82.6	Old to new	40.5	60.1
Years avg.	78.3			50.4	
trans. Insert[6]	6.12	2.84		1.10	0.59
Years avg.	4.48			0.85	
Cap to new, %	94.3	95.1	Cap to new	None	
Years avg.	94.0				
trans. 2/4[7]. %	76.5	70.0			
Years avg.	73.3				

[1]New data from Table 23 (Precht et al., 1999). Determined by the Ag-TLC/GLC method, Wolff et al. (1998). Total *trans* is 2+3+16*t*-18:1.

[2]Old data that would be determined by short packed GLC columns. Sums of 1 to 5.

[3]Cap data that would be determined with a capillary GLC column alone. Sum of 1 and 2 for 18:1. 12-15*t* coelutes with 9*c*-18:1.

[4]*Trans* 2 only. Isomers in 3 coelute with 18:1 peak. No changes in 18:2s.

[5]Correction factor, e.g., 1/old total, 19.44/26.29 for Summer 18:1 = 73.9%.

[6]Amounts of *trans* to be inserted.

[7]2 Divided by 4 = 76.5% for Summer *trans*-18:1. Divide amount obtained by capillary column alone by correction factor to obtain correct quantity.

presented in four graphs; 14:0-8:0, 10:0-12:0, 14:0-20:0, and 18:1, 18:2, and 18:3. Another paper from Australia describes the effect of season on the physical properties, dropping point and solid fat content, of MF (Papalois et al., 1996).

Granelli et al. (1998) sought why five herds of Swedish dairy cattle had serious problems with the development of spontaneous oxidized flavor, on five control herds. They investigated factors that might be affected by feeding. They determined the amounts of volatiles, copper, α-tocopherol, β-carotene, and FA in milk TAG and PL. They were unable to find a specific reason for the cause of the off-flavor, since the only difference was more *trans*-18:1 in the control milks. The development of the flavor appeared to be related to fewer antioxidants (α-tocopherol and β-carotene) and more 18:2 and 18:3 in the milk. In one herd, a high content of 18:2 in the PL may have been responsible. In another, high levels of copper were found. The copper and the vitamins came from the water and the feed. No data on the contents were given. As for the PUFA, the differences in amounts are caused by variations in rumen fermentations (wt%) as indicated by the greater amounts of *trans*-18:1 in control TAG; 2.06, compared with the experimental no flavor, 1.52, and flavor, 1.54. These could probably be altered, but unless the feedstuffs are shown to be high in copper and low in the vitamins, the only remedy would be to replace the cattle. Conjugated linoleic acids were detected in all milks, 0.31 to 0.58%, but were not correlated with the development of the flavor.

Shiratsuchi et al. (1994) analyzed the volatile flavor compounds in spray-dried skim milk powder with GLC-MS. Of the 196 peaks detected, they identified 187. The major compounds were 48 hydrocarbons, 16 aldehydes, 20 ketones, 27 alcohols, 29 FA, 8 esters, 2 furans, 7 phenolic compounds, 10 lactones, and 14 nitrogenous compounds. Most of them originated from MF. Free FA were 79% of the total. The authors discussed the origin of the compounds and their contribution to the flavor of the product. They employed distillation-extraction with diethyl ether under reduced pressure to recover the "vola-

Table 28. Amounts (wt%) of long-chain (sphingoid) bases and fatty acids (FA) in milk sphingomyelins.

Long-chain bases				FA		
Component	Nyberg (1995)	Ollson et al. (1997)	Ollson et al. (1997)br[1]	Component	Nyberg (1995)	Valeur et al. (1994)
16:0	7.0	3.6	7.1	14:0	1.0	0.4
				15:0		0.1
16:1	23.0	27.2	2.5[1]	16:0	49.0	7.4
				17:0	. . .	0.2
17:0	3.0	0.7	. . .	18:0	1.0	2.8
17:1	. . .	8.1	. . .	20:0	. . .	0.8
				20:1	3.0	. . .
18:0	3.0	0.5		22:0	18.0	21.8
18:1	64.0	33.0	14.2	23:0	20.0	24.4
19:1	. . .	. . .	2.8[2]	23:1	. . .	3.7
				24:0	8.0	24.8
				25:0	. . .	0.9
				26:0	. . .	0.9
				27:0	. . .	0.4
				28:0	. . .	0.4
				18:1 *n*-9	. . .	1.9
				23:1	. . .	3.4
				24:1 *n*-9	. . .	4.5
				25:1 *n*-9	. . .	1.3
				26:1 *n*-9	. . .	0.2

[1]Total of branched-chain FA (%): i, 1.5; ai, 1.0.
[2]Total of branched-chain FA (%): i, 2.3; and ai, 0.5.

tiles." The method extracted high molecular weight compounds, e.g., 16:0 and 18:0.

Determination of FA with Short-Packed GLC Columns

I have included these papers because they are important. The first paper, which I had not seen, was brought to my attention by Robert L. Wolff. It is by J.-P. Gallacier et al. (1984) and is in French. The analyses were done with a packed column, 3.4 m in length × 2 mm diameter and contain comprehensive data on the FA in butter from three dairies in the Western region of France during each of the 12 mo of the year. They obtained quantities for 33 FA including *trans*-18:1 and conjugated 18:2. We know now that the amounts of that

Table 29. Combinations of long-chain (sphingoid bases) and fatty acids (FA) in milk sphingolipids.

	16:0		16:1		17:0	17:1		18:1[1]		19:1
FA	Ref.[1]	Ref.[2]	Ref.[1]	Ref.[2]	Ref.[2]	Ref.[1]	Ref.[2]	Ref.[1]	Ref.[2]	Ref.[2]
14:0	. . .	. . .	. . .	. . .	. . .	. . .	. . .	1	**	. . .
16:0	. . .	. . .	6	**	. . .	. . .	**	40	***	. . .
16:1	. . .	. . .	. . .	. . .	*	. . .	*	. . .	. . .	. . .
17:1	. . .	*	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
18:0	3	. . .	. . .	***	. . .	. . .	. . .	1	*	. . .
18:1	. . .	**	. . .	. . .	. . .	. . .	. . .	. . .	. . .	*
20:0	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .		. . .
20:1	3		. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .
21:0	. . .	. . .	. . .	. . .	. . .	. . .	. . .	. . .	***	. . .
22:0	4	**	6	***		1	***	7	. . .	. . .
23:0	. . .	. . .	8	***		1	**	11	***	*
23:1	. . .	. . .	. . .	. . .	1	. . .	**	. . .	. . .	. . .
24:0	. . .	. . .	3	***		1	***	4	***	. . .
24:1	. . .	**	. . .	. . .		. . .	. . .	. . .	. . .	. . .
25:0	. . .	**	. . .	**		. . .	. . .	. . .	. . .	. . .
HO-21:0	. . .	*	. . .	. . .		. . .	. . .	. . .	*	. . .
HO-22:0	. . .	. . .	. . .	. . .		. . .	. . .	. . .	. . .	. . .
HO-23:0	. . .	*	. . .	. . .		. . .	. . .	. . .	*	. . .

[1]Nyberg (1995). Major sphingoid base. Numbers are wt% of FA.
[2]Karlsson et al. (1997). Asterisks denote relative amounts: *, low; **, medium; and high, ***, respectively. Data from Tables 1 and 2 included.

Table 30. Molecular ions of glucosyl ceramide (GlcCer), lactosyl ceramide (LacCer), and sphingomyelin (SM) in bovine milk, represented by their respective ceramide units.[1]

GlcCer Ceramide	Abundance[2]	LacCer Ceramide	Abundance	SM Ceramide
				32:1
				32.0
33:1	**			33:1
				33:0
34:1	***	34:1	***	34:1
		34:0	*	34:0
				35:1
36:1	**			36:1
				37:1
38:1	***	38:1	**	38:1
		38:0	*	38:0
39:1	***	39:1	***	39:1
		39:0	*	39:0
40:1	***	40:1	***	40:1
40:0	**			40:0
41:1	***	41:1	***	41:1
41:0	**			
42:1	***	42:1	***	42:1
43:1	**	43:1	*	43:1
43:0	**			
				44:1
				45:1

[1]Adapted from Karlsson et al. (1997).

[2]The asterisks: *, **, and *** are low, medium, and high amounts, respectively.

FA are incorrect. I will not present the data because they are too voluminous.

The other papers (Palmquist et al., 1993; Barbano, 1990) contain the reference data on FA in milks from 50 cheese plants in 10 regions of the United States in February, May, August, and November. The pooled results, shown in Table 36, were obtained with short-packed GLC columns, so the contents of 18:1 and 18:2 were overestimated and *trans*-FA were not detected. I have provided the corrected quantities using a total amount of *trans*-FA from another source (Table 23) that is approximate, but probably near the actual amount. It

Table 31. Fatty acid (FA) composition (wt%) of two milk phospholipids.[1]

FA	Phosphatidylethanolamine (%)	Phosphatidylcholine (%)
12:0		1
14:0	1	7
16:0	10	28
18:0	10	6
18:1	58	41
18:2	15	13
18:3		
18:3	2	2
20:3	1	1
20:4	1	1
20:5		

[1]Adapted from Nyberg (1995). Phospholipids obtained from a commercial preparation, SMR.

Table 32. Hydroxy fatty acids (FA) identified in skim milk.[1]

FA	Location of hydroxyl group
10:0	C2, C3
11:0	C3
12:0	C2, C3
14:0	C2, C3, C8[2], C9[2]
15:0	C2, C3
16:0	C2, C3, C7-C13[2]
18:0	C18-C16[2]
20:0	C2, C3
21:0	C2, C3
22:0	C2, C3
23:0	C2, C3
24:0	C2
25:0	C2

[1]Adapted from Chance et al. (1998). The methods employed prevented identification of 3- and 4-hydroxy 6:0 - 16:0 plus the 18:0 and 19:0 - 4-OH FA. These were found in butter.

[2]Previously unidentified.

Table 33. Composition of the saturated oxo fatty acids found in Cheddar cheese, expressed as mg/kg cheese and as weight percent of the fraction.[1]

Component	Concentration, mg/kg	wt%	Confirmed	Found[2] previously
4-oxo-9:0	trace	trace		*
5-oxo-10:0	0.29	0.30		*
4-oxo-12:0	0.77	0.79	*	*
5-oxo-12:0	0.26	0.27	*	*
7-oxo-12:0	0.28	0.29	*	*
4-oxo-13:0	0.09	0.09		
4-oxo-14:0	0.21	0.22		
5-oxo-14:0	0.51	0.52	*	*
6-oxo-14:0	1.42	1.45	*	*
9-oxo-14:0	1.66	1.70	*	*
11-oxo-14:0	0.19	0.19		
5-oxo-15:0	trace	trace		*
4-oxo-16:0	trace	trace		*
5-oxo-16:0	0.49	0.50	*	*
7-oxo-16:0	trace	trace	*	*
8-oxo-16:0	16.59	17.00	*	*
11-oxo-16:0	3.24	3.32	*	*
13-oxo-16:0	0.59	0.60	*	
14-oxo-16:0	0.59	0.60	*	
15-oxo-16:0	0.63	0.65	*	
9-oxo-17:0	0.44	0.45	*	
9-oxo-18:0	trace	trace	*	*
10-oxo-18:0	66.07	67.69	*	*
13-oxo-18:0	2.99	3.06	*	*
15-oxo-18:0	0.27	0.28	*	
16-oxo-18:0	0.64	0.66	*	*
17-oxo-18:0	0.27	0.28	*	
11-oxo-19:0	2.67	2.74	*	*
4-oxo-20:0	0.12	0.12		
5-oxo-20:0	0.14	0.14		
11-oxo-20:0	0.52	0.53	*	*
15-oxo-20:0	0.21	0.22	*	*
4-oxo-22:0	0.08	0.08		
5-oxo-22:0	trace	trace		
7-oxo-22:0	trace	trace		
13-oxo-22:0	0.22	0.23	*	*

[1]Adapted from Brechany and Christie (1992).

[2]From Weihrauch (1974).

Table 34. Composition of the unsaturated oxo fatty acids of Cheddar cheese, measured as methyl esters and expressed as g/kg of total fraction and as mg/kg of cheese.[1]

Fatty acid	g/kg of Total fraction	mg/kg of Cheese	wt%[2]	Found[3] previously
9-oxo-tetradec-5-enoate	4	0.1	Trace	*
11-oxo-hexadec-7-enoate	34	0.95	3.4	*
?-oxo-hexadec-?-enoate	4	0.11	0.4	
8-oxo-hexadec-11-enoate	6	0.18	0.6	
8-oxo-hexadec-12-enoate	6	0.16	0.6	
?-oxo-hexadec-?-enoate	1	0.03	0.1	
8-oxo-hexadec-13-enoate	8	0.23	0.8	
8-oxo-heptadec-?-enoate	7	0.21	0.8	
9-oxo-octadec-12-enoate	16	0.45	1.6	*
9-oxo-octadec-13-enoate	90	2.52	9.0	*
10-oxo-octadec-12-enoate	trace			
10-oxo-octadec-13-enoate	85	2.39	8.6	
10-oxo-octadec-14-enoate	147	4.13	14.8	
10-oxo-octadec-15-enoate	161	4.51	16.1	
13-oxo-octadec-5-enoate	129	3.62	3.0	
13-oxo-octadec-9-enoate	220	6.19	22.0	*
16-oxo-octadec-9-enoate	57	1.60	5.7	
17-oxo-octadec-9-enoate	24	0.67	2.4	

[1]Adapted from Brechany and Christie (1994).
[2]Calculated by author.
[3]From Weihrauch (1974).

is a year's average, so the effect of season is eliminated. I have given detailed descriptions of the calculations used to obtain the corrected values. We have prepared a paper on the overestimates of 18:1 and 18:2 in bovine and human MF and margarines (Precht et al., 2001).

Factors Affecting the FA Composition of Milk

These factors have been reviewed by Palmquist et al. (1993), German et al. (1997), Doreau et al. (1997), Demeyer and Doreau (1999), Mansbridge and Blake (1997), and Annison and Bryden, (1998, 1999). The papers published since can be assigned to animal factors; which includes genetics (breed and selection), stage of lactation, mastitis, and feed factors, which comprises grain and energy intakes, dietary fats, and seasonal and regional effects (Palmquist et al., 1993). The purpose of many investigations was to alter the FA composition with emphasis on PUFA, *trans* isomers, and CLA.

Biosynthesis of milk FA. The esters in the dietary lipids are hydrolyzed primarily by microbial lipases, and the unsaturated FA are extensively hydrogenated with decreases in total unsaturated FA, increases in saturates, and formation of *cis* and *trans* isomers, mostly of 18:1. Some lipids are contributed by the rumen microorganisms. Cellulose and related materials are converted to acetate, propionate, and hydroxybutyrate, which enter the circulation and butyrate is changed to hydroxybutyrate in the rumen wall. The longer FA move into the intestine and through the intestinal wall.

Table 35. Fatty acid (FA) composition (g/kg of FA) and content of vitamins (μg/g of FA) in cream and milk fat globule membrane (MFGM) from individual cows.[1]

	Cow 1 (n = 10)			Cow 2 (n = 11)		
FA	Cream	MFGM	$P <$	Cream	MFGM	$P <$
4:0	44	36	0.01	33	47	0.05
6:0	20	7	0.001	16	6	0.001
8:0	10	8	0.001	10	6	0.05
10:0	19	10	0.001	16	7	0.01
12:0	22	13	0.001	18	10	0.01
14:0	85	62	0.001	79	52	0.001
14:1	11	6	0.001	12	6	0.001
15:0	7	5	0.001	9	7	0.001
16:0	279	236	0.001	299	233	0.001
16:1	28	25	0.01	32	28	0.01
17:0	9	+		10	+	
18:0	105	101	NS	116	100	NS
18:1	325	395	0.001	315	409	0.001
18:2	22	56	0.001	16	43	0.001
18:3	7	12	0.001	7	10	0.05
20:0	1	2	0.05	2	2	0.05
20:1	3	4	0.001	3	5	0.05
20:2	1	1	0.05	1	4	0.001
20:3	1	5	0.001	0	4	0.001
20:4	1	11	0.001	3	12	0.001
20:5	1	3	0.001	0	2	0.05
22:5	1	4	0.001	2	6	0.001
Saturated	599	479	0.001	603	461	0.001
Monounsaturated	367	430	0.001	363	447	0.001
Polyunsaturated	34	91	0.001	34	89	0.001
Total fatty acid, g/kg sample	441	12	0.001	420	9	0.001
α-Tocopherol	17.0	14.9	NS	38.6	75.0	0.01
γ-Tocopherol	1.9	ND		1.6	ND	
Retinol	11.2	ND		10.8	ND	
β-Carotene	0.6	ND		0.6	ND	

[1]Adapted from Jensen and Nielsen (1996). NS = Contents in cream, and MFGM were not significantly different ($P > 0.05$); ND = Not detectable. Cows 1 and 2 were given a feed poor in vitamin E. Cow 2 received an intraperitoneal injection of emulsified DL-α-tocopherol at the start of the experiment.

They are transported in lipoproteins to the mammary gland. Some of the dietary fat is deposited in and mobilized from adipose tissue. The TAG in the lipoproteins are hydrolyzed to FA to facilitate entry into the gland.

De novo synthesis of most of the 4:0 to 14:0 and half of the 16:0 from circulating acetate and β-hydroxybutyrate occurs in the mammary gland (Mansbridge and Blake, 1997). These precursors arise from the microbial fermentation of cellulose and hemicellulose in the rumen.

The longer FA entering the gland, mostly 18:0, are extensively desaturated, primarily to 18:1. The FA are synthesized into organ- and species-specific TAG, with the short-chain FA located at the *sn*-3 position of the molecule in decreasing amounts as the chain-length increases. The TAG must be liquid at body temperature or liquid in the bulk TAG. See the section on TAG for information.

Table 36. Fatty acid (FA) composition of a US reference milk fat with corrected amounts of 18:1 and 18:2.[1]

FA	Amounts, wt%		
	Reported–old		Corrected–new
4:0	3.32		None
6:0	2.34		None
8:0	1.19		None
10:0	2.81		None
12:0	3.39		None
14:0	11.41		None
14:1	2.63		None
16:0	29.53		None
16:1	3.38		None
18:0	9.84		None
18:1	27.39	9*c*-18:1	21.45[2]
		trans-18:1	4.48[3]
		cis except 9-18:1	1.46[4]
18:2	2.78	9*c*, 12*c*-18:2	1.40[5]
		t, t -18:2	0.85[6]
		other FA in 18:2	0.44[7]

[1]Adapted from Palmquist et al. (1993); Barbano (1990).

[2]Reported amount, 27.39%, times correction factor, 78.3% from Table 23, gives 21.45. Contains 9*c*-18:1, *trans-18:1* and other *cis* isomers.

[3]Contains 5-11*t* and 12-15*t* from Table 27.

[4]Reported minus corrected 18:1 = 5.85 which minus *trans*-18:1, 4.84 = 1.46.

[5]Reported 18:2 corrected by 50.4% from Table 27.

[6]*trans*, *trans*-18:2 from Table 27.

[7]Sum and average of 18:2 isomers from Table 27.

Animal Factors

While evaluating the data in these sections, readers should remember that, unless noted, all of the FA analyses were made with capillary GLC columns only. Nevertheless, as I described earlier (Table 27), the 18:1 contents were overestimated and *trans*-18:1 underestimated. Some of the investigators employed columns that would separate a portion of the *trans* isomers, but did not report them. I will provide data from papers that I believe to be representative and give references for the remainder.

Breed. The FA profiles of milks from Holstein, Jersey, and Brown Swiss were determined with butyl esters (DePeters et al., 1995), and some of the differences were small. I will not reproduce the data because they would be eliminated by the pooling of milk that occurs before processing. This applies to all of the factors except season.

Stage of lactation. The milks from early lactation (30 d) contained less 4:0 to 12:0 than those from middle (120 d), and late (210 d) lactation periods in New Zealand cows (Auldist et al., 1998). The difference was independent of seasonal (feed) effect and was attributed to the physiological inability of cows in early lactation to consume enough DM to meet energy requirements. The synthesis of 4:0 to 12:0 in the mammary gland increased during early lactation then decreases and the mobilization of FA from adipose tissue increases (Palmquist et al., 1993). Seasonal and stage of lactation affect the CLA contents, but the differences were small.

Rowney and Christian (1996) evaluated the effects of diet and stage of lactation on milks for cheeses as sources of butter, etc. They found that diet quality had the greatest effect. The authors did not report their complete FA data, presenting the results as classes of FA, e.g., short chain. They detected CLA, but not other *trans*-FA. More information is available in Thomas and Rowney (1996).

Mastitis. Clinical mastitis reduces the amount of milk and the contents of protein and fat by varying amounts (Hortet and Seegers, 1998). The average loss in milk yield was estimated at 4 to 6% or 300 to 400 kg/lactation. Losses of fat were 0.1% and protein, 0.05%. However, the milk is withdrawn from use, so the data are mostly of academic value.

Somatotropin. While bST is not strictly an animal factor, it is included here because it affects the whole animal. Bovine somatotropin and lactation were reviewed by Bauman (1999), whose group has done much of the basic and applied research on this peptide hormone, which is secreted by bovine pituitary glands. Its use results in increased milk yield with much greater efficiency. It is a homeorhetic control, which unites many physiological processes to produce, in this case, milk. Homeorhesis is a concept developed earlier by Bauman and Currie (1980). Recombinant bST is available as a prolonged-release formulation, Posilac, from the Monsanto Co. (St. Louis, MO) and sales began in 1994.

Although some groups and individuals have expressed concern about the use of bST, its safety has been certified by all relevant medical and safety organizations. In a later study (Chalupa et al., 1998), cows were given bST daily beginning at d 28 to 35 of lactation. More milk was produced with greater efficiency. Amounts of fat, protein, and TS in milk were not affected. Bauman et al. (1999) evaluated the production responses of dairy herds in the Northeast US and obtained the same results as the group above.

Ionophores. These compounds-monensin, laslocid, nigericin, and tetrosin-are feed additives that alter ion transfer across all membranes (Duffeld and Bagg, 2000). The use of ionophores in dairy cattle 1) improves energy metabolism and reduces the effects of ketosis, 2) increases milk production, and 3) causes small decreases in milk protein and fat contents. Sauer et al. (1998) determined that the addition of monensin to the diet of dairy cattle decreased the production of methane and feed intake. The total and percentage fat decreased temporarily. Milk production and the amounts of all

Table 37. Seasonal variation in fatty acid (FA) composition (wt%) of milk fat in the United States[1] with corrected amounts of 18:1, *trans*-18:1, and 18:2.[2]

	FA														
Month	4:0	6:0	8:0	10:0	12:0	14:0	14:1	16:0	16:1	18:0	18:1	9*c*-18:1[2]	*t*-18:1[3]	18:2	9*c*,12*c*-18:2[2]
February	3.48	2.44	1.24	2.95	3.52	11.63	2.57	29.89	3.32	9.68	26.51	21.90	3.64	2.77	1.66
May	3.42	2.36	1.20	2.82	3.38	11.20	2.58	28.40	3.36	10.14	28.10	20.77	. . .	3.05	1.24
August	3.07	2.28	1.12	2.55	3.10	10.92	2.66	28.76	3.41	10.28	29.00	21.13	6.73	2.86	1.46
November	3.33	2.31	1.20	2.90	3.54	11.80	2.69	30.78	3.37	9.37	26.19	22.00	. . .	2.53	1.52

[1]Adapted from Palmquist et al. (1993); Barbano (1990).

[2]Adapted from Precht et al. (1999). Calculated as described in Table 27 using correction factors (%) for 18:1 and 18:2 from Ref. above: February and November 0.826 and 0.601, May and August, 0.739 and 0.405.

[3]Calculated by adjusting amounts (%) of *trans*-18:1, summer, 6.12 and winter, 2.84 from Table 23 by ratios of *c*-9-18:1 in the table and 2 above.

unsaturated including *t*-18:1 and CLA increased. The quantities of all saturated FA decreased. A controlled release form of monensin has been approved for use in Canada, but not in the United States, as of November 2000 (personal communication, D. L. Palmquist) for treatment of subclinical ketosis. The effects of several ionophores on biohydrogenation in continuous flow through rumen fermenters was examined by this group (Fellner et al., 1997, 1999). The amount of 9*c*, 11*t*-18:2 was increased.

Feed Factors

Milk fat is hypercholesterolemic compared with polyunsaturated oils (Noakes et al., 1996). Lauric acid, 14:0, and 16:0 are the major culprits. The challenge that confronted the dairy industry was, and is, to replace these FA in MF with unsaturated FA. The factors that affect FA composition of milk can be altered, some much more than others. The factors are: 1) seasonal and regional, 2) feeding various fats and oils, 3) supplemental oils, 4) protected oils, and 5) miscellaneous. Noakes et al. (1996) modified MF by feeding a protected unsaturated lipid to cows. The milk and some of its products were fed to humans and the impact on plasma lipids measured. I will present some of these data in section 4) protected fats.

When evaluating the forthcoming data, the reader should remember that weight percents are relative. If, for example, the 18:2 content shows a real increase of 5 to 15%, then the sum of the remaining acids will be lowered by the same amount, an artificial decrease. Data on the actual weights are needed. These are easily acquired with the use of internal standard during GLC analyses or from the fat yields and percents.

Bobe et al. (1999) added another factor, associations with individual proteins as determined by correlations and factor analyses. They grouped FA, all milk proteins, and β-lactoglobulin into seven families with factor analyses of shared pathways of synthesis or genetic origins of proteins and FA. The authors suggested that results from the analyses could be employed to develop hypotheses for synthesis of milk components or related metabolic processes. McNamara and Baldwin (2000) developed and tested a model that estimated the parameters describing lipid metabolism in lactation. Model simulations on yields of milk components were within 5% of observed means. The authors found that more data describing energy-utilizing reactions are required. Nevertheless, the model may be useful for obtaining information on metabolic interactions in cows. I suggest that investigators planning to conduct feeding trials read this paper carefully. It might help them design a more productive study.

Seasonal and regional. The only comprehensive information on FA in US milks are the pooled data in Table 36 and the seasonal profiles in Table 37 (Barbano, 1990; Palmquist et al., 1993). These were obtained with short-packed GLC columns, so I've corrected the contents of 18:1 and 18:2 and added the trans-FA contents. Jahreis et al. (1996) determined the variations in milk FA depending on season and farm management systems. Their findings are given in Tables 38 and 39. Graphs showing monthly changes in 16:0, cis-18:1s, and 11t-18:1 (vaccenic acid) are presented in the paper. Contents of fat, protein, and ash were significantly lower in the ecological group, but the amounts of 9c, 11t-18:2 (a CLA) were higher than in the other groups. The ecological group, described only as typical grassland, were unable to obtain significant energy and protein-rich rations for the cattle. This deficiency reduced the fat and protein contents in the milk.

Various fats and oils. Data on milks from Holstein cows fed tallow or soybeans and diets low in copper are given in Table 40 (Morales et al., 2000b). Feeding soybeans increased the amounts of 4:0 to 14:0, 18:0, 18:2, and 18:3, while 16:0, t-18:1, and 18:1 were decreased. The authors in this and later papers identified 18:1 as *c*-18:1. This is incorrect, because we know (Table 26) that the GLC columns used do not resolve

Table 38. Effects of season and management systems on amounts (wt%) of saturated and *cis* monounsaturated fatty acids in German milks.[1]

Fatty acid	Group[2]		
	Indoor	Pasture	Ecological
8:0	1.59[a] ± 0.20	1.55[a] ± 0.23	2.06[b] ± 0.14
10:0	4.86[b] ± 0.24	4.01[a] ± 0.11	4.59[c] ± 0.31
12:0	5.01[b] ± 0.16	4.55[a] ± 0.23	5.10[b] ± 0.11
14:0	13.62[b] ± 0.19	12.61[a] ± 0.45	13.47[b] ± 0.27
15:0	1.51[b] ± 0.06	1.26[a] ± 0.09	1.51[b] ± 0.07
16:0	33.09[b] ± 0.64	30.18[a] ± 0.83	30.45[a] ± 1.24
17:0	0.77[a] ± 0.06	0.77[a] ± 0.06	0.82[a] ± 0.05
18:0	8.84[b] ± 0.31	11.60[a] ± 0.53	9.40[c] ± 0.60
20:0	0.15[b] ± 0.02	0.19[a] ± 0.02	0.18[c] ± 0.02
22:0	0.07[ab] ± 0.02	0.09[a] ± 0.02	0.06[b] ± 0.02
24:0	0.04[a] ± 0.02	0.03[a] ± 0.01	0.04[a] ± 0.02
14:1 7	0.53[b] ± 0.03	0.59[a] ± 0.06	0.64[c] ± 0.03
14:1 9	1.23[b] ± 0.07	1.04[a] ± 0.08	1.12[a] ± 0.08
16:1 7	0.22[a] ± 0.02	0.25[ab] ± 0.03	0.26[b] ± 0.05
16:1 9	2.09[a] ± 0.06	1.96[ab] ± 0.11	1.91[b] ± 0.18
17:1 10	0.25[a] ± 0.03	0.24[a] ± 0.02	0.22[a] ± 0.04
18:1 7-10	19.21[b] ± 0.44	20.55[a] ± 0.72	18.51[c] ± 0.30
18:1 11	0.59[a] ± 0.05	0.54[a] ± 0.05	0.40[b] ± 0.13
18:1 12	0.26[a] ± 0.02	0.23[a] ± 0.04	0.20[b] ± 0.04
18:1 13	0.09[a] ± 0.01	0.09[a] ± 0.01	0.20[b] ± 0.04
18:1 15	0.08[b] ± 0.01	0.11[a] ± 0.02	0.10[a] ± 0.02
20:1 11	0.10[b] ± 0.02	0.12[a] ± 0.03	0.09[b] ± 0.02

[a,b,c]Different letters indicate significant differences.

[1]Adapted from Jahreis et al. (1996).

[2]Indoor = Corn (maize) and grass silages throughout year. Pasture = During summer, silages in winter. Ecological = Typical grassland. Eleven samples taken once a month for a year from bulk tanks on their farms. N = 396.

Table 39. Effects of seasonal management systems on amounts (wt%) of *cis* polyunsaturated and *trans* fatty acids in German milks.[1]

Fatty acids	Groups[2]		
	Indoor	Pasture	Ecological
18:2 9, 12	1.80[ab] ± 0.19	1.89[a] ± 0.14	1.63[b] ± 0.22
18:3 6, 9, 12	0.04[a] ± 0.01	0.04[a] ± 0.01	0.05[a] ± 0.02
18:3 9, 12, 15	0.27[b] ± 0.04	0.43[a] ± 0.05	0.89[c] ± 0.06
20:2 11, 14	0.03[a] ± 0.01	0.04[a] ± 0.01	0.04[a] ± 0.01
20:3 8, 11, 14	0.09[b] ± 0.02	0.09[b] ± 0.02	0.07[a] ± 0.02
20:4 5, 8, 11, 14	0.15[a] ± 0.03	0.12[ab] ± 0.02	0.10[b] ± 0.03
20:3 11, 14, 17	0.05[a] ± 0.01	0.04[a] ± 0.01	0.04[a] ± 0.02
20:5 5, 8, 11, 14, 17	0.03[b] ± 0.01	0.05[a] ± 0.01	0.05[a] ± 0.01
22:4 7, 10, 13, 16	0.03[a] ± 0.01	0.03[a] ± 0.01	0.03[a] ± 0.01
14:1 9	0.24[b] ± 0.02	0.29[a] ± 0.03	0.32[c] ± 0.04
16:1 7	0.05[a] ± 0.00	0.06[a] ± 0.01	0.06[a] ± 0.01
16:1 9	0.36[b] ± 0.02	0.46[a] ± 0.03	0.43[a] ± 0.04
18:1 9	0.32[a] ± 0.03	0.33[a] ± 0.03	0.37[b] ± 0.04
18:1 11	1.21[b] ± 0.08	2.21[a] ± 0.43	2.67[c] ± 0.64
18:1 14*c* + 16*c*	0.28[a] ± 0.03	0.32[a] ± 0.03	0.39[b] ± 0.11
18:2 9*t*, 12*t* + 18:1 16*c*	0.28[a] ± 0.03	0.24[a] ± 0.04	0.31[b] ± 0.07
18:2 9*c*, 12*t*	0.18[b] ± 0.02	0.11[a] ± 0.03	0.13[a] ± 0.04
18:2 9*t*, 12*c*	0.06[a] ± 0.02	0.10[a] ± 0.02	0.30[b] ± 0.07
18:2 9*c* + 11*t* + 18:2 9*t*, 11*c* 9*t*, 11*c*	0.34[b] ± 0.04	0.61[a] ± 0.08	0.80[c] ± 0.77

[a,b,c]Different letters indicate significant differences ($P < 0.05$).

[1]Adapted from Jahreis et al. (1996).

[2]Indoor = Corn (maize) and grass silages throughout the year. Pasture = During summer, silages in winter. Ecological = Typical grassland. Eleven samples taken once a month for a year from bulk tanks on their farms. N = 396.

the 12 to 15 *trans* isomers from the *c*-18:1 peak. I have deleted the prefix *c* from 18:1. Milk from Jersey cows was also analyzed, and the effects of copper status were examined. More information is available in Morales et al. (2000a, 2000b). Copper was included because dairy processors in Ohio where the study was done have noted an increase in spontaneous oxidized flavor during the winter. Copper is a known prooxidant. Feeding higher copper and soybeans increased the 18:2 and 18:3 contents and potential development of spontaneous oxidized flavor. Injection of the antioxidant, α-tocopherol, increased the amounts of the vitamin in the blood and milk from the cows, but effects on oxidized flavor were not reported. Also, according to the authors, copper influences the activity of Δ^9 desaturase. This enzyme converts vaccenic acid (11*t*-18:1) to 9*c*, 11*t*-18:2, a bioactive CLA. A deficiency of copper increased the amount of CLA, probably because the desaturase was not inhibited and tallow, a ruminant fat, would contain more vaccenic acid than soybean oil. Focant et al. (1998) found in another study that a daily oral supplement of α-tocopherol in the diets of cows fed rapeseed and linseed improved the resistance of fat to oxidation. The depression of MF that accompanies the feeding of unsaturated oils was also prevented. The amounts and FA composition of oils influence rumen metabolism. Beam et al. (2000) found in an in vitro study of rumen contents that added 18:2 in soybean oil decreased the rate of lipolysis. The greater quantities of 18:2 in the oil decreased the rate of biohydrogenation.

Crocker et al. (1998) studied the effects of feeding processed corn grain on nutrient digestion and milk composition in Holstein cows. Lactating dairy cows require diet components high in energy such as starch. However, processing of corn is required to improve starch digestion. Steam flaking and dry rolling of corn were used in this trial. The results are presented in Table 40. Milk composition and yield were similar with both types and changes in FA composition except for 18:1 and 18:2. The fermentable starch intake was below (18.6 to 19:9%; calculated by author) the level, >50% of feed quoted by Palmquist et al. (1993) as depressing fat percentage. In this study, fat percentage was decreased but fat yield was not.

The effects of different forms of canola oil FA plus canola protein on milk composition and physical properties of butter were investigated (Bayourthe et al., 2000). The results of feeding an extruded blend of canola meal and canola seeds to Holstein cattle are depicted in Table 40. The fat depressing effect of the diet is shown. Canola oil contains about 58% *c*-18:1, 20% 18:2, and only 6% 16:0. The canola preparation reduced the percentages of 12:0 to 16:0 and increased 18:1. The canola oil preparation reduced the amount of solid fat in butter at 30°C.

Table 40. Effects of tallow and soybeans, corn, and canola on the fatty acid (FA) composition (wt%), milk and fat yield and amounts.

	Tallow[1] copper		Soybean[1] copper		Corn[2]		Canola oil[3]	
FA	Control	Low	Control	Low	Dry rolled	Steam flaked	Control	Meal + seeds
12:0	2.08	1.50	3.17	2.83	2.59	3.15	4.41	–0.96
14:0	9.27	7.12	10.75	10.13	10.09	10.88	13.54	–1.96
16:0	31.95	30.29	25.58	25.63	26.69	29.11	32.75	–7.23
18:0	9.66	12.13	12.63	12.27	15.77	15.50	10.82	+2.69
t-18:1	5.39	5.00	4.18	5.27	1.63[b]	1.62[b]	1.62	+1.49
18:1	24.31	28.03	21.60	20.55	23.74[c]	21.65[c]	18.75	+5.62
18:2	2.23	2.50	5.09	5.73	2.97[d]	4.01[d]	2.46	+0.02
18:3	0.42	0.48	0.97	1.03[a]	1.02	1.05	0.54	+0.25
Conjugated linoleic acid	0.83	0.84	1.02	1.45[a]	NR	NR	NR	NR
Yield, kg/d								
Milk	28.70	27.3	29.20	29.30	29.50	30.1	39.6	+1.8
Fat	0.72	0.66	0.75	0.86	1.13	1.14	gm/d 1519	–239
Fat %	2.53	2.45	2.52	3.01	3.86[e]	3.58[e]	3.86	–0.72
Protein	0.92	0.86	0.94	0.92	0.86	0.97	gm/d 1258	–12

[1]Adapted from Morales et al. (2000b). Holstein cows. a = Difference between fats significant ($P < 0.05$). NR = Not reported.

[2]Adapted from Crocker et al. (1998). b = Includes 9 and 11*t* isomers, 0.38 and 1.25%; 0.30 and 1.32%, c = Includes 9- and 10 isomers, d = Differences between diets significant ($P < 0.05$), e = Significant decrease ($P < 0.05$).

[3]Adapted from Bayourthe et al. (2000). The trial diet was an extruded blend of canola meal and seeds. Meal and seeds + and – are changes from control.

Table 41. Important properties of fat supplements.[1]

Properties	Comment
Lipid composition: relative proportions of triacylglycerols/FFA	The rate of ruminal lipolysis and accumulation of *trans*-18:1s in the biohydrogenation sequence is influenced by the proportion of triacylglycerols and degree of protection.
Fatty acids (FA)	The C18 unsaturated FA tend to inhibit cellulolytic activity more than their saturated counterparts and produce *trans* isomers.
Degree of protection or inertness in the rumen	In general terms, the potentially deleterious effect on ruminal metabolism and milk synthesis is less as the degree of protection or inertness increases.
Digestibility	Intestinal digestion and absorption of long-chain FA increase as the proportion of unsaturated FA is enhanced.
Transfer into milk	Approximately 50 to 60% of the FA are transferred, but this amount is dependent on the quantity, composition, and degree of ruminal protection and digestibility.
Effects on mammary gland lipogenesis	Supplements containing 16:0 to 18:0 FA decrease the synthesis of 6:0 to 14:0. Increased amounts of *trans*-18:s may inhibit lipogenesis and Δ9-desaturase activity.

[1]Adapted from Ashes et al. (1997). Includes protected fats.

Supplemental fats. Ashes et al. (1997) reviewed the potential of supplemental fats to alter the content and composition of MF. They included protected fats in the supplemented category. Their list of supplements includes whole, extruded, or exploded oilseeds, and these rumen-protected fats; Ca salts of long-chain FA, pelleted, or prilled fats containing TAG and starch, animal, and vegetable-blended fats, yellow grease, oil protected by formaldehyde-treated protein and protein supplements, and butylsoyamides or oleamides (last added by the author). The supplements increase the energy density of diets and modify the FA profile of milk. Ashes et al. (1997) listed the properties of fat supplements in Table 41. They did not separate supplemental and protected fats. I have. Some recent data on supplemental fats are shown in Table 42.

Avila et al. (2000) stated that the enhanced milk yield in modern cows requires energy in their diets without sacrificing fiber content. Supplemental fats provide the needed energy density. Tallow and yellow grease are available at relatively low cost. Tallow is an effective fat supplement, but there are few data on yellow grease, which contains about 48% 18:1. These researchers fed both to Holstein cattle to investigate their effects on digestion and milk yield. Their results can be seen in Table 42. Supplementation increased yields of milk, MF, and vaccenic acid (11t-18:1). Supplementation diets containing whole cottonseed increased milk yield, increased milk production without disturbing rumen function. This group did not report detection of CLA, although they used a GLC column, which should have resolved some of these FA. There are many others in which the effects of supplemental fats on milk FA, etc., are described, but this paper shows the results. I will present data from some of these papers in the section on CLA.

Rumen-protected fats. Pure fats and oil seeds have been treated to avoid interactions between them and ruminal digestion (Mansbridge and Blake, 1997). These products are called rumen-protected fats. Early investigations utilized oils encapsulated with formaldehyde treated casein. However, the products were only partially protected against rumen metabolism. Nevertheless, Noakes et al. (1996) noted a modest decrease in human plasma LDL-cholesterol; 4.49 to 4.25 mmol/L by including modified MF in their diets. They obtained an unsaturated MF by feeding oils protected with an unspecified protein, presumably denatured by treatment with formaldehyde. Data on changes in percent or amounts of MF were not given.

Wright et al. (1998a, 1998b) fed a custom designed nondegradable protein supplement containing fishmeal as a source of 22:6 n-3, an FA that is considered to be conditionally essential for human infants. The content of the FA in milk was increased from about 0.15 to 0.33% of long-chain FA. The method has been patented, US Patent number; 5,932,257. I question the applicability of the method because it is much easier and less expensive to incorporate fish oil containing 22:6 n-3 into infant formulas with standard manufacturing procedures.

Conversion of dietary oils to calcium salts has been proposed as a method for their protection from ruminal biohydrogenation (Mansbridge and Blake, 1997; Chouinard et al., 1998b). The salts were believed to be insoluble in rumen fluid. The extent of biohydrogenation is dependent on the pH of the fluid. If the pH rises to 6.0 or above, hydrogenation of the calcium

Table 42. Fatty acid (FA) composition of milk fat and yields of individual FA from lactating Holstein cows fed diets containing supplemental fat differing in degrees of saturation.[1]

		Supplemental fat				Contrasts[2]		
	Control	Tallow	Blend[3]	YG[4]	SE	Added fat	Linear tallow	Blended fat
	(g/100 g)					*P* <		
				SE				
Composition								
4:0	4.39	4.90	4.87	4.31	0.19	0.22	0.07	0.31
6:0	2.96	2.71	2.57	2.58	0.03	<0.01	0.03	0.11
8:0	1.61	1.38	1.29	1.30	0.03	<0.01	0.13	0.21
10:0	3.50	2.82	2.62	2.63	0.08	<0.01	0.14	0.35
12:0	3.60	2.90	2.70	2.71	0.09	<0.01	0.18	0.39
14:0	11.96	10.87	10.13	9.96	0.19	<0.01	0.02	0.26
16:0	30.10	30.06	29.35	29.36	0.23	0.10	0.08	0.25
16:1 *trans*	0.42	0.49	0.44	0.43	0.02	0.11	0.05	0.36
16:1 *cis*	1.41	1.53	1.51	1.49	0.02	0.01	0.31	0.80
18:0	14.20	15.27	15.41	15.93	0.30	<0.01	0.17	0.62
18:1 *trans*-9	0.32	0.42	0.45	0.46	0.01	<0.01	<0.01	0.78
18:1 *trans*-11	1.30	1.05	1.21	1.53	0.06	0.63	<0.01	0.31
18:1 *cis* 9 and 10	20.15	22.29	24.13	23.69	0.35	<0.01	0.03	0.04
18:2	3.32	2.65	2.67	2.92	0.09	<0.01	0.07	0.33
18:3	0.78	0.66	0.67	0.70	0.04	0.04	0.45	0.89
SCFA[5]	8.95	8.99	8.73	8.19	0.19	0.19	0.02	0.58
MCFA[6]	50.98	48.67	46.75	46.59	0.42	<0.01	0.01	0.14
LCFA[7]	40.07	42.34	44.53	45.22	0.33	<0.01	<0.01	0.11
	(g/d)							
4:0	52.16	61.37	58.16	55.39	34.00	0.23	0.33	0.97
6:0	34.98	33.86	30.71	33.04	1.51	0.21	0.71	0.19
8:0	18.93	17.17	15.37	16.66	0.73	0.02	0.63	0.13
10:0	41.17	35.15	31.33	33.59	1.41	<0.01	0.47	0.13
12:0	42.39	36.12	32.28	34.71	1.44	<0.01	0.51	0.13
14:0	141.16	135.72	120.95	128.10	5.18	0.08	0.34	0.14
16:0	356.17	374.71	350.37	375.65	17.30	0.61	0.97	0.29
16:1 *trans*	4.90	6.05	5.24	5.49	0.29	0.08	0.21	0.18
16:1 *cis*	16.69	19.05	17.98	19.26	0.89	0.09	0.88	0.32
18:0	167.74	190.76	183.72	202.89	10.77	0.09	0.46	0.36
18:1 *trans*-9	3.75	5.24	5.31	5.92	0.29	<0.01	0.15	0.48
18:1 *trans*-11	15.21	13.02	14.36	19.42	1.37	0.82	0.07	0.31
18:1 *cis* 9 and 10	238.66	277.84	288.07	304.17	15.01	0.03	0.26	0.88
18:2	39.19	32.90	31.87	37.34	2.07	0.07	0.18	0.25
18:3	9.25	8.16	8.02	8.90	0.60	0.24	0.41	0.51
SCFA	106.07	112.41	104.24	105.08	5.81	0.87	0.41	0.55
MCFA	602.48	606.81	558.14	596.80	25.67	0.63	0.79	0.21
LCFA	473.80	527.92	531.34	578.63	28.42	0.07	0.25	0.55

[1]Adapted from Avila et al. (2000).

[2]Contrasts: Effect of supplemental fat (control vs. others); linear effect of tallow among supplemental diets; effect of blended fat (blended vs. single source of fat).

[3]60% tallow and 40% yellow grease blend.

[4]Yellow grease.

[5]Short-chain FA = sum of 4:0 to 8:0.

[6]Medium-chain FA = sum of 10:0 to 16:1 *cis*.

[7]Long-chain FA = sum of 18:0 to 18:3.

salts of 18:2 and 18:3 is much lower than of these FA in soybean oil (Van Nevel and Demeyer, 1994). Results from feeding calcium salts of unsaturated oils to Holstein cows are presented in Table 43 (Chouinard et al., 1998b). Feeding these protected oils increased the amounts 11*t*-18:1 and 18:1. The thermal properties of the MF were improved. Other recent papers about feeding Ca salts are (Enjalbert et al., 1997; Kowalski et al., 1999). Enjalbert et al. (1997) presented data from feeding of the more widely used Ca salts of palm oil FA.

Aigster et al. (2000) used milk from cows fed calcium salts of high-oleic sunflower oil to make cheese. The 18:1 contents of the original and modified milks were 26.3 and 40.2%. The amounts of 12:0, 14:0, and 16:0 decreased from 40 to 33%. These acids are believed to

Table 43. Contents of selected fatty acids (FA; wt%) in oils and milks from cows fed calcium salts of various oils.[1]

FA	Control	Ca COA		Ca SOFA		Ca LOFA	
16:0	26.21	4.75	19.26	10.96	19.25	6.37	19.09
18:0	11.37	1.91	15.04	3.81	14.76	3.14	14.99
11*t*-18:1	1.84	. . .	9.42	. . .	12.59	. . .	10.18
18:1	19.76	58.55	26.94	23.31	25.99	20.05	22.92
18:2	2.45	22.06	2.32	54.31	2.45	18.16	3.36
18:3	0.24	7.71	0.20	5.00	0.17	51.52	0.31
20:4	0.17	. . .	0.09	. . .	0.69	. . .	0.01
Yield kg/d							
Milk	35.90		36.2		37.00		38.4
Fat	1.43		1.02		1.11		1.32
Fat %	4.05		2.67		2.98		3.56
Protein	1.13		1.17		1.11		1.13

[1]Adapted from Chouinard et al. (1998b). 18:1 is mostly, 95% 9*c*, remainder 11*c*. Ca COA = Canola oil FA; Ca SOFA = soybean oil, and Ca LOFA = linseed oil. Contrasts between control and treatment significant ($P < 0.05$) except for 18:2 and 18:3. No significant contrasts in yield components.

be hypercholesterolemic. The Latin American white cheese, queso blanco, made from the milks, were similar in firmness and flavor.

The effects of altering the rumen pH have been investigated. The addition of Na and K bicarbonates to the diets of cows fed calcium salts of canola oil increased milk yield, protein, and the amounts of *t*-18:1, 18:1, and 18:2 in the milk (Chouinard et al., 1997). This group confirmed their results above, but found that the addition of MgO as an alkalinizing agent decreased the milk contents of *t*-18:1, 18:2, and 18:3 and increased 18:1 (Thivierge et al., 1998).

Kennelly et al. (1999) looked at the influence of concentrate-to-forage rations and buffer on the various parameters of interest. Cows fed a high-concentrate diet had a lower rumen pH, which increased the quantity of *t*-18:1 and decreased the MF content. The depression associated with the intake of high concentrate diets was again confirmed.

Another mode of protection involves the formation of fatty acyl amides with, for example, the FA in soybean oil and butylamine. The resulting butyl soyamides resist biohydrogenation and caused less ruminal disruption. Results from an investigation of feeding the preparation are presented in Table 44 (Jenkins et al., 1996). The linoleic acid content was increased Lauric acid (12:0), 14:0, and were increased relative to soybean oil, but decreased compared with the control. The quantities of *t*-18:1, 18:1, and protein were decreased. These changes appear to negate the increase in 18:2. Cost and availability of the amide were not discussed.

Jenkins (1999, 2000) investigated the influence of feeding oleamide to lactating cows. His results are presented in Table 44. He prepared the synthetic oleamide from 18:1 and urea. Feeding oleamide to Jersey cows in this study increased milk 18:1, did not affect *t*-18:1 contents, and decreased feed intake and milk yield. Jenkins (1998) obtained essentially the same results earlier but did not analyze *trans*-18:1 contents.

Jenkins (1998) also determined the FA profiles in milk from Holstein cows fed 0 to 5% oleamide. He employed GLC for analysis and found that the content of 18:1 increased in parallel with the quantities of oleamide fed. Contents of *t*-18:1 would not be resolved by the GLC method. Oleamide was not detected in the milks.

Summary

These attempts to make MF more "heart friendly" have achieved a modest success. However, in my opinion, it is less expensive and arduous in theory for consumers to modify their diets. In practice, this is extremely difficult, as shown by the recent pandemic of obesity. An expansion of the program of understandable and persuasive education, as is being conducted by the National Dairy Council, should be done.

TRANS-FATTY ACIDS

Ruminal Bypass Studies

Results from several papers confirm that unsaturated FA infused into the duodenum (Wagner et al., 1998; Hermansen et al., 1995; Kalscheur et al., 1997b; Christensen et al., 1998; Enjalbert et al., 2000; or abomasum, Bremmer et al., 1998) of cows are efficiently transferred to MF after further metabolism of, for example, 18:3 n-6 (Hermansen et al., 1995). In one of these papers (Christensen et al., 1998), the cows had ruminal and duodenal cannulas. The effects of low and high fat diets and nicotinic acid were evaluated. The 18:0 content in milk and the yields of 6:0 to 16:0 were decreased by fat, but nicotinic acid had no effect.

Enjalbert et al. (2000) infused 16:0, 18:0, and 18:1 and increased the amounts of these FA in milk. The milk FA profiles were not reported by Bremmer et al. (1998), but abomasal infusions of unsaturated FA decreased nutrient intake.

Factors Affecting *trans*-FA Contents in Milk

Data in Table 23 (Precht et al., 1999; Precht and Molkentin, 2000a) show that the contents of *trans*-FA, primarily 11*t*-18:1 (vaccenic acid) vary with the season or changes from summer pasture feeding, 5.08% (2.87% 11*t*) transition feeding, 3.80% (1.78% 11*t*) winter barn feeding, 2.65% (0.93% 11*t*) for a yearly average of 3.82% (1.72% 11*t*). However, when cows are fed diets high in concentrate, the depression in MF is accompanied by an increase in *t*-18:1 content (Kalscheur et al., 1997a). Production of *trans*-18:1s is the result of incomplete biohydrogenation of dietary PUFA. *Trans*-FA were again associated with decreased fat percentage in milk (Wonsil et al., 1994). Abomasal infusion of *t*-18:1 depressed MF and yield possibly by decreasing synthesis of FA and activity of acyl transferase in mammary tissue (Gaynor et al., 1994). These results instigated the development of two theories, glucogenic and lipogenic (author's designation) about the cause of the fat depression. The glucogenic theory proposes that diets high in concentrates suppress the mobilization of FA from adipose tissue and increase the competition between this compartment and mammary tissue for glucose, NEFA, and TAG. Gaynor et al. (1994) discredited the glucogenic theory because they did not observe an increase in serum glucose and insulin in cows with the greatest drop in MF percentage. They also noted that cows with high *trans*-FA contents in milk also had increases in serum thyroid hormones. They later provided evidence that *trans*-18:1s affected synthesis of MF in the mammary gland (Gaynor et al., 1996; Romo et al., 1996). In another study, they again confirmed the association of *t*-18:1s with MF depression, but increased incorporation into MF was associated with a proportional decrease in MF (Kalscheur et al., 1997b).

Griinari et al. (1997a, 1997b) discussed the theories of MF depression. These were: 1) glucogenic-insulin and 2) inhibition by compounds produced during the synthesis of MF. They based their discussion on new information about the amounts of *cis* and *trans* isomers of 18:1 and 18:2 in MF. With these data and knowing that MF depression was not related to high circulating insulin levels, Griinari et al. (1997a, 1997b) selected the inhibition theory with *trans*-FA as the likely compounds. Using comparisons of FAME retention times (Molkentin and Precht, 1995b; Wolff and Bayard, 1995), not the Ag-TLC/GLC procedure, they found that the *t*-18:1 contents were associated with the MF depression induced by high-concentrate, low-fiber diets. They also found that an infusion of CLA isomers inhibited MF synthesis. Griinari et al. (1998) confirmed that the low-fiber, unsaturated FA (corn oil) diet increased the content of 10*t*-18:1 and

Table 44. Effects of feeding butylsoyamide and oleamide to cows on contents (wt%) of selected fatty acid (FA) and yield of milk.

	Butylsoyamide[1]			Oleamide[2]			
FA	Control	Soybean oil	Butylsoyamide	Control	Canola	Commercial	Synthetic
12:0	50.4[a]	3.05[c]	4.42[b]	4.32	3.32	2.50	2.76
14:0	14.38[a]	10.15[c]	13.53[b]	12.42	10.78	9.33	9.53
16:0	37.45[a]	27.00[c]	33.91[b]	34.28	25.70	21.44	24.61
18:0	9.84[b]	14.31[a]	10.19[b]	9.24	15.97	14.56	14.17
t-18:1	1.69[b]	9.48[a]	2.72[b]	1.72	4.22	4.06	4.21
18:1	37.45[b]	25.00[a]	19.55[b]	17.44	22.13	30.00	27.12
18:2	3.68[c]	4.77[a]	6.28[a]	2.30	1.58	2.08	1.62
CLA	. . .	. . .	. . .	0.36	0.27	0.43	0.42
18:3	. . .	. . .	. . .	0.32	0.16	0.36	0.30
Yield kg/d							
Milk	29.7	30.10	28.0	26.6	27.8	25.8	25.00
Fat	0.95[a]	0.75[b]	0.87[a]	1.24	1.22	0.97	1.00
Fat %	3.19[a]	2.52[b]	3.12[a]	4.79	4.45	3.77	4.10
Protein	0.97[a]	0.98[b]	3.19	0.94	0.97	0.87	0.81

[a,b,c]Means in FA within a row lacking a common superscript differ ($P < 0.05$). [a,b] in Yield same as Jenkins, FA.

[1]Adapted from Jenkins et al. (1996).

[2]Adapted from Jenkins (2000). Contrasts. Control versus canola, significant ($P < 0.15$) except conjugated linoleic acids (CLA). NS. Canola versus both oleamides, significant (0.15) except t-18:1 NS Both oleamides, NS except 16:0 and 18.2.

Table 45. Dry matter intake, milk production, and milk composition of cows fed control or milk fat-depressing (MFD) diets.[1]

Item	Control	MFD	SED[2]	P[3]
	kg/d			
DMI	20.4	19.7	0.21	<0.090
Milk	27.6	27.6	0.98	<0.987
3.5% FCM[4]	28.6	23.4	0.98	<0.002
	g/100 g			
Milk components				
Fat	3.28	1.88	0.09	<0.001
Protein	3.24	3.58	0.06	<0.003
	g/dl			
Milk production				
Fat	945	536	36.8	<0.001
Protein	930	1,055	52.8	<0.002
SCC/L, n	2098	1986	722	<0.914

[1]Adapted from Piperova et al. (2000). Values are least square means, n = 10.

[2]Standard error of the difference.

[3]Probability that treatments are not different.

[4]Energy corrected yield corresponding to milk containing 3.5% fat.

Table 46. Milk fatty acid (FA) composition of cows fed control or milk fat-depressing (MFD) diets.[1]

Fatty acid	Control	MFD	SED[3]	P[4]
	g/100 g FAME			
4:0	3.8	3.2	0.17	0.051
6:0	2.3	1.4	0.08	0.001
8:0	1.6	0.9	0.14	0.002
10:0	3.5	2.4	0.15	0.001
12:0	4.2	3.2	0.18	0.002
14:0	12.0	9.3	0.40	0.001
14:1	1.0	1.0	0.09	0.884
16:0	30.7	21.3	0.59	0.001
16:1	2.1	1.9	0.09	0.235
18:0	9.7	8.8	0.56	0.238
trans-18:1	1.9	15.6	0.81	0.001
cis-18:1	19.7	19.3	0.63	0.607
18:2 (n-6)	3.1	6.3	0.39	0.001
18:2i[5]	0.9	1.5	0.08	0.001
18:3	0.6	0.5	0.04	0.251
Total PUFA[6]	4.5	8.3	0.49	0.001
Total SFA[7]	68.1	50.4	1.46	0.001
Total de novo FA	39.2	27.7	1.03	0.002
Total LCFA[7,8]	68.7	75.2	1.48	0.010
	g/d			
4:0	34.8	16.3	1.61	0.001
6:0	21.4	7.3	0.92	0.001
8:0	15.6	4.9	1.40	0.001
10:0	34.0	12.5	1.51	0.001
12:0	40.5	16.7	1.71	0.001
14:0	112.3	48.5	3.90	0.001
14:1	8.6	5.1	0.92	0.023
16:0	279.8	107.7	9.95	0.001
16:1	18.2	9.5	0.95	0.001
18:0	88.0	44.4	5.42	0.001
trans-18:1	16.9	75.6	6.04	0.001
cis-18:1	170.1	95.7	1.18	0.001
18:2 (n-6)	26.5	31.1	1.90	0.126
18:2i[4]	7.2	7.4	0.05	0.724
18:3	5.1	2.5	0.30	0.001
Total PUFA[6]	38.8	41.0	9.40	0.864
Total SFA[1]	626.4	242.0	22.65	0.001
Total de novo FA	223.8	89.9	12.03	0.001
Total LCFA[7,8]	611.8	373.9	21.00	0.001

[1]Adapted from Piperova et al. (2000).

[2]Values are least square means (n = 10). Other FA, including odd and branch chain, representing less than 3% of the total FA methyl ester are not included.

[3]Standard error of the difference.

[4]Probability that treatments are not different.

[5]Conjugated and nonconjugated.

[6]Total polyunsaturated FA.

[7]Total saturated FA.

[8]Long-chain FA (16 carbon and greater).

that this combination was associated with a significant decrease in MF content and yield and the contents of 4:0 to 16:0. The amount of fiber did not affect the CLA contents of milk when saturated FA was fed. The probable role of CLA as an inhibitor was mentioned but not discussed further. The *trans* isomers were identified by comparisons of FAME retention times, as mentioned above, not the Ag-TLC/GLC method.

In what I consider to be one of the definitive papers, Piperova et al. (2000) examined the problem of MF depression by examining the changes in: 1) the activities of mammary acetyl-CoA carboxylase (ACC) and FA synthase, 2) relative abundance of ACC mRNA, and 3) distributions of CLA and *trans*-18:1s in milk. They fed two diets: control, 60:40% forage/concentrate and a MF-depressing diet (**MFD**), 25:70% forage/concentrate supplemented with 5% soybean oil. All of their findings can be seen in Tables 45 to 49. Their results show that the MFD diet decreased the fat content, ACC and FA synthase activities, and the relative abundance of ACC mRNA. De novo synthesis of FA decreased, but the amount of 10*t*-18:1 increased. The results suggested that the CLA isomer, 10*t*, 12*c* might be involved in MFD. I will discuss this in the section on CLA.

Amounts of *trans*-FA in Bovine Milk Fats

Precht and Molkentin (2000b) collated the contents of *trans*-FA in MF from European countries in Table 50. References are in the footnotes of the table. I added the amounts of *trans* 18:1 and 18:2 from US milks (Piperova et al., 2000). Most of these data were obtained by analyses with the Ag-TLC/GLC or comparable methods.

Health Effects of Milk *trans*-FA

I mentioned this aspect earlier, because *trans*-FA have been implicated as a causative factor of heart disease. This has been questioned (Ackman, 2000). In

Table 47. *Trans*-18:1 content and isomer distribution in milk fat of cows fed control or milk fat-depressing (MFD) diet.[1]

	Control	MFD	SED[2]	*P*
	— g/100 g of FAME[3] —			
Total *trans* -18:1	1.9	15.6	0.81	<0.001
	· g/100 g total *trans*-18:1 ·			
Double bond position				
6 + 7 + 8	2.6	6.9	0.7	<0.002
9	5.5	6.1	0.7	NS[4]
10	13.9	59.2	1.7	<0.001
11	28.5	10.9	0.8	<0.001
12	12.2	4.3	0.6	<0.001
13 + 14	22.6	8.6	0.9	<0.001
15	7.1	2.2	0.3	<0.001
16	7.4	1.4	0.6	<0.001
	g/d			
6 + 7 + 8	0.5	6.1	0.9	<0.002
9	1.0	5.4	0.9	<0.001
10	2.5	50.0	0.6	<0.001
11	5.1	8.4	0.8	<0.001
12	2.2	3.1	0.2	0.02
13 + 14	4.0	7.2	0.7	<0.01
15	1.3	1.6	0.9	NS
16	1.3	1.0	0.6	NS

[1]Adapted from Piperova et al. (2000). Values are least square means (n = 10).

[2]Standard error of the difference.

[3]Fatty acid methyl ester.

[4]NS, $P \geq 0.05$.

Table 48. Conjugated linoleic acids (CLA) and isomer distribution in milk fat of cows fed control or milk fat-depressing diets (MFD).[1]

	Control	MFD	SED[2]	*P*
	— g/100 g of FAME[3] —			
Total CLA	0.56	0.95	0.11	<0.001
	– g/100 g of total CLA –			
CLA isomers				
trans-7, *cis*-9	7.8	23.4	1.66	<0.001
cis-8, *trans*-10	1.5	1.8	0.06	<0.01
cis-9, *trans*-11	79.7	56.7	1.23	<0.001
trans-10, *cis*-12	1.0	10.1	0.49	<0.001
cis-11, *trans*-13	0.2	0.1	0.05	NS[3]
trans-11, *cis*-13	0.7	0.2	0.05	<0.001
cis-12, *trans*-14	0.7	0.7	0.19	NS
	g/d			
Total CLA	5.1	4.5	1.13	NS
trans-7, *cis*-9	0.4	1.1	0.04	<0.001
cis-8, *trans*-10	0.1	0.1	0.01	NS
cis-9, *trans*-11	4.1	2.6	0.36	<0.01
trans-10, *cis*-12	0.05	0.5	0.04	<0.001
cis-11, *trans*-13	0.01	0.004	0.001	<0.03
trans-11, *cis*-13	0.04	0.01	0.004	<0.001
cis-12, *trans*-14	0.04	0.03	0.005	NS

[1]Adapted from Piperova et al. (2000). Values are least square means (n = 10); *trans*/*trans* - and *cis*/*cis* - isomers are not included.

[2]Standard error of the difference.

[3]Fatty acid methyl ester.

[4]NS, $P \geq 0.05$.

Table 49. Effect of milk fat-depressing diet (MFD) on acetyl-CoA carboxylase (ACC) and fatty acid (FA) synthase activity and ACC mRNA abundance in cow mammary gland tissue.[1]

	Control	MFD	SED[2]	*P*[3]
ACC[4]	9.8	3.8	1.1	<0.001
FA synthase[5]	13.2	7.4	1.9	<0.001
ACC mRNA[6]	0.22	0.07	0.01	<0.001

[1]Adapted from Piperova et al. (2000). Values are least square means (n = 10).

[2]Standard error of the difference.

[3]Probability that treatments are not different.

[4]Values are expressed as nmol [^{14}C]HCO_3 fixed (min^{-1} (mg $protein^{-1}$.

[5]Values are expressed as nmol NADPH oxidized (min^{-1}(mg $protein^{-1}$.

[6]Values are expressed as densitometric units, normalized against bovine ribosomal protein S4 mRNA.

addition, much of the *trans*-FA consumed in the United States are in products that contain partially hydrogenated vegetable oils and these have a different profile of *trans*-18:1 isomer than MF (Chen et al., 1995). In addition, humans have consumed *trans*-FA in MF for millennia since ruminants were domesticated and their products utilized. Our metabolic systems may have adjusted positively to their presence (Ackman, 2000). Otherwise, primitives who consumed ruminant products would not have survived. However, the possibility of potential harm has not been eliminated. A variety of deleterious effects have been noted in animals (Teter et al., 1990). One of the problems is how much *trans*-FA are consumed in the United States? Amounts have varied, but Allinson et al. (1999) recently estimated the mean individual intake to be 5.3g/d and this was 7.4% of the total fat with about 15 to 20% from milk and butter (Allinson et al., 1999; Craig-Schmidt et al., 1998). Data on the intake of other FA and stratified by age groups and sex are available in the paper by Allinson et al. (1999). Additional information can be found in the review by Craig-Schmidt (1998). Because almost all of these estimates were based on data obtained with capillary GLC columns only, they were underestimated. See Tables 26 and 27. This raises questions about the validity of the amounts related to health concerns, since the subjects were consuming more *trans*-FA than estimated.

Katan (2000) has long implicated consumption of *trans*-FA as a cause of cardiovascular disease (**CVD**). This has been questioned and relatively speaking, MF is such a minor source it shouldn't be excluded from the diet for this reason. This statement also applies to the concerns of Koletzko and Decsi (1997) about the conversion of 18:2 n-6 to 20:4 n-6 and impairment of early human growth. These concerns should be reexamined when *trans*-FA contents determined by the

Table 50. Mean values (mean), minimal (min), and maximal (max) contents of *trans* fatty acids (TFA) in (n) milk fats from different countries (wt% of fatty acids).[1]

Mean	Min	Max	n	TFA	Analysis	Year	Country	Ref[2]
0.03			1	C15:1	Ag-TLC/GC	1983	Denmark	1
0.18			1	C16:1	Ag-TLC/GC	1983	Denmark	1
0.08	0.04	0.13	12 (autumn)	C16:1	Ag-TLC/GC	1994	France	2
0.13	0.10	0.16	12 (spring)	C16:1	Ag-TLC/GC	1994	France	2
0.13	0.05	0.25	27	C16:1	Ag-TLC/GC	1992	Germany	3
5.53	4.52	7.31	17	C18:1	TLC/Densitometer	1972	New Zealand	4
2.7			1	C18:1	Ag-TLC/GC	1981	Sweden	5
3.8	2.46	5.10	24	C18:1	Ag-TLC/GC	1994	France	3
3.33	1.75	5.20	31	C18:1	Ag-TLC/GC	1994	Austria	6
3.62	1.29	6.75	1756	C18:1	Ag-TLC/GC/TR[1]	1995	Germany	7
3.83	1.91	6.34	100	C18:1	Ag-TLC/GC	1995	Germany	7
4.24	3.19	5.91[3]	111	C18:1	Ag-TLC/GC/TR[1]	1995	EU[4]	7
2.65	1.29	4.21	927 (winter)	C18:1	Ag-TLC/GC/TR[1]	1997	Germany	8
3.80	2.71	4.94	236 (transition)	C18:1	Ag-TLC/GC/TR[1]	1997	Germany	8
5.08	3.28	6.75	593 (summer)	C18:1	Ag-TLC/GC/TR[1]	1997	Germany	8
4.35	2.18	5.18	58	C18:1	Ag-TLC/GC/TR[1]	1997	Germany	8
0.5	0.1	2.2	10	C18:2	GC	1982	Germany	9
0.53	0.3	0.8	10	C18:2	GC	1993	Germany	10
0.99	0.56	1.58	100	C18:2	Ag-TLC/GC	1997	Germany	11
0.46	0.11	0.95	927 (winter)[5]	C18:2	Ag-TLC/GC/TR[1]	1997	Germany	12
0.66	0.40	1.14	236 (transition)[5]	C18:2	Ag-TLC/GC/TR[1]	1997	Germany	12
0.87	0.54	1.41	593 (summer)[5]	C18:2	Ag-TLC/GC/TR[1]	1997	Germany	12
3.70[6]	1.29	7.31	2098	C18:1	Ag-TLC/GC	2000	All	13
1.9				C18:1	Ag-TLC/GC	2000	US	14[7]
3.1				C18:2	Ag-TLC/GC	200l	US	14[7]

[1]Adapted from Precht and Molkentin (2000a). TR derived from triglyceride formulas being based on silver ion (Ag)-TLC/GC analyses.

[2]References.1. Lund and Jensen (1983); 2. Wolff (1994); 3. Molkentin and Precht (1997a); 4. Gray (1973); 5. Akesson et al. (1981); 6. Henninger and Ulberth (1994); 7. Precht and Molkentin (1996); 8. Precht and Molkentin (1997a); 9. Renner and Yoon (1982); 10. Pfalzgraf et al. (1993); 11. Precht and Molkentin (1997b); 12. Precht and Molkentin (1997b); 13. Precht and Molkentin (2000b); 14. Piperova et al. (2000).

[3]Without t, t, - NMID (*trans*, *trans* nonmethylene interrupted dienes).

[4]Minimal and maximal mean content of individual countries.

[5]Ten different countries in the European Union excluding Germany.

[6]Weighted average of 2098 samples, European samples from p. 636, ref. 13.

[7]Added by author.

Ag-TLC/GLC method are available. It is possible that individual *trans* isomers may alter metabolism, e.g., the desaturation of 11*t*-18:1 to 9*c*,11*t*- 18:2 or rumenic acid (**RA**).

Conjugated Linoleic Acids

Conjugated linoleic acids are a group of conjugated or nonmethylene interrupted dienoic 18:2s with double bonds at 9 and 11 or 10 and 12. Each double bond can be *cis* or *trans*, but those with one *trans* bond are bioactive and occur in MF primarily (80 to 90%) as the 9*c*, 11*t* isomer.

The presence of CLA in MF has been known for years (Parodi, 1999a), but they were ignored until pioneering efforts by M. W. Pariza and colleagues showed that they were anticarcinogenic and that the bioactive isomers were present in milk and meats from ruminants (Pariza, 1999). Their discovery unleashed a burst of activity on the analysis, properties, amounts, benefits, chemical synthesis, and biosynthesis of CLA. The results have been summarized in a book, which contains the references by Parodi and Pariza above and many more. Other short useful reviews are in Molkentin (1999), Parodi (1999b), Pariza et al. (1999, 2000), and MacDonald (2000). A review by Kritchevsky undated is available from the web site: http://www.nationaldairycouncil.com/medcent/mnews/CLA.html. A bibliography of publications about CLA (1999) is on a the web site. To illustrate the proliferation of papers about CLA, there were 62 listed in 1998, 98 in 1999, and 126 in 2000. The stimuli for the research reported in these papers and many more in 2000 were, that in addition to their anticarcinogeneity, the FA also beneficially affected 1) atherosclerosis, 2) the immune system, and 3) lipid metabolism and body composition (Pariza, 1999), and a shift in the transfer of nutrients from adipose tissue to lean body mass (Pariza et al., 1999, 2000). Bone formation

(Watkins et al., 1999) and diabetes (Belury and Vanden Neuvel, 1999) are also affected.

The importance of CLA stimulated Kramer et al. (1998a) to suggest the trivial name, rumenic acid, for 9*c*, 11*t*-18:2.

I will concentrate on analysis, contents in milk and its products, factors affecting the amounts, and effects of CLA on lactation. Readers who want additional information on the health and medical aspects can use the references above. It is useful to remember that results about humans are limited to epidemiological surveys and studies on groups who are willing to use themselves as subjects, e.g., body builders.

Analysis

Readers should consult Christie et al. (2001) and Banni and Martin (1998) for information on analysis. Both are excellent and, while short, and the paper by Banni and Martin (1998) gives details for some of the procedures.

Derivatization. The analysis of CLA usually requires their conversion to derivatives, mostly FAME, before separation by GLC or HPLC (Yurawecz et al., 1998). I mentioned earlier that base-catalyzed methanolysis is the preferred method for the preparation of FAME. Acid catalysts decrease the quantities of CLA by isomerization and formation of allylic methoxy artifacts are formed (Kramer et al., 1997). If the product to be analyzed contains significant amounts of FFA, e.g., cheeses, Yurawecz et al. (1999) recommended that the lipids be separated into their components by TLC or HPLC with an appropriate methylation procedure before separation. Free FA will form Na soaps when reacted with Na methoxide and cannot be converted to FAME. An acid catalyzed procedure must be employed.

Ostrowska et al. (2000) compared the Ag-HPLC quantification of free and methylated CLA. They found that acid (HCl and BF_3) and base (tetramethyguanidine/MeOH) catalyzed methylation procedures resulted in intraisomerization of CLA and losses in total conjugated diene content. They did not test Na-methoxide catalyzed methylation. They suggested CLA should be analyzed as FFA when Ag-HPLC is employed. However, Nikolova-Damyanova et al. (2000) evaluated the use of *p*-methoxyphenacyl esters for the resolution of CLA isomers by Ag-HPLC. Using a single instead of the multiple columns required by others, they were able to separate saturated FA; *t,t -, c,t*/*t,c,* and *c,c*-CLA positional isomers; *cis*-monoenes, methylene interrupted *cis*, *trans*; *trans*, *cis*-, and *cis*, *cis*- dienes in a single run. These esters appear to be the preferred derivative for resolution of CLA.

Separation and esterification. Kramer et al. (1999) reviewed the separation of conjugated FA or CLA from biological matrices and from each other. They prefer this term instead of CLA because some of the conjugated FA are not biologically derived from 18:2, e.g., delta-9 desaturation of 11*t*-18:1 to produce 9*c*, 11*t*-18:2 in the human liver and mouse adipose tissue (Palmquist and Santora, 1999). However, most investigators will employ the acronym CLA. Absorptions of UV at 230 nm and of infrared at 948 and 982 cm^{-1} are characteristic identifiers of CLA. Using these and chromatographic techniques, Parodi (1999a) isolated CLA from MF and identified it as primarily 9*c*, 11t-18:2. Among the techniques he used was Ag-TLC, which aided in the isolation of the fraction.

Kramer et al. (1999) stated that if the CLA in MF are being determined routinely, a 60-m Supelcowax-10 fused silica column or equivalent is satisfactory. However, if a detailed analysis is needed, a 100-m column should be used. They presented a list of GLC parameters for analysis of CLA. Kramer et al. (1999) provided lists of 20 *cis*/*trans* CLA that have been identified in cheese by GLC and Ag-HPLC. However, a few of these had not been confirmed. They mentioned that Ag-HPLC separated isomers that coeluted with 9*c*, 11*t*-18:2 on GLC columns. They recommended the complementary application of the techniques. The book, which contains their chapter, above, includes chapters on other methods, e.g., infrared spectroscopy, which have been employed for the identification of CLA. According to Roach (1999), identification of the isomers in CLA required Ag-HPLC to fractionate the mixture. The fractions from pig tissues lipids were converted to derivative and analyzed by GLC-MS and GLC/Fourier transform infrared spectroscopy by Kramer et al. (1998b). Ag-HPLC of the derivatized components in the CLA mixture resolved the compounds more completely than GLC alone (Sehat et al., 1998b, 1999). Identification of the isomers may require their conversion to and resolution of dimethyloxazoline derivatives Yurawecz et al. (1997) or triazoline diene adducts (Christie et al., 1997; Dobson, 1998).

Roach et al. (2000) recommended that separation of minor CLA isomers from natural products include identification of the isomers with gas chromatography high-resolution selected-ion mass spectrometry. They found that trace amounts of 20:1 and 20:2 FA eluted in the CLA region of the cheese FA that they analyzed. Other papers that contain information about Ag-HPLC analysis of CLA isomers are in references by Adlof and Lamm (1998), Sehat et al. (1999), and Rickert et al. (1999).

Fritsche et al. (1999a) presented a figure that summarizes the protocol that they recommend for the

Table 51. Conjugated linoleic contents (CLA; wt%) of milks and dairy products. Amounts are 80 to 90% 9*c*, 11*t*-18:2 unless noted.

Source-Treatment	Amount			Range	Method
Pooled milk					
German n = 238[1]	0.81	0.38	SD	0.25-1.75	Ag-TLC/GLC[10]
German, seasonal[2]					
All, n = 1756	0.75	0.38	SD	0.10-1.89	Ag-TLC/GLC
Barn feeding, n = 927	0.45	0.34	SD	0.10-1.05	Ag-TLC/GLC
Transition, n = 236	0.76	0.15	SD	0.19-1.19	Ag-TLC/GLC
Pasture, n = 593	1.20	0.20	SD	0.49-1.89	Ag-TLC/GLC
United States n = 1[3]				0.97-1.02	Ag-HPLC
Canadian n = 4[4]					
Skim milk powder, 0.1% fat	0.18	0.02	SEM		GLC - 100 m
Whole milk, 3.2% fat	0.34	0.02	SEM		GLC - 100 m
Half/half cream, 12.1% fat	0.55	0.04	SEM		GLC - 100 m
Butter, 81.0% fat	0.49	0.19	SEM		GLC - 100 m
European milk[6]					
n = 78[5]	0.70			0.26-1.43	GLC - 50 m
n = 8[6]	1.01	0.25	SD		GLC - 50 m
German products[7]					
Raw milk, n = 7	1.16	0.10	SD		GLC - 50 m
Pasteurized milk, n = 2	0.98				
UHT milk	0.80				
Butter, n = 12	0.94	0.48	SD		GLC - 50 m
Condensed milk	0.63				GLC - 50 m
Yoghurt, n = 4	0.69	0.30	SD		GLC - 50 m
Probiotic yoghurt, n = 2	1.05				GLC - 50 m
Milk					
Mean[8], n = 1738	0.75	0.38	SD		GLC - 50 m
Minimum[8]	0.10				GLC - 50 m
Maximum[8]	1.89				GLC - 50 m
New Zealand milk[9]	1.20				Ag-HPLC

[1]From Precht and Molkentin (1997c).

[2]From Precht and Molkentin (1997a).

[3]From Yurawecz et al. (1998). Isomer is 9*c*, 11*t* - 18:2. Amounts calculated by author. Amounts of total CLA as percentages of FA not given.

[4]From Ma et al. (1999).

[5]From Jahreis et al. (1999a).

[6]From Jahreis et al. (1999b).

[7]From Fritsche and Steinhart (1998).

[8]From Precht and Molkentin (2000a). Isomer is 9*c*, 11*t* - 18:2.

[9]From Robinson and MacGibbon (2000).

[10]Silver-ion TLC/GLC.

analysis of CLA. More instructions and references are available in the paper. The table is meant for milk and its products, but it can be employed as a guide for analysis of CLA in other materials.

Amounts in milk and its products. The employment of GLC methods by Precht and Molkentin (1997a, 1997c, 1998a, 1999) is an example of the information that can be obtained with the procedures. Yurawecz et al. (1998) analyzed milk, cheese, and other natural material with Ag-HPLC and other methods. In addition to about 82% 9*c*, 11*t*-18:2 and the other minor isomers, they identified a new CLA: 7*t*, 9*c*-18:2. I have collated recent data from these and other papers on the amounts of CLA and relevant information in milk and dairy products in Table 51, European milks in Table 52 (plus related FA), and cheeses in Table 53. All references are given in the tables. Unless noted, the CLA contents are 80 to 90% 9*c*, 11*t*-18:2. Information about methods is included. Data on the CLA contents of dairy foods and factors affecting them are available in references by Fritsche et al. (1998, 1999a, 1999b), Kramer et al. (1999), Griinari and Bauman (1999), Lin et al. (1995), and other references listed in the tables. Lin et al. (1995) presented the amounts of CLA in a wide variety of US dairy products. For example, they found 0.45% CLA in the fat of whole milk containing 3.2% fat. Heat treatment and other processing including aging of cheese had little effect on the CLA contents.

Robinson and MacGibbon (2000) identified the TAG, which contained CLA in New Zealand MF. They separated the TAG with RP-HPLC. With this technique,

Table 52. Mean contents (g/100 g) of conjugated linoleic acids and related fatty acids (FA) from 14 European countries.[1]

FA	Mean	SD	Min	Max	n	Country
c9t11	0.75	0.38	0.10	1.89	1738	DE[2]
c9t11	0.45	0.13	0.10	1.05	909	DE[3]
c9t11	0.76	0.15	0.19	1.19	236	DE[4]
c9t11	1.20	0.20	0.49	1.89	593	DE[5]
c9t11	0.92	0.26	0.52	1.44	13	AU
c9t11	0.76	. . .	. . .	. . .	1	BE
c9t11	0.87	0.19	0.59	0.99	4	DK
c9t11	0.95	0.09	0.82	1.11	10	ES
c9t11	0.74	0.27	0.21	1.56	198	FR
c9t11	1.03	0.28	0.60	1.40	21	UK
c9t11	0.87	0.02	0.85	0.89	4	GR
c9t11	0.94	0.31	0.63	1.67	12	IT
c9t11	1.41	0.31	0.56	1.82	23	IR
c9t11	0.67	. . .	. . .	. . .	1	LUX
c9t11	0.73	0.28	0.36	1.33	63	NL
c9t11	0.56	. . .	. . .	. . .	1	SV
c9t11	0.93	0.35	0.36	1.82	153	EU 12[6]
c9t11	0.76	0.37	0.10	1.89	2089	EU 14
t-$C_{18:1}$	3.62	1.22	1.29	6.75	1756	DE[1]
t-$C_{18:1}$	2.65	0.45	1.29	4.21	927	DE[2]
t-$C_{18:1}$	3.80	0.41	2.71	4.94	236	DE[3]
t-$C_{18:1}$	5.08	0.65	3.28	6.75	593	DE[4]
t-$C_{18:1}$	3.58	0.92	1.80	6.26	201	FR
t-$C_{18:1}$	4.37	1.13	2.43	7.17	153	EU 12[6]
t-$C_{18:1}$	3.67	1.21	1.29	7.17	2110	EU 14
t11	1.72	0.98	0.35	4.43	1705	DE[2]
t11	0.93	0.30	0.35	1.96	876	DE[3]
t11	1.78	0.35	0.85	2.73	236	DE[4]
t11	2.87	0.55	1.45	4.43	593	DE[5]
t11	1.71	0.70	0.36	3.81	201	FR
t11	2.26	0.94	0.78	4.46	153	EU 12[6]
t11	1.76	0.96	0.35	4.46	2110	EU 14
t-$C_{18:2}$	1.11	0.29	0.30	2.04	1756	DE[2]
t-$C_{18:2}$	0.89	0.15	0.30	1.41	927	DE[3]
t-$C_{18:2}$	1.15	0.14	0.72	1.74	236	DE[4]
t-$C_{18:2}$	1.44	0.15	1.03	2.04	593	DE[5]
t-$C_{18:2}$	1.16	0.19	0.76	1.65	201	FR
t-$C_{18:2}$	1.22	0.27	0.71	1.87	153	EU 12[6]
t-$C_{18:2}$	1.12	0.28	0.30	2.04	2110	EU 14
t11c15	0.30	0.14	0.01	0.67	1750	DE[2]
t11c15	0.19	0.05	0.01	0.34	921	DE[3]
t11c15	0.31	0.06	0.10	0.47	236	DE[4]
t11c15	0.46	0.08	0.25	0.67	593	DE[4]
t11c15	0.27	0.11	0.05	0.59	201	FR
t11c15	0.37	0.13	0.16	0.68	153	EU 12[6]
t11c15	0.30	0.13	0.01	0.68	2104	EU 14
t-total[7]	4.86	1.51	1.71	8.70	1756	DE[2]
t-total	3.65	0.57	1.71	5.59	927	DE[3]
t-total	5.06	0.52	3.55	6.44	236	DE[4]
t-total	6.66	0.78	4.49	8.70	593	DE[5]
t-total	4.87	1.08	2.79	8.03	201	FR
t-total	5.71	1.30	3.47	8.47	153	EU 12[6]
t-total	4.92	1.48	1.71	8.70	2110	EU 14
c9c12	1.24	0.12	0.45	1.76	1756	DE[2]
c9c12	1.24	0.12	0.45	1.76	2110	EU 14
c9c12c15	0.68	0.13	0.32	1.02	1756	DE[2]
c9c12c15	0.69	0.13	0.32	1.11	2110	EU 14

[1]Adapted from Precht and Molkentin (2000a). c9t11 = 18:2. t11 = 18:1. t11c15 = 18:2.

[2]Germany = All feeding periods.

[3]Germany = Barn feeding.

[4]Germany = Transition period.

[5]Germany = Pasture feeding.

[6]12 European countries without DK, FR.

[7]*trans* - C16:1, *trans* - C18:1, *trans* - C18:2.

the TAG are separated by molecular weight only, positional distributions of FA cannot be determined. Their results are shown in Table 54. As stated in footnote c in their paper, no specific regioisomers are implied, but since 4:0 and 6:0 are located almost completely at *sn*-position 3, and using the data in Table 5, the presence of several TAG is obvious. For example, *sn*-CLA-12:0-4:0 is one.

Alteration of the CLA Content by Processing

Considerable effort has been expended on the analysis of CLA in cheese in part to determine whether processing affects the contents. Ha et al. (1989) detected 9*c*, 11*t*-18:2, and other CLA in several cheeses. They noted that Cheese Whiz, a processed spread, contained much more CLA than the other cheeses and suggested that heat, aging, and protein as initiators of a free radical type oxidation of 18:2 may be the cause. Shantha et al. (1992) and Shantha and Decker (1993) confirmed that oxidation is involved in the production of CLA during the manufacture of processed cheese. Werner et al. (1992) had earlier noted that the total CLA concentration in Cheddar cheese was not affected by the starter cultures used, processing conditions, and aging period used. These factors did alter the distribution of the CLA isomers. They stated that CLA is a stable component. The results on cheddar were confirmed by data reported by Shantha et al. (1995) and Lin et al. (1999a). They suggested that although processing conditions had a minor influence on CLA contents, more information is needed to assess the effects of cheese components. The effects of pasteurization and other processing methods on milk are academic since pasteurization of most milk is required and other procedures must be used to produce the desired product.

Jiang et al. (1998) investigated 19 dairy starter cultures for their ability to convert 18:2 to CLA. Three strains of *Propionibacter* spp. produced extracellular CLA from free 18:2. The 9*c*, 11*t*-18:2 represented about 70% of the CLA formed. Most of the other cultures were inhibited by 18:2. Because the *Propionibacter* spp. are used in the manufacture of Swiss cheese, their metabolic activities could increase the CLA content somewhat. *Lactobacillus acidophilus*, one of the microorganisms used to make yogurt, was inhibited by 18:2.

Lin (2000) tested three cultures of *Lactobacillus* spp., two of *Lactococcus* spp., and one of *Streptococcus* spp. for the effects of sucrose, lactose, fructose, and NaCl added to a skim milk medium. This was done because many fermented milks are sweetened or salted in the Middle East. The CLA content in the

Table 53. Conjugated linoleic acid contents (wt%) of fatty acids in cheeses. Amounts are 80 to 90% 9*c*, 11*t*-18:2 unless noted.

Type	wt%-Amount		Range	Method
French[1]				
Type I. No treatment	0.6	0.01 SEM		GLC–HPLC
Type II. Mechanical	1.1	0.05 SEM		GLC–HPLC
Type III. Mechanical, heat	1.3	0.03 SEM		GLC–HPLC
United States				
Unidentified[2]	1.0		0.99-1.02	Ag–HPLC
Identified[3]		9*c*, 11*t*		
American processed	0.46	0.37		Combined
Cheddar, sharp	0.54	0.45		Combined
Cheddar, extra sharp	0.52	0.40		Combined
Colby	0.40	0.33		Combined
Cream cheese	0.71	0.64		Combined
Feta	0.49	0.40		Combined
Monterey jack	0.47	0.38		Combined
Mozzarella	0.47	0.37		Combined
Parmesan	0.30	0.31		Combined
Prepared cheese product	0.36	0.46		Combined
Processed, Cheddar	0.50	0.42		Combined
Canadian[4]				
Goat	0.27	0.02 SEM		GLC–100 m
Brie	0.38	0.05 SEM		GLC–100 m
Italian Parmesan	0.42	0.05 SEM		GLC–100 m
Imperial Cheddar	0.47	0.02 SEM		GLC–100 m
Cheez Whiz	0.47	0.07 SEM		GLC–100 m
German[5]				
Gouda	0.40			
Munster	0.62			
Emmentaler	1.16			
Blue cheese	0.55			
Gorgonzola	0.69			

[1]From Lavonniere et al. (1998). Type I = No physical treatment (includes Camembert). Type II = Some physical treatment (includes Tomme). Type II = Mechanical and heat treatment (includes Emmentaler).

[2]From Yurawecz et al. (1998). Amounts calculated by author.

[3]From Sehat et al. (1998a).

[4]From Ma et al. (1999). Selected cheeses.

[5]From Fritsche and Steinhart (1998). Selected cheeses.

central medium of *Lactobillus acidophilus* was the highest of all cultures tested. The NaCl-treated skim milk medium under aerobic conditions for 24-h incubations was most effective in maintaining a high level of CLA. However, all of the additives reduced the production of CLA by cell cultures except the *Lactococcus* spp. Lin et al. (1999b) had determined earlier that the yield of CLA in skim milk was dependent on the amounts of 18:2 added. This is not commercially feasible, since pure 18:2, which is required, is expensive, $25/g. The amount of 9*c*, 12*c*-18:2 in whole milk is low, 1.05% (Table 24). The quantity of free 18:2 would be much lower, dependent on the extent of lipolysis.

Garcia et al. (1998) increased the CLA content of anhydrous BO from 0.6 to 0.15 g/100 g of fat by interesterification with a CLA mixture, using the lipase from *Candida cylindracea*. Other lipases were not as effective. Solvents were not employed, as had been done by earlier investigators. Arcos et al. (1998) observed that an immobilized *Candida antarctica* lipase catalyzed the esterification of glycerol with CLA. About 95% of the original CLA was incorporated into a mixture of mono, -di-, and triesters after 7 h. The mixture could be used as a food-grade emulsifier. The procedure appears to be a relatively inexpensive method of increasing the CLA content of foods, which use emulsifiers. Recently, Garcia et al. (2000) observed that the immobilized lipase from *C. antarctica* increased the CLA content of the C46 to C54 TAG in anhydrous MF when incubated with a CLA mixture. This procedure may be commercially useful. In another study with lipases, Haas et al. (1999) employed strains of *Geotrichum candidum* that were highly selective for 9*c*, 11*t*-18:2. The FFA released by one of the lipases, contained 94% of this isomer. The 9*t*, 11*c*-18:2 was not hydrolyzed from the commercial mixture of CLA. Analogous results were obtained with esterification reactions. The lipases can be used to produce high purity 9*c*, 11*t*-18:2, and CLA fractions reduced in this isomer.

Table 54. Conjugated linoleic acid triacylglycerols (CLA-TAG) in milk fat.[1]

Peak number	Mass % of sample[2]	Identity	Typical ret. time (min)
1	Trace[3]	CLA, 6:0, 4:0[4]	3.7
2	Trace	CLA, 8:0, 4:0	4.9
3	0.02	CLA, 10:0, 4:0	6.8
4	0.03	CLA, 12:0, 4:0	9.8
5	0.09	CLA, 14:0, 4:0	14.7
6	0.08	CLA, 18:1[5], 4:0	20.6
7	0.19	CLA, 16:0, 4:0	22.2
8	0.07	CLA, 18:1, 6:0	28.4
9	0.09	CLA, 12:0, 10:0	30.2
10	0.02	CLA, 14:0, 8:0	31.7
11	0.05	CLA, 16:0, 6:0	33.0
12	0.03	CLA, 18:1, 8:0	38.1
13	0.05	CLA, 12:0, 12:0; CLA, 14:0, 10:0	39.4
14	0.05	CLA, 16:0, 8:0	40.3
15	0.03	CLA, 18:0, 6:0	41.8
16	0.04	CLA, 18:1, 10:0	48.8
17	0.12	CLA, 14:0, 12:0; CLA, 16:0, 10:0	50.9
18	0.06	CLA, 18:1, 12:0	59.7
19	0.05[2]	CLA, 18:1*t*, 12:0	60.7
20	0.15	CLA, 14:0, 14:0; CLA, 16:0, 12:0	61.9
21	0.22	CLA, 18:1, 14:0	70.0
22	0.06[2]	CLA, 18:1*t*, 14:0	71.3
23	0.26	CLA, 16:0, 14:0; CLA, 18:0, 12:0	72.4
24	0.11	CLA, 18:1, 18:1	77.4
25	0.04[2]	CLA, 18:1*t*, 18:1	78.5
26	0.28	CLA, 18:1, 16:0	79.5
27	0.09[2]	CLA, 18:1*t*, 16:0	80.8
28	0.30	CLA, 16:0, 16:0; CLA, 18:0, 14:0	82.0
29	0.08	CLA, 18:1, 18:0	88.4
30	0.03[6]	CLA, 18:1*t*, 18:0	89.6
31	0.15	CLA, 16:0, 18:0	90.9
32	0.03	CLA, 18:0, 18:0	99.4
Total	1.2% FAME		

[1]Adapted from Robinson and MacGibbon (2000).

[2]Calculated from 100 × (observed area/1.42×10^6) × (molecular weight of the CLA-TAG)/(total injection mass in ng).

[3]Indicates 0.01% or less.

[4]Fatty acid composition: No specific regioisomers are implied by the nomenclature, although it is well known that the short-chains 4:0 and 6:0 are almost exclusively at *sn*-position 3.

[5]18:1 is *cis*-9 oleic acid; vaccenic acid is indicated as 18:1*t*.

[6]An impure CLA-TAG peak. The concentration of the CLA-TAG listed is therefore overestimated when measured from a trace of the total milk fat. Argentation thin-layer chromatography indicated that there are unidentified CLA-TAG with coincident retention times underlying the CLA-TAG described.

This may be the best method to use for the production of relatively large quantities of 9*c* 11*t*-18:2.

Romero K et al. (2000) utilized a supercritical fluid CO_2 processing system to separate anhydrous MF into five fractions. One of the fractions, S1, 8.8% of the contained (mg/g): 110 C and 7.8 CLA. The original MF had 273 and 4.3. The saturated FA content was decreased and the unsaturated FA increased. This technique may also be commercially applicable. See Rizvi and Bhaskar (1999) for more information. Bauman et al. (2000a) produced butter rich in CLA by feeding cows a low forage diet supplemented with sunflower oil and selective collection of milk. Kim and Liu (1999) increased the CLA content of milk FA from 0.5 to 1.3% with urea complex crystallization. Urea forms complexes with saturated FA and they can be easily removed by crystallization.

Kim et al. (2000) improved the oxidative stability of CLA by microencapsulation in cyclodextrins. Oxidation was measured by determination of peroxide values. Oxidation was prevented completely by encapsulation in α-cyclodextrin. beta-Cyclodextrin was also effective and is lower in cost than the other cyclodextrins. O'Shea (2000) found that dry fractionation of MF produced a soft fraction, which contained CLA, 2.22 g/100 g of fat compared with 1.36 in the control MF. The process may not be industrially practical. However, they did find that four butters contained 1.26 to 1.42% CLA.

Alteration of CLA Content by Diet

The CLA content of milk and dairy products depends on ruminal production of CLA and 11*t*-18:1 and the activity of Δ^9 desaturase in tissue. The substantial variations of CLA contents in MF between herds observed earlier suggested that diet has a major influence. Bauman et al. (2000b) compiled a list in Table 55 of the many factors known to affect the CLA content of milk. Relevant references are in the Table. I've added six newer references as shown and more information is available in Tables 40, 44, and 48.

Bauman et al. (2000b) divided the factors into three groups: 1) diets that provide lipid substrates for the biosynthesis of CLA or 11*t*-18:1 in the rumen, 2) factors that alter the rumen environment, and 3) combinations of 2) and 3). More information is available in the reference above; however, I will discuss the topic of CLA supplements further. When evaluating the results in these papers readers should know that almost none of the CLA supplements employed were pure 9*c*, 11*t*- or 10*t*, 12*c*-18:2*s*. Pure isomers are available in the United States from Matreya, Inc. (Pleasant Gap, PA) and Nu-Chek Prep, Inc. (Elysian, MN). However, the large quantities needed would be prohibitively expensive. Most of the CLA supplements used contained relatively large quantities of 8, *ct*/*tc*-;10, *ct*/*ct* and 11*c*,13*t*-18.2 (Sehat et al., 1998b).

A portion of the CLA in milk is produced in the rumen by isomerization of 9*c*, 12*c*-18:2 to 9*c*, 11*t*. The rest results from desaturation of 11*t*-18:1 in the mammary gland and adipose tissue (Griinari and Bauman, 1999) as described below. Abomasal infusion of CLA increased the contents in milk and body fat. However, the fat contents and yields were markedly reduced

Table 55. Summary of dietary factors that affect concentrations of conjugated linoleic acid (CLA) in milk fat.[1]

Dietary factor	Content of CLA in milk fat	References[2]
a. Lipid substrate		
Unsaturated vs. saturated fat	Increased by addition of unsaturated fat	1,2,3
Plant oils		
Type of plant oil	Increased with oils high in unsaturated fatty acids	4,5,6,7,8,9,10,11
Level of plant oil	Dose-dependent increase	3,4,10,11
Ca salts of plant oils	Increased	6
High-oil plant seeds		
Raw seeds	No effect	6,10
Processed seeds	Increased	6,12,13,14
High-oil corn grain and silage	Minimal effect	6,10
Animal fat by-products	Minimal effect	6
b. Modifiers of rumen environment		
Forage:concentration ratio	Variable effect	15,16,17,18
Nonstructural carbohydrate level	Minor effect	19,20
Restricted feeding	Variable effect	3,21,22
Fish oil/fish meal	Increased	3,6,17, 23,24
Marine algae	Increased	25
Ionophores	Variable effect	17,19,26
Dietary buffers	Little effect with sufficient fiber	3
Copper concentration	Low Cu increased	1,4,19
c. Combination of a and b		
Pasture	Higher than on conserved forages	22,26,27,28-1,29,30
Growth stage of forage	Increased with less mature forage	19,27
CLA supplement	Dose-dependent increase	16,18,30,31,32,33,34,35,36

[1]From Bauman et al. (2000b). Adapted by Bauman et al. from Griinari and Bauman (1999).

[2]References from Bauman et al. (2000b). 1. Morales et al. (2000a); 2. Griinari et al. (1998); 3. Jones et al. (2000); 4. Tesfa et al. (1991); 5. Dhiman et al. (2000); 6. Chouinard et al. (2001); 7. Kelly et al. (1998a); 8. Offer et al. (1999); 9. Chilliard et al. (2000); 10. Dhiman et al. (1999a); 11. McGuire et al. (1996); 12. Stanton et al. (1997); 13. Dhiman et al. (1999b); 14. Lawless et al. (1998); 15. Griinari et al. (1998); 16. Piperova et al. (2000); 17. Jiang et al. (1996); 18. Loor and Herbein (1998); 19. Morales et al. (2000b) 20. Solomon et al. (2000); 21. Fritsche and Steinhart (1998); 22. Timmen and Patton (1989); 23. Chilliard et al. (1999); 24. Donovan et al. (2000); 25. Franklin et al. (1999); 26. Sauer et al. (1998); 27.Zegarska et al. (1996); 28. Precht and Molkentin (1997a); 29. Jahreis et al. (1996); 30. Kelly et al. (1998b); 31. Chouinard et al. (1999a); 32. Chouinard et al. (1999b); 33. Giesy et al. (1999b); 34. Gulati et al. (2000); 35. Baumgard et al. (2000); 36. Griinari et al. (2000). Author added (1,2,3,6,8,9,19,20,24,35,36).

(Loor and Herbein, 1998; Chouinard et al., 1999a) Supplements can be protected when fed as calcium salts (Chouinard et al., 1998b, 2001; Giesy et al., 1999), encapsulated in denatured protein (Gulati et al., 2000) and as oleamides (Table 44). We know that a diet high in grain and low in forage depresses the content of fat in milk (Piperova et al., 2000). See Tables 45 to 49. As I discussed earlier, Piperova et al. (2000) decreased de novo FA synthesis in the mammary gland. This mode apparently involves changes in the relative abundance of mammary acetyl-CoA carboxylase and of FA synthase. The CLA isomer, 10*t*, 12*c*-18:2 may affect fat depression. The 9*c*, 11*t*-18:2 produced was not affected by the diet but 10*t*, 12*c*-18:2 increased from 0.15 to 0.5. The content of 11*t*-18:1, a precursor of 9*c*, 11*t*-18:2 in the mammary gland, was also increased. Baumgard et al. (2000) confirmed with abomasal infusions that this isomer is responsible for the inhibition of MF synthesis. The mechanism is unknown, but their results suggest inhibition of de novo (10:0 to 14:0) synthesis and the desaturase system in the gland. Recently, Griinari et al. (2000) found that 64% of the CLA in MF is synthesized endogenously from 11*t*-18:1 by Δ^9 desaturase. To summarize, the mammary gland and adipose tissue in cows have substantial desaturase activity. In the tissues, the desaturase also converts 18:0 to 9*c*-18:1 (Bauman et al., 2000b). In the rumen, high dietary linoleic acid contents are converted to a high yield of 9*c*, 11*t*-18:2 (Dhiman et al., 1999a). Incidentally, Bauman and Griinari (2000) have summarized information about the low-MF syndrome. Research on the causes of the syndrome lead to the information we have about production of 9*c*, 11*t*, and 10*t*, 12*c*-18:2.

The effects of CLA on lactation in ruminants seem to be known, but the role of what appears to a complex negative feedback loop involving 10*t*, 12*c*-18:2 in the mammary gland is not. Bovine milk is intended for the nutrition of calves, so what is the function of this loop? Finally, are CLA required or needed by the calf? If so, why? As noted above, de novo synthesis of FA and the desaturase system in the gland are involved.

CLA Consumption by Humans

CLA have many potential benefits for humans. How much should be consumed to achieve these effects?

This question has been addressed by McGuire et al. (1999). Most of the CLA is found in foods derived from ruminants; milk, dairy products, beef, and lamb. Almost all of the CLA in these foods is 9*c*, 11*t*-18:2 or RA. Although RA is formed in humans, probably by desaturation of 11*t*-18:1, the amount is small (Adlof et al., 2000). However, the amount of RA in human adipose tissue, 0.50% of total FA, was positively correlated (r = 0.42) with milk intake (Jiang et al., 1999). The amount in serum was 0.25%. The quantities in both tissues were strongly correlated with 14:1. CLA was shown to inhibit earlier carcinogenesis in animals (Ip et al., 1991, 1994) and more recently in rats (Ip et al., 1999). This paper contains definitive evidence that 9*c*, 11*t*-18:2 is an effective anticarcinogen, and I will describe their findings in greater detail.

Ip et al. (1999) noted that all of the in vivo research with CLA as a cancer preventative agent had been done with commercial preparations of FFA containing in greatest quantities, 9*c*, 11*t*; 10*t*, 12*c*, and 11*c*, 13*t* isomers. CLA in food is mostly (80 to 90%) the 9*c*, 11*t* isomer in TAG. They prepared butter with a high CLA content, fed this, the control butter, and two commercial preparations of CLA to young rats. They evaluated the effects of CLA on the development of mammary gland cancer induced by methylnitrosourea. The CLA will inhibit mammary chemical carcinogenesis. Pubescent rats were fed diets that contained 0.1 or 0.8 g/100 g of total CLA in the diet as triglycerides (**TG**) or the FFA preparations, 0.7 g/100 g, in the control butter. Feeding butter fat CLA reduced the terminal end buds of the mammary alveoli and inhibited mammary tumor yield by 53% ($P < 0.05$). The FFA mixtures caused the same magnitude of changes. The rats fed CLA enriched butter accumulated more total CLA in mammary and other tissues than those given the CLA as FFA. The authors suggested that the greater contents of 11*t*-18:1 in butter serving as a precursor for 9*c*, 11*t* by desaturation may have been responsible. The presence of this precursor of CLA is another reason for the consumption of MF as a possible procedure for the reduction or prevention of breast cancer.

Because CLA have been proven to inhibit carcinogenesis in animals, it is important to derive the dosages used in these studies to amounts that could be used for humans.

The amounts consumed by humans have been estimated to be (mg/d): men in the United States, aged 20 to 39 yr, 120; and women, 75 (McGuire et al., 1999). Ip et al. (1994) calculated that 0.1% dietary CLA, the amount that prevented breast cancer in rats, would be equivalent to a daily intake of 3.5 g for humans. Because this quantity is apparently based on trials in which commercial mixtures of CLA containing 42.5% 9*c*/*t* 11*t*/*c*-18:2 (Ip et al., 1991), the anticarcinogenic isomer, the 3.5 g can be reduced to 1.48 g. Consumption of CLA earlier by girls may help inhibit breast cancer. However, there are few data from trials with humans and, as seen, the dietary intake in humans is low (Banni et al., 1999; McGuire et al., 1999). Jiang et al. (1999) observed that the consumption of 25 g of MF containing 0.16 g of 9*c*,11*t*-18:2 per day by Swedish men produced 0.16 g of the FA in their adipose tissue. The consumption of MF was significantly related, r = 0.42, to the amount in their adipose tissue, but the relative anticarcinogenicity is unknown. Knekt and Järvinen (1999) surveyed the risk of breast cancer and intake of dairy products. The results were contradictory, with low, high, and no associations. It was almost impossible to eliminate confounding influences.

MILK LIPIDS AND HUMAN HEALTH

The Handbook of Dairy Foods and Nutrition (Miller et al., 1999) contains information about ML and cardiovascular or CVD. Lefevre (2000) also discussed the relation between MF and CVD. They emphasize that results, indicating risks resulting from consuming MF in studies on populations have been applied to individuals. Those who have family histories or other indicators of CVD should regulate their diets, others may not need to restrict their intake of the atherogenic fats. All should seek the advice of their physician and probably a dietitian. We must remember that consumers eat many different fats and oils. Pentadecanoic acid in serum has been found to be a marker for intake of MF by elderly men in Sweden (Snedmen et al., 1999). This could be more helpful than dietary recalls in assessing food consumption. Also, a low intake of n-3 polyunsaturated FA, 300 mg of 22:5 n-3 and 22:6 n-3 in 500 ml of special milk preparation, reduced plasma TG and increased HDL C in healthy subjects (Visioli et al., 2000). Further information was not given about the preparation, but consumption of fish would provide these acids. Svahn et al. (2000) fed milk containing low fat or 50 and 100% vegetable fat to children 12 to 18 mo of age. The children receiving the altered milks had less saturated FA and more PUFA in their plasma than those fed MF only. Another milk in which the hypercholesterolemic FA, 12:0,14:0, and 16:0 were reduced from 52.98 to 34.66% of total FA by feeding the cows a diet lower in soybean meal and containing crushed rape seeds (Tholstrup et al., 1998) was employed. Feeding the modified milk did not lower the LDL C of the subjects, possibly because the contents of the FA were still high and the trans-FA content was increased form 1.1 to 6.4%. The CLA contents were also increased. However, in general,

adherence to recommended and diverse diets is the best choice. The positive benefits of milk consumption by healthy older adults aged 55 to 85 yr was evaluated (Barr et al., 2000). The subjects drank three cups of skim or 1% milk per day. The authors concluded that older adults can increase their intake of milk, and improve their nutrient intakes without altering total and LDL levels and the ratios of total C to HDL were unchanged. The TAG levels increased, but were within the normal range. The quality of life remained unchanged throughout the study. Milk can be consumed by older adults with beneficial results and without increasing CVD indicators. I recommend the book by Gurr (1999) for a balanced discussion of the role of lipids in nutrition and the paper by Maijala (2000) for an assessment of cow milk relative to human development and well being. Finally, I recommend that readers check the publication on Dairy Foods and Cardiovascular Health by various authors. (IDF, 2000b). Many relevant topics are discussed including the role of homocysteine in atherogenesis.

FATTY ACIDS IN BOVINE MILK

The FA that have been identified in milk through December 2000 are listed in Table 56. The identities were determined by GLC-MS and appropriate methods. These are the corrected numbers from (Jensen, 2000). The Table 11 in this paper contains an error in the column of saturates, Normal, 14:2 instead of 2-28. This error also occurs in Table 20 (Jensen and Newberg, 1995). Another error, mentioned in footnote

Table 56. Fatty acid composition of bovine milk as of December 2000.[1]

Type	No	Identity	References
Saturates			
Normal	27	2-28,even; 3-28 odd	Jensen and Clark (1988); Strocchi and Holman (1971); Kurtz (1974); Iverson et al. (1965); Iverson and Sheppard (1986)
Monobranched	56	Me br:4-26, Et br.5	Jensen and Clark (1988); Strocchi and Holman (1971); Iverson et al. (1965); Iverson and Sheppard (1986); Ha and Lindsay (1990); Massart-Leen et al. (1981); Attaie et al. (1993)
Multibranched	17	16-28, three or more positional isomers	Same as monobranched
Monoenes			
cis	65	10-26. Positional isomers of 14.1, 16.1 -25.1	Same as Normal plus Precht et al. (1999); Hay and Morrison (1970); Precht et al. (1999)
trans	64	10-26. Positional isomers of 14.1, 16.1 -25.1	Jensen and Clark (1988); Strocchi and Holman (1971); Precht et al. (1999); Precht et al. (2000b); Destaillats et al. (2000); Iverson et al. (l965); Hay and Morrison (1970)
Dienes	50	14-26, evens; *cis,cis,trans,trans* or *trans,cis; cis, trans*; con- and nonconjugated	Jensen and Clark (1988); Strocchi and Holman (1971); Precht et al. (1999); Kramer (1999)
Polyenes			
Tri	12	18,20,22, and 24. Geometric, positional, con- and nonconjugated isomers	Strocchi and Holman (1971); Precht et al. (1999); Offer et al. (1999); Iverson et al. (1965)
Tetra-	5	18,20,22 positional isomers	Same as above
Penta-	2	20, 22	Strocchi and Holman (1971); Offer et al. (1999); Iverson et al. (1965)
Hexa-	1	22	Offer et al. (1999)
Keto (oxo)			
Saturated	45		Weihrauch et al. (1974); Brechany and Christie (1992)
Unsaturated	19		Weihrauch (1974); Brechany and Christie (1994)
Hydroxy			
2-position	27		Chance et al. (1998) Weihrauch et al. (1974); Morrison and Hay (1990); Kurtz (1974)
4 and 5 position	24	R isomers	
4 and 5 position	5	S isomers	
Other positions	5[2]		
Cyclic			
Hexyl	1	11;terminal cyclohexyl	Schoght and Havercamp Begemann (1965); Guth and Grosch (1992)
Furan	9		
Total	416		

[1]Corrected from Jensen (2000), Patton and Jensen, (1975).

[2]Number given as 60 in Jensen (2000). Change explained in text.

2, Table 56 and now deleted, was the 60 hydroxy FA listed in Table 11 (Jensen, 2000). The amount was apparently given in a paper presented at the 1972 AOCS meeting in Ottawa, but was never published except as an abstract (Schwartz, 1972) and an in-house USDA paper, which did not contain data on FA (Schwartz, 1970). The other relevant references are listed in Table 56.

Our first publication in which the numbers of FA were listed as 437 was Patton and Jensen (1975). The number should have been about 370 because of the erroneous inclusion of 60 hydroxy acids described above. There are certainly more FA in milk than those listed in Table 56. For example, there are probably more tri-and tetraenes yet to be identified.

Related Compounds

There are many others, alcohols, aldehydes, ethers, and conjugates that occur in MF. Information about these is available in Jensen and Clark (1988) and Kurtz (1974). Schwartz (personal correspondence, 2001) identified nine saturated and separated sixteen unsaturated glyceryl ethers.

Flavor Compounds

There are a large number of flavor compounds, some desirable and some not. Some are secreted in milk, others are the result of processing and storage of milk and its products. The components produced by the deterioration of MF have been discussed by Weihrauch (1988).

Fatty Acids and the Flavor of Dairy Products

The free volatile short-chain FA, n and branched, contribute to the characteristic flavor of cheeses (Ha and Lindsay, 1990). I found these references which contain data about cheese flavors: surface mold ripened types (Molimard and Spinnler, 1996), Brevibacter linens, smear surface ripened cheeses (Rattray and Fox, 1999), soft cheeses Sable and Cottenceau, 1999), and Cheddar cheese (Fox and MacSweeney, 1998).

SUMMARY

I have presented what I believe are the most recent reliable data on the lipids in bovine milk. While much has been done, research on the composition of market milk and dairy products in the United States is and has been almost nonexistent. I have mentioned many areas that should be investigated. Probably the most important is a repeat of the FA analyses on many milk samples as done by Palmquist et al. (1993) and Barbano (1990), but using the Ag-TLC/GLC method (Wolff et al., 1998). We do not have, as of March 2001, detailed data about the kinds and amounts of FA in the milks and dairy products we drink and eat in the United States.

ACKNOWLEDGMENTS

I thank Ross Products Division, Abbott Laboratories; Wyeth-Ayerst International Inc., and Mead Johnson Research Center for funds to pay for typing and purchase reprints. I am grateful to Martina M. Long and to my wife, Helene C. Jensen for typing portions of the manuscript. I thank Sheila Andrew, PhD, Extension Specialist in the Department of Animal Sciences, Univ of Conn., for reviewing relevant sections of the Review.

REFERENCES

Ackman, R. G. 2000. The dichotomy of the *trans* ethylenic bond in our foods. 2000. Eur. J. Lipid Sci. Technol. 102:630–632.

Ackman, R. G., and E. J. Macpherson. 1994. Coincidence of *cis* and *trans*-monoethylenic fatty acids simplifies the open-tubular gas-liquid chromatography of butyl esters of butter fatty acids. Food Chem. 50:45–52.

Adlof, R. O., S. Duval, and E. A. Emken. 2000. Biosynthesis of conjugated linoleic acid in humans. Lipids 35:131–135.

Adlof, R., and T. Lamm. 1998. Fractionation of *cis* and *trans*-oleic, linoleic, and conjugated linoleic fatty acid methyl esters by silver-ion high-performance liquid chromatography. J. Chromatogr. A 799:329–332.

Ahrne, L., L. Bjorck, T. Razniekiewicz, and O. Claesson. 1979. Glyceryl ethers in colostrum and milk from cow, pig, and sheep. J. Dairy Sci. 63:741–745.

Aigster, A., C. Sims, R. Schmidt, and S. F. O'Keefe. 2000. Comparison of cheeses made from milk having normal and high oleic fatty acid compositions. J. Food Sci. 65:920–924.

Akesson, B., B.-M. Johansson, M. Svensson, and P.-A. Öckerman. 1981. Content of *trans*-octadecenoic acid in vegetarian and normal diets in Sweden, analyzed by the duplicate portion technique. Am. J. Clin. Nutr. 34:2517–2520.

Allinson, D. B., S. K. Egan, L. M. Barraj, C. Caughman, M. Infante, and J. T. Heimbach. 1999. Estimated intakes of *trans* fatty and other fatty acid in the US population. J. Am. Diet. Assoc. 99:166–174.

Alonso, L., J. Fontecha, L. Lozada, and M. Juarez. 1997. Determination of mixtures in vegetable oils and milk fat by analysis of sterol fraction by gas chromatography. J. Am. Oil Chem. Soc. 74:131–135.

Alonso, L., L. Lozada, J. Fontecha, and M. Juarez. 1995. Determination of cholesterol in milkfat by gas chromatography with direct injection and sample saponification. Chromatographia 41:23–28.

Angers, P., E. Tousignant, A. Boudreau, and J. Arul. 1998. Regiospecific analysis of fractions of bovine milk fat triacylglycerols with the same partition number. Lipids 33:1195–1201.

Annison, E. F., and W. L. Bryden. 1998. Perspectives on ruminant nutrition and metabolism. I. Metabolism in the rumen. Nutr. Res. Rev. 11:173–198.

Annison, E. F., and W. L. Bryden. 1999. Perspectives on ruminant nutrition and metabolism. II. Metabolism in ruminant tissues. Nutr. Res. Rev. 12:147–177.

Appelqvist, L.-A. 1996. Oxidized sterols. Bull. IDF 315, Brussels, Belgium.

Arcos, J. A., C. Otero, and C. G. Hill, Jr. 1998. Rapid enzymatic production of acylglycerols from conjugated linoleic acid and glycerol in a solvent free system. Biotechnol. Lett. 20:617–621.

Ashes, J. R., S. K. Gulati, and T. W. Scott. 1997. Potential to alter the content and composition of milk fat through nutrition. J. Dairy Sci. 80:2204–2212.

Association of Official Analytical Chemists. 1995. AOAC official method 989. 05, Modified Mojonnier. Pages 18–19 in Official Methods of Analysis. 16th ed, Vol II. P. Cunnif, ed. AOAC Intl., Arlington, VA.

Atal, S., M. J. Zarnowski, S. W. Cushman, and J. Sampugna. 1994. Comparison of body weight and adipose tissue in male C57BI/6J mice fed diets with and without *trans* fatty acids. Lipids 29:319–325.

Attaie, R., R. L. Richter, and A. H. Reine. 1993. Low molecular weight branched chain and n-chain fatty acids in caprine and bovine colostrum. J. Dairy Sci. 76:62–69.

Auldist, M. J., B. J. Walsh, and N. A. Thomson. 1998. Seasonal and lactational influences on bovine milk composition in New Zealand. J. Dairy Res. 65:401–411.

Avila, C. D., E. J. De Peters, H. Perez-Mont, S. J. Taylor, and R. A. Zinn. 2000. Influences of saturation ratio of supplemental dietary fat on digestion and milk yield in dairy cows. J. Dairy Sci. 83:1505–1519.

Bachman, K. C., J-H. Lin, and C. J. Wilcox. 1976. Sensitive colorimetric determination of cholesterol in dairy products. J. AOAC 59:1146–1149.

Banni, S., E. Angioni, G. Carta, V. Casu, M. Driaha, M. A. Dessi, L. Lucchi, M. P. Melis, A. Rosa, S. Vargiolu, and F. D. Corongiu. 1999. Influence of dietary conjugated linoleic acid on lipid metabolism in relation to its anticarcinogenic activity. Pages 307–318 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds., Am. Oil Chem. Soc. Press, Champaign, IL.

Banni, S., and J. C. Martin. 1998. Conjugated linoleic acid and metabolites. Pages 261–302 in *Trans* Fatty Acids in Human Nutrition. J. L. Sébédio, and W. W. Christie, eds. Oily Press, Dundee, U. K.

Barbano, D. M. 1990. Seasonal and regional variation in milk composition in the U. S. Proc. Cornell Nutr. Conf. Feed Manuf., Cornell Univ., Ithaca, NY.

Barr, S. I., D. A. McCarron, R. B. Heaney, B. Dawson-Hughes, S. L. Berga, J. S. Stern, and S. Oparil. 2000. Effects of increased consumption of fluid milk on energy and nutrient intake, body weight, and cardiovascular risk factors in healthy older adults. J. Am. Diet. Assoc. 100:810–817.

Barrefors, P., L.-A. Granelli, and L. Björck. 1995. Chemical characterization of raw milk samples with and without oxidative off-flavour. J. Dairy Sci. 78:2691–2699.

Battelli, G., and L. Pellegrino. 1994. Detection of non-dairy fat in cheese by gas chromatography of triglycerides. Ital. J. Food Sci. 4:407–419.

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebo, and D. E. Bauman. 2000. Identification of the conjugated linoleic isomer that inhibits milk fat synthesis. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278:R179–R184.

Bauman, D. E. 1999. Bovine somatotropin and lactation: From basic science to commercial application. Domest. Anim. Endocrinol. 17:101–116.

Bauman, D. E., D. M. Barbano, D. A. Dwyer, and J. M. Griinari. 2000a. Technical note: Production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. J. Dairy Sci. 83:2422–2425.

Bauman, D. E., L. H. Baumgard, B. A. Corl, and J. M. Griinari. 2000b. Biosynthesis of conjugated linoleic acid in ruminants. Proc. Am. Soc. Anim. Sci., 1999. Available at: http://www. asas.org/jas/symposia/proceedings/0937.pdf.

Bauman, D. E., and W. B. Currie. 1980. Partitioning of nutrients during pregnancy and lactation: A review of mechanisms including homeostasis and homeorhesis. J. Dairy Sci. 63:1514-1529.

Bauman, D. E., R. W. Everett, W. H. Weiland, and R. J. Collier. 1999. Production responses to bovine somatotropin in Northeast dairy herds. J. Dairy Sci. 82:2564–2573.

Bauman, D. E., and J. M. Griinari. 2000. Regulation and nutritional manipulation of milk fat. Low-fat milk syndrome. Pages 209–216 in Biology of the Mammary Gland. J. A. Mol and R. A. Class, eds. Academic/Plenum Publishers, New York.

Bayourthe, C., F. Enjalbert, and R. Moncoulon. 2000. Effects of different forms of canola oil fatty acids plus canola meal on milk composition and physical properties of butter. J. Dairy Sci. 83:690–696.

Beam, T. A., T. C. Jenkins, P. J. Moate, R. A. Kohn, and D. L. Palmquist. 2000. Effects of amount and source of fat on the rates of lipolysis and biohydrogenation in ruminal contents. J. Dairy Sci. 83:2564–2573.

Belury, M. A., and J. P. Vanden Heuvel. 1999. Modulation of diabetes by conjugated linoleic acid. Pages 404–411 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.

Bitman, J., and D. L. Wood. 1990. Changes in milk phospholipids during lactation. J. Dairy Sci. 73:1208–1216.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917.

Bobe, G., D. G. Beitz, A. E. Freeman, and G. J. Lindberg. 1999. Associations among individual proteins and fatty acids in bovine milk as determined by correlation and factor analysis. J. Dairy Res. 66:523–528.

Bonafede, D. M., L. J. Macala, M. Constantine-Paton, and R. K. Yu. 1989. Isolation and characterization of ganglioside 9-O-acetyl-GD3 from bovine buttermilk. Lipids 24:680–684.

Boyd, L. C., N. C. Drye, and A. P. Hansen. 1999. Isolation and characterization of whey phospholipids. J. Dairy Sci. 82:2550–2557.

Brechany, E. Y., and W. W. Christie 1992. Identification of the saturated oxo fatty acids in cheese. J. Dairy Res. 59:57–64.

Brechany, E. Y., and W. W. Christie. 1994. Identification of the unsaturated oxo fatty acids in cheese. J. Dairy Res. 62:111–115.

Breitschuh, B., and E. J. Windhab. 1996. Direct measurement of thermal fat crystal properties for milk-fat fractionation. J. Am. Oil Chem. Soc. 73:1603–1610.

Bremmer, D. R., L. D. Ruppert, J. H. Clark, and J. K. Drackley. 1998. Effects of chain length and unsaturation of fatty acid mixtures infused into the abomasum of lactating dairy cows. J. Dairy Sci. 81:176–188.

Bushway, A. H., and T. W. Keenan. 1978. Composition and synthesis of three higher ganglioside homologs in bovine mammary tissue. Lipids 13:59–65.

Casirighi, E., M. Lucisano, C. Pompei, and C. Dellea. 1994. Cholesterol determination in butter by high performance chromatography. Milchwissenschaft 49:194–196.

Chalupa, W., B. Vecchiarelli, D. T. Galligan, J. D. Ferguson, L. S. Baird, R. W. Hemken, J. R. Harmon, C. G. Soderholm, D. E. Otterby, R. J. Annexstad, J. G. Linn, W. P. Hansen, F. R. Ehle, D. L. Palmquist, and R. G. Eggert. 1998. Responses of dairy cows supplemented with somatotropin during weeks 5 through 25 of lactation. J. Dairy Sci. 79:800–812.

Chance, D. L., K. O. Gerhardt, and T. P. Mawhinney. 1998. Gas-liquid chromatography-mass spectrometry of hydroxy fatty acid as their methyl esters *tert*-butyl dimethyl silyl ethers. 1998. J. Chromatogr. A 793:91–98.

Chen, Z. -Y., G. Pelletier, R. Hollywood, and W. M. N. Ratnayake. 1995. *Trans* fatty acid isomers in Canadian human milk. Lipids 30:15–21.

Chilliard, Y., J. M. Chardigny, J. Chabrot, A. Ollier, J. L. Sebedio, and M. Doreau. 1999. Effects of ruminal or postruminal fish oil supply on conjugated linoleic acid (CLA) content of cow milk fat. Proc. Nutr. Soc. 58:70A. (Abstr.)

Chilliard, Y., A. Ferlay, R. M. Mansbridge, and M. Doreau. 2000. Ruminant milk plasticity: Nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. Ann. Zootech. 49:181–205.

Chouinard, P. Y., L. Corneau, D. M. Barbano, L. E. Metzger, and D. E. Bauman. 1999a. Conjugated linoleic acids alter milk fatty acid

composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129:1579–1584.
Chouinard, P. Y., L. Corneau, W. P. Butler, Y. Chilliard, J. K. Drackley, and D. E. Bauman. 2001. Effect of dietary lipid source on conjugated linoleic acid concentrations in milk fat. J. Dairy Sci. 84:680–690.
Chouinard, P. Y., L. Corneu, M. L. Kelly, J. M. Griinari, and D. E. Bauman. 1998a. Effect of dietary manipulation on milk conjugated linoleic acid concentrations. J. Dairy Sci. 81(Suppl. 1):233. (Abstr.)
Chouinard, P. Y., L. Corneau, A. Saebo, and D. E. Bauman. 1999b. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci. 82:2737–2745.
Chouinard, P. Y., V. Girard, and G. J. Brisson. 1997. Lactational response of cows to different concentrations of calcium salts of canola oil fatty acids with or without bicarbonates. J. Dairy Sci. 80:1185–1193.
Chouinard, P. Y., V. Girard, and G. J. Brisson. 1998b. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with varying unsaturation. J. Dairy Sci. 81:471–481.
Christensen, R. A., J. H. Clark, J. K. Drackley, and S. A. Blum. 1998. Fatty acid flow to the duodenum and in milk from cows fed diets that contained fat and nicotinic acid. J. Dairy Sci. 81:1078–1088.
Christie, W. W. 1995. Composition and structure of milk lipids. Pages 1–36 in Advanced Dairy Chemistry. 2: Lipids. 2nd ed. P. F. Fox, ed. Chapman and Hall, New York, NY.
Christie, W. W., G. Dobson, and F. G. Gunstone. 1997. Isomers in commercial samples of conjugated linoleic acid. J. Am. Oil Chem. Soc. 74:1231.
Christie, W. W., R. C. Noble, and C. Davies. 1987. Phospholipids in milk and dairy products. J. Soc. Dairy Technol. 40:10–12.
Christie, W. W., J. L. Sébédio, and P. Juaneda. 2001. A practical guide to the analysis of conjugated linoleic acid. INFORM 12:149–152.
CLA. 1999. http://www.wisc.edu/fri/clarets.htm.
Craig-Schmidt, M. C. 1998. Worldwide consumption of *trans* fatty acids. Pages 59–113 in *trans* Fatty Acids in Human Nutrition. J. L. Sebedio and W. W. Christie, eds. The Oily Press, Dundee, Scotland.
Creamer, L. K., and A. H. K. MacGibbon. 1996. Some recent advances in the basic chemistry of milk proteins and lipids. Int. Dairy J. 6:539–568.
Crocker, L. M., E. J. DePeters, J. G. Fadel, H. Perez-Monti, S. J. Taylor, J. A. Wycoff, and R. A. Zinn. 1998. Influence of processed corn grain in diets of dairy cows on digestion of nutrients and milk composition. J. Dairy Sci. 81:2394–2407.
Dalgleish, D. G., S. M. Tosh, and S. West. 1996. Beyond homogenization: the formation of very small emulsion droplets during the processing of milk by a microfluidizer. Neth. Milk Dairy J. 50:135–148.
Demeyer, D., and M. Doreau. 1999. Targets and procedures for altering ruminant meat and milk lipids. Proc. Nutr. Soc. 59:593–607.
DePeters, E. J., J. F. Medrano, and B. A. Reed. 1995. Fatty acid composition of milk fat from three breeds of dairy cattle. Can. J. Anim. Sci. 75:267–269.
Destaillats, F., R. L. Wolff, D. Precht, and J. Molkentin. 2000. Study of individual *trans* and *cis*-16:1 isomers in cow, goat, and ewe cheese fats by gas-liquid chromatography with emphasis on the *trans*-Δ3 isomer. Lipids 36:1027–1032.
Dhiman, T. R., G. R. Anand, L. D. Satter, and M. W. Pariza. 1999a. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci. 82:2146–2156.
Dhiman, T. R., E. D. Helmink, D. J. McMahon, R. L. Fite, and M. W. Pariza. 1999b. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82:412–419.
Dhiman, T. R., L. D. Satter, M. W. Pariza, M. P. Galli, K. Albright, and M. X. Tolosa. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83:1016–1027.
Dirk, W., and E. H. Reimerdes. 1988. Analysis of gangliosides with particular reference to milk. Z. Unters Forsch. 186:99–107.
Dobson, G. 1998. Identification of conjugated fatty acids by gas chromatography-mass spectrometry of 4-methyl-1,2,4-triazoline-3,5-diene adducts. J. Am. Oil Chem. Soc. 75:137–142.
Donovan, D. C., D. J. Schingoethe, R. J. Baer, J. Ryall, A. R. Hipppen, and S. T. Franklin. 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci. 83:2620–2628.
Doreau, M., D. T. Demeyer, and C. J. Van Nevel. 1997. Transformation and effects of unsaturated fatty acids in the rumen. Consequences on milkfat secretion. Pages 73–92 in Milk Composition, Production and Technology. R. A. S. Welch, D. J. W. Burns, S. R. Davis A. I. Popay, and C. G. Prosser, eds. CAB International, New York.
Duffield, T. F., and R. N. Bagg. 2000. Use of ionophores in lactating dairy cattle: A review. Can. Vet. J. 41:388–394.
Enig, M. G., L. A. Pallansch, J. Sampugna, and M. Keeney. 1983. Fatty acid composition of the fat in selected food items with emphasis on *trans* components. J. Am. Oil Chem. Soc. 60:1788–1795.
Enjalbert, F., M. C. Nicot, C. Bayourthe, and R. Moncolon. 2000. Effects of duodenal infusions of palmitic, stearic, or oleic acids on milk composition and physical properties of butter. J. Dairy Sci. 83:1428–1433.
Enjalbert, F., M. C. Nicot, C. Bayourthe, M. Vernay, and R. Moncoulon. 1997. Effects of dietary calcium soaps of unsaturated fatty acids on digestion, milk composition and physical properties of butter. J. Dairy Res. 64:181–195.
Fellner, V., F. D. Sauer, and J. K. G. Kramer. 1997. Effect of nigericin, monensin, and tetronasin on biohydrogenation in continuous flow-through fermenters. J. Dairy Sci. 80:921–928.
Fellner, V., F. D. Sauer, and J. K. G. Kramer. 1999. Effect of ionophores on conjugated linoleic acid in ruminal cultures and in the milk of dairy cows. Pages 209–214 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and C. J. Nelson, eds. Am. Oil. Chem. Soc. Press, Champaign, IL.
Firestone, D., and A. Sheppard. 1992. Determination of *trans* fatty acids. Pages 273–322 in Advances in Lipid Methodology-One. W. W. Christie, ed. The Oily Press, Ayr, Scotland.
Fletouris, D. L, N. A. Botsoglou, J. E. Psomas, and A. I. Mantis. 1998. Rapid determination of cholesterol in milk and milk products by direct saponification and capillary gas chromatography. J. Dairy Sci. 81:2833–2840.
Focant, M., E. Mignolet, M. Marique, F. Clabots, T. Breyne, D. Dalemans, and V. Larondelle. 1998. The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention milk fat oxidation. J. Dairy Sci. 81:1095–1101.
Fontecha, J., V. Diaz, M. J. Fraga, and M. Juarez. 1998. Triglyceride analysis by gas chromatography in assessment of authenticity of goat milk fat. J. Am. Oil Chem. Soc. 75:1893–1896.
Foote, R. H. 1999. The need to be a student of history. J. Dairy Sci. 82:453.
Fox, P. F., and P. L. H. McSweeney. Milk lipids. 1998. Pages 67–149 in Dairy Chemistry and Biochemistry. Blackie Academic Profession/ Chapman and Hall, New York.
Franklin, S. T., K. R. Martin, R. J. Baer, D. J. Schingoethe, and A. R. Hippen. 1999. Dietary marine algae (*Schizochytrium* sp.) increases concentration of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J. Nutr. 129:2048–2052.
Fritsche, J., R. Rickert, and H. Steinhart. 1999a. Formation, contents, and estimation of daily intake of conjugated linoleic acid isomers and *trans* fatty acids in foods. Pages 378–396 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil. Chem. Soc. Press, Champaign, IL.
Fritsche, J., R. Rickert, H. Steinhart, M. P. Yurawecz, M. M. Mossoba, N. Sehat, J. A. G. Roach, J. K. G. Kramer, and Y. Ku. 1999b. Conjugated linoleic acid (CLA) isomers: Formation, analysis, amounts in foods, and dietary intake. Fett/Lipid 8:272–278.
Fritsche, J., and H. Steinhart. 1998. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z. Lebensm. Unters. Forsch. A 206:77–82.

Gallacier, J.-P., J.-P. Barbier, and S. Kuzdal-Savoire. 1984. Seasonal variations of the fatty acid composition of butter from three dairies in the Western region of France. Tech Laitiere 993:13–29.

Garcia, H. S., J. M. Storkson, M. W. Pariza, and C. G. Hill, Jr. 1998. Enrichment of butter oil with conjugated linoleic acid via enzymatic interesterfication (acidolysis) reactions. Biotechnol. Lett. 20:393–395.

Garcia, H. S., K. J. Keough, J. A. Arcos, and C. G. Hill, Jr. 2000. Interesterfication (acidolysis) of butterfat with conjugated linoleic acid in a batch reactor. J. Dairy Sci. 83:371–377.

Gaynor, P. J., R. A. Erdman, B. B. Teter, A. V. Capuco, and D. R. Waldo. 1996. Glucose and norepinephrine challenges during abomasal infusion of *cis* and *trans* octadecenoates in Holstein cows. J. Dairy Sci. 79:1590–1595.

Gaynor, P. J., R. A. Erdman, B. B. Teter, J. Sampugna, A. V. Capuco, D. R. Waldo, and M. Hamosh. 1994. Milk fat yield and composition during abomasal infusion of *cis* or *trans* octadecenoates in Holstein cows. J. Dairy Sci. 77:157–165.

Gaynor, P. J., D. B. Waldo, A. V. Capuco, R. A. Erdman, L. W. Douglass, and B. B Teter. 1995. Milk fat depression, the glucogenic theory and *trans*-$C_{18:1}$ fatty acids. J. Dairy Sci. 78:2008–2015.

German, J. B., and C. J. Dillard. 1998. Fractionated milk fat: Composition, structure, and functional properties. Food Technol. 52:33–38.

German, J. B., L. Morand, C. J. Dillard, and R. Xu. 1997. Milk fat composition: Targets for alteration of function and nutrition. Pages 39–72 in Milk Composition, Production and Biotechnology. R. A. S. Welch, D. J. W. Burns, S. R. Davis, A. I. Popay, and C. G. Prosser, eds. CAB International, New York, NY.

Giesy, J. G., S. Viswanadha, T. W. Hanson, L. R. Falen, M. A. McGuire, C. H. Skaric, and A. Vinci. 1999. Effects of calcium salts of conjugated linoleic acid (CLA) on estimated energy balance in Holstein cows early in lactation. J. Dairy Sci. 82(Suppl. 1):74. (Abstr.)

Granelli, K., P. Barrefors, L. Björk, and L.-A. Applequist. 1998. Further studies on lipid composition of bovine milk in relation to spontaneous oxidised flavour. J. Sci. Food Agric. 77:161–171.

Gray, I. K. 1973. Seasonal variations in the composition and thermal properties of New Zealand milk fat. J. Dairy Res. 40:207–214.

Gresti, J., M. Bugaut, G. Maniogui, and J. Bezard. 1993. Composition of molecular species of triacylglycerols in bovine milk fat. J. Dairy Sci. 76:1850–1869.

Griinari, J. M., and D. E. Bauman. 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Pages 180–200 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and C. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.

Griinari, J. M., P. Y. Chouinard, and D. E. Bauman. 1997a. *Trans* fatty acid hypothesis of milk fat depression revised. Pages 208–216 in Proc. Nutr. Conf. Feed Manuf., Rochester, NY, Cornell Univ. Ithaca, NY.

Griinari, J. M., B. A. Corl, S. H. Lacy, P. Y. Chouinard, K. V. V. Nurmela, and D. E. Bauman. 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ9 desaturase. J. Nutr. 130:2285–2291.

Griinari, J. M., D. A. Dwyer, M. A. McGuire, D. E. Bauman, D. L. Palmquist, and K. V. V. Nurmela. 1998. *Trans*-Octadecenoic acids and milk fat depression in lactating dairy cows. J. Dairy Sci. 81:1251–1261.

Griinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. L. Palmquist. 1997b. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076–1084.

Gulati, S. K., S. M. Kitessa, J. R. Ashes, E. Fleck, E. B. Byers, Y. G. Byers, and T. W. Scott. 2000. Protection of conjugated linoleic acids from ruminal hydrogenation and their incorporation into milk fat. Anim. Feed Sci. Technol. 86:139–148.

Gurr, M. I. 1999. Lipids in Nutrition and Health: A Reappraisal. The Oily Press, P. J. Barnes & Bridgewater, England.

Guth, H., and W. Grosch. 1992. Furan fatty acids in butter and butteroil. Z. Lebensm. Unters. Forsch. 194:360–362.

Ha, J. Kim and R. C. Lindsay. 1990. Method for the volatile free and total branched chain fatty acids in cheese and milk fat. J. Dairy Sci. 73:1988–1999.

Ha, Y. L., N. K. Grimm, and M. W. Pariza. 1989. Newly recognized anticarcinogenic fatty acids: Identification in natural and processed cheeses. J. Agric. Food Chem. 37:75–81.

Haas, M. J., J. K. G. Kramer, G. McNeill, K. Scott, T. A. Foglia, N. Sehat, J. Fritsche, M. M. Mossoba, and M. P. Yurawecz. 1999. Lipase-catalyzed fractionation of conjugated linoleic acid isomers. Lipids 34:979–987.

Hamosh, M. 1990. Pregastric esterase. Pages 107–126 in Lingual and Gastric Lipases: Their Role in Fat Digestion. CRC Press, Inc., Boca Raton, FL.

Harmer, W. R., and C. Wijesundera 1996. Heat stability of milkfat in relation to vegetable oils. Austral. J. Dairy Technol. 51:108–111.

Hawke, J. C., and M. W. Taylor. 1995. Influence of nutritional factors on the yield, composition and physical properties of milk fat. Pages 37–88 in Advanced Dairy Chemistry. 2. Lipids. 2nd ed. P. F. Fox, ed. Chapman and Hall, New York, NY.

Hay, J. D., and W. R. Morrison. 1970. Isomeric monenoic fatty acids in bovine milk fat. Biochim. Biophys. Acta 202:237–243.

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

Hermansen, J. E., F. Jonsbo, J. O. Andersen, K. F. Michaelsen, and M. R. Weisbjerg. 1995. On the transfer of γ-linoleic acid into milk fat and its possible elongation to arachidonic acid by cows. Milchwissenschaft 50:3–5.

Hladick, J., and C. Michaelec. 1966. Ceramide monohexosides and ceramide-dihexosides in lipoproteins in the membranes of fat globules in bovine milk. Acta Biol. Med. Ger. 16:696–699.

Hortet, P., and H. Seegers. 1998. Loss in milk yield and related composition changes resulting from clinical mastitis in dairy cows. Prev. Vet. Med. 37:1–20.

Huber, W., A. Molero, C. Pereyra, and E. Martinez de la Ossa. 1995. Determination of cholesterol in milkfat by supercritical fluid chromatography. J. Chromatogr. A 715:333–336.

IDF. International Dairy Federation. 1992. Milkfat and Milkfat Products. Determination of cholesterol contents. IDF Std. 159:1992, Brussels, Belgium.

IDF. International Dairy Federation. 1995. Milk and Milk Products. Extraction Methods for Lipids and Liposoluble Compounds. IDF Std. 172: 1995, Brussels, Belgium.

IDF. International Dairy Federation. 1999a. Milkfat. Preparation of Fatty Acid Methyl Esters. IDF Std, 182:1999, Brussels, Belgium.

IDF. International Dairy Federation. 1999b. Milkfat. Determination of the Fatty Acid Composition by Gas-Liquid Chromatography. IDF Std, 184:1999, Brussels, Belgium.

IDF. International Dairy Federation. 2000a. Inventory of IDF/ISO/AOAC International Adopted Methods of Analysis and Sampling for Milk and Milk Products. 6th ed. Bull. 350/2000, Brussels, Belgium.

IDF. International Dairy Federation. 2000b. Dairy Foods and Cardiovascular Health, Bull. 353/2000, Brussels, Belgium.

Ip, C., B. Banni, E. Angioni, G. Carta, J. McGinley, H. J. Thompson, D. Barbano, and D. Bauman. 1999. Conjugated linoleic acid enriched butterfat alters mammary gland morphogenesis and reduces cancer risk in rats. J. Nutr. 129:2135–2142.

Ip, C., S. F. Chin, J. A. Scimeca, and M. W. Pariza. 1991. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res. 51:6118–6124.

Ip, C., J. A. Scimeca, and H. J. Thompson. 1994. Conjugated linoleic acid. A powerful anticarcinogen from animal fat sources. Cancer (Suppl.) 74:1050–1054.

Iverson, J. L., J. Eisner, and D. Firestone. 1965. Detection of trace fatty acids in fats and oils by urea fractionation and gas-liquid chromatography. J. Am. Oil Chem. Soc. 47:1063–1068.

Iverson, J. L., and A. J. Sheppard. 1986. Determination of fatty acids in butterfat using temperature programmed gas chromatography of the butyl esters. Food Chem. 21:223–234.

Jahreis, G., J. Fritsche, and J. Kraft. 1999a. Species-dependent, seasonal, and dietary variation of conjugated linoleic acid in milk. Pages 215–225 in Advances in Conjugated Linoleic Acid Research.

Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and C. J. Nelson, eds. Am. Oil. Chem. Soc. Press, Champaign, IL.

Jahreis, G., J. Fritsche, P. Mockel, F. Schone, U. Mäller, and H. Steinhart. 1999b. The potential anticarcinogenic conjugated linoleic acid, *cis*-9, *trans*-11 C18. 2 in milk of different species. Cow, goat, ewe, sow, mare, woman. Nutr. Res. 19:1541–1549.

Jahreis, G., J. Fritsche, and H. Steinhart. 1996. Monthly variations of milk composition with special regard to fatty acids depending on season and farm management systems-Conventional versus ecological. Fett/Lipid 98:356–369.

Jahreis, G., J. Fritsche, and H. Steinhart. 1997. Conjugated linoleic acid in milk fat: High variation depending on production system. Nutr. Res. 17:1479–1484.

Jenkins, T. C. 1998. Fatty acid composition of milk from Holstein cows fed oleamide or canola oil. J. Dairy Sci. 81:794–800.

Jenkins, T. C. 1999. Lactation performance and fatty acid composition of milk from Holstein cows fed 0 to 5% oleamide. J. Dairy Sci. 82:1525–1531.

Jenkins, T. C. 2000. Feeding oleamide to lactating Jersey cows. I. Effects on lactation performance and milk fatty acid composition. J. Dairy Sci. 83:332–337.

Jenkins, T. C., H. G. Bateman, and S. M. Block. 1996. Butylsoyamide increases unsaturation of fatty acids in plasma and milk of lactating dairy cows. J. Dairy Sci. 79:585–590.

Jenneman, R., and H. Wieghandt. 1994. A rapid method for the preparation of ganglioside $G_{lac}2$ (GD3). Lipids 29:365–368.

Jensen, R. G. 1999. Lipids in human milk. Lipids 34:1243–1271.

Jensen, R. G. 2000. Fatty acids in milk and dairy products. Pages 109–123 in Fatty Acids in Foods and their Health Implications. 2nd ed. C. K. Chow, ed. Marcel Dekker, Inc., New York, NY.

Jensen, R. G., and R. M. Clark. 1988. Lipid composition and properties. Pages 171–213 in Fundamentals of Dairy Chemistry. 3rd ed. N. Wong, ed. Van Nostrand Reinhold Company, New York, NY.

Jensen, R. G., C. J. Lammi-Keefe, and B. Koletzko. 1997. Representative sampling of human milk and the extraction of fat for analysis of environmental lipophilic contaminants. Toxicol. Environ. Chem. 62:229–247.

Jensen, R. G., and D. S. Newberg. 1995. Bovine milk lipids. Pages 543–575 in Handbook of Milk Composition. R. G. Jensen, ed. Academic Press, San Diego, CA.

Jensen, S. K., and K. N. Nielsen. 1996. Tocopherols, retinol, β-carotene, and fatty acids in the globule membrane and fat globule core in cows' milk. J. Dairy Res. 63:566–574.

Jiang, J., L. Bjórck, and R. Fonden. 1998. Production of conjugated linoleic acid by dairy starter cultures. J. Appl. Microbiol. 85:95–102.

Jiang, J., L. Bjoerck, R. Fonden, and M. Emanuelson. 1996. Occurrence of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk: Effects of feed and dairy regimen. J. Dairy Sci. 79:438–445.

Jiang, J., A. Wolk, and B. Vessby. 1999. Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue. Am. J. Clin. Nutr. 70:21–27.

Jimenez-Flores, R. 1997. Trends in research for alternate uses of milk fat. J. Dairy Sci. 80:2644–2650.

Jones, D. F., W. P. Weiss, and D. L. Palmquist. 2000. Short communication: Influence of dietary tallow and fish oil in milk fat composition. J. Dairy Sci. 83:2024–2026.

Kallio, P., A. Kempinnen, and I. Kilpelaeinen. 1996. Determination of positional distribution of butyryl groups in milk fat triacylglycerols, triacylglycerol mixtures, and isolated positional isomers of triacylglycerols by gas chromatography and ^{1}H nuclear magnetic resonance spectroscopy. Lipids 31:331–336.

Kalscheur, K. F., B. B. Teter, L. S. Piperova, and R. A. Erdman. 1997a. Effect of dietary forage concentration and buffer addition on duodenal flow of *trans* $C_{18:1}$ fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2104–2114.

Kalscheur, K. F., B. B. Teter, L. S. Piperova and R. A. Erdman. 1997b. Effect of fat source on duodenal flow of *trans*-$C_{18:1}$ fatty acids and milk fat production in dairy cows. J. Dairy Sci. 80:2115–2120.

Karlsson, A. A., K. C. Arnoldsson, G. Westerdahl, and G. Odham. 1997. Common molecular species of glucosyl ceramides, lactosyl ceramides and sphingomyelins in bovine milk determined by high-performance liquid chromatography-mass spectrometry. Milchwissenschaft 52:554–559.

Karlsson, A. A., P. Michelsen, and G. Odham. 1998. Molecular species of sphingomyelin: Determination by high-performance liquid chromatography/mass spectrometry with electrospray and high-performance liquid chromatography/ tandem mass spectrometry with pressure chemical ionization. J. Mass Spectrom. 33:1192–1198.

Katan, M. B. 2000. *Trans* fatty acids and plasma proteins. Nutr. Rev. 58:188–191.

Kaylegian, K. E., and R. C. Lindsay. 1995. Milk fat usage and modification. Pages 1-18 in Handbook of Milkfat Fractionation Technology and Application. Am. Oil Chem. Soc. Press, Champaign, IL.

Keenan, T. W., I. H. Mather, and D. P. Dylewski. 1988. Physical equlibria: Lipid phase. Pages 461–582 in Fundamentals of Dairy Chemistry. 3rd ed. N. Wong, ed. Van Nostrand Reinhold Company, New York, NY.

Keenan, T. W., and S. Patton. 1995. The milk fat globule membrane. Pages 5–49 in Handbook of Milk Composition. R. G. Jensen, ed. Academic Press, San Diego, CA.

Kelly, M. L., J. R. Berry, D. A. Dwyer, J. M. Griinari, P. Y. Chouinard, M. E. Van Amburgh, and D. E. Bauman. 1998a. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128:881–885.

Kelly, M. L., E. S. Kolver, D. E. Bauman, M. E. Van Amburgh, and L. D. Muller. 1998b. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating dairy cows. J. Dairy Sci. 81:1630–1636.

Kemppinen, A., and P. Kalo. 1998. Analysis of *sn*-1 (3)- and *sn*-2-short-chain acyl isomers of triacylglycerols in butteroil by gas-liquid chromatography. J. Am. Oil Chem. Soc. 75:91–100.

Kennelly, J. J., B. Robinson, and G. R. Khorasani. 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early-lactation Holstein cows. J. Dairy Sci. 82:2486–2496.

Kim, S. J., G. B. Park, C. B. Kang, S. D. Park, M. Y. Jung, J. O. Kim, and Y. L. Ha. 2000. Improvement of oxidative stability of conjugated linoleic acid (CLA) by microencapsulation in cyclodextrins. J. Agric. Food Sci. 48:3922–3929.

Kim, Y.-Y., and R. H. Liu. 1999. Selective increase in conjugated linoleic acid in milk fat by crystallization. J. Food Sci. 64:792–795.

King, R. A., M. M. Mano, and R. J. Head. 1998. Assessment of isoflavanoid concentrations in Australian bovine milk samples. J. Dairy Res. 65:479–489.

Kisza, J., B. Stanlewski, and M. Juskiewicz. 1994. Determination of cholesterol in milk fat by gas chromatography (GC). Polish J. Food Nutr. Sci. 3:75–81.

Klatt, L. V., B. A. Mitchell, and R. L. Smith. 1995. Cholesterol analysis in foods by direct saponification-gas-chromatographic method-collaborative study. J. AOAC Int. 78:75–79.

Klump, B., H. U. Melchert, and K. Rubach. 1982. Extraction of lipids from bovine and human serum and milk by means of Extrelut for GLC of triglycerides, cholesterol, and fatty acids. Fresnius Z. Anal. Chem. 313:553–560.

Knekt, P., and R. Järvinen. 1999. Intake of dairy products and breast cancer risk. Pages 444–470 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds., Am. Oil Chem. Soc. Press, Champaign, IL.

Koletzko, B., and T. Decsi. 1997. Metabolic aspects of *trans* fatty acids. Clin. Nutr. 16:229–237.

Kowalski, Z. M., P. M. Pisulewski, and M. Spanghero. 1999. Effects of calcium soaps of rapeseed fatty acids and protected methionine on milk yield and composition in dairy cows. J. Dairy Res. 66:475–487.

Kramer, J. K. G., V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, and M. P. Yurawecz. 1997. Evaluating acid base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total *trans* fatty acids. Lipids 32:1219–1228.

Kramer, J. K. G., P. W. Parodi, R. G. Jensen, M. M. Mossoba, M. P. Yurawecz, and R. O. Adlof. 1998a. Rumenic acid: A proposed common name for the major conjugated linoleic acid found in natural products. Lipids 33:835.

Kramer, J. K. G., N. Sehat, M. E. R. Dugan, M. M. Mossoba, M. P. Yurawecz, J. A. G. Roach, K. Eulitz, J. L. Aalhus, A. L. Schaefer, and Y. Ku. 1998b. Distributions of conjugated linoleic acid (CLA) isomers in tissue classes of pigs fed a commercial CLA mixture determined by gas chromatography and silver ion-high performance liquid chromatography. Lipids 33:549–558.

Kramer, J. K. G., N. Sehat, J. Fritsche, M. M. Mossoba, K. Eulitz, M. P. Yurawecz, and Y. Ku. 1999. Separation of conjugated fatty acid isomers. Pages 83–109 in Advances in Conjugated Linoleic Acid Research, Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.

Kritchevsky (Undated), subject. CLA-A good news story. http//www.nationaldairycouncil. com/medcent/mnews/CLA. html. Accessed Mar. 10, 2000.

Kummerow, F. A., R. M. Olinescu, L. Fleischer, B. Handler, and S. V. Shinkareva. 2000. The relationship of oxidized lipids to coronary artery stenosis. Atherosclerosis 149:181–190.

Kurtz, F. E. 1974. The lipids of milk: Composition and properties. Pages 125–219 in Fundamentals of Dairy Chemistry. 2nd ed. B. H. Webb, A. H. Johnson, and J. A. Alford, eds. The Avi Publishing Company, Westport, CT.

Laakso, P., and P. Manninen. 1997. Identification of milk fat triacylglycerols by capillary supercritical fluid chromatography-atmospheric pressure chemical ionization mass spectrometry. Lipids 32:1285–1295.

Laakso, P., P. Manninen, J. Maekinen, and H. Kallio. 1996. Postparturition changes in the triacylglycerols of cow colostrum. 1996. Lipids 31:937–943.

Laegrid, H., H.-B. Kolsto-Otnaess, and J. Fugelsang. 1986. Human and bovine milk: Comparison of ganglioside composition and enterotoxin-inhibitory activity. Pediatr. Res. 20:416–421.

Lai, H-C., and D. M. Ney. 1998. Gastric digestion modifies absorption of butterfat into lymph chylomicrons in rats. J. Nutr. 128:2403–2410.

Lanza, E., and H. T. Slover. 1981. The use of SP-2340 glass capillary columns for the estimation of the *trans* fatty acid content of foods. Lipids 16:260–267.

Lavillonnière, F., J. C. Martin, P. Bougnoux, and J.-L. Sébédio. 1998. Analysis of conjugated linoleic acid isomers and content in French cheese. J. Am. Oil Chem. Soc. 75:343–352.

Lawless, F., J. J. Murphy, D. Harrington, R. Devery, and C. Stanton. 1998. Elevation of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk because of dietary supplementation. J. Dairy Sci. 81:3259–3267.

Lawler, P. J., and P. S. Dimick. 1998. Crystallization and polymorphism of fats. Pages 229–250 in Food Lipids: Chemistry, Nutrition, and Biotechnology, C. C. Akoh and D. B. Min, eds. Marcel Dekker, New York, NY.

Lee, D. K., J. Aha, and H. S. Kwak. 1999. Cholesterol removal from homogenized milk with β-cyclodextrin. J. Dairy Sci. 82:2327–2330.

Lefevre, M. 2000. Nutrition issues update. Is milkfat the scapegoat for CHD? National Dairy Council: Calcium Info Kit. Available from:http://www.nationaldairycouncil.com/medcent/mnews/CHP.htm/

Lehman, D., B. Mass, and A. Mosandi. 1985. Stereoisomeric flavour compounds LXIX: stereodifferentiation of δ (γ)-lactones C8-C18 in dairy products, margarine and coconut. Z. Lebens. Unters. Forsch. 201:55–61.

Lenck, R. W., N. Swink, H. Snelting, and J. Arul. 1998. Increasing short-chain fatty acid yield during lipase hydrolysis of a butterfat fraction with periodic aqueous extraction. J. Am. Oil. Chem. Soc. 75:1195–2000.

Limb, J-K., Y-H. Kim, S-Y. Han, and G-J. Jhon. 1999. Isolation and characterization of monoacetyldiglycerides from the bovine udder. J. Lipid Res. 40:2169–2176.

Lin, H., T. D. Boylston, M. J. Chang, L. G. Ludecke, and T. D. Shultz. 1995. Survey of the conjugated linoleic acid contents of dairy products. J. Dairy Sci. 78:2358–2365.

Lin, H., T. D. Boylston, L. O. Luedecke, and T. D. Shultz. 1999a. Conjugated linoleic acid content of Cheddar-type cheeses as affected by processing. J. Food Sci. 64:874–878.

Lin, M. P., C. A. Sims, C. R. Staples, and S. F. O'Keefe. 1996. Flavor quality and texture of modified fatty acid high monoene, low saturate butter. Food Res. Int. 29:367–371.

Lin, T. Y. 2000. Conjugated linoleic acid concentration as affected by lactic cultures and additives. Food Chem. 69:27–31.

Lin, T. Y., C. W. Lin, and C. H. Lee. 1999b. Conjugated linoleic acid concentration as affected by lactic cultures and added linoleic acid. Food Chem. 67:1–5.

Lipp, M. 1995. Review of methods for the analysis of triglycerides in milk fat: Application for studies of milk quality and adulteration. Food Chem. 54:213–221.

Lobb, K., and C. K. Chow. 1999. Fatty acid classification and nomenclature. Pages 1–15 in Fatty Acids in Foods and Their Health Implications. 2nd ed. C. K. Chow, ed. Marcel Dekker, Inc., New York, NY.

Loor, J. J., and J. H. Herbein. 1998. Exogenous conjugated linoleic acid isomers reduce bovine milk fat concentration and yield by inhibiting de novo fatty acid synthesis. J. Nutr. 128:2411–2419.

Lozada, L., M. H. de La Fuente, J. Fontecha, and M. Juarez. 1995. Considerations of the quantitative aspect of the determination of milk fat triglycerides with split PTV and on-column injection. J. High Resol. Chromatogr. 18:771–775.

Lund, P., and F. Jensen. 1983. Isomeric fatty acids in milk fat. Milchwissenschaft 38:193–196.

Lyons, A. L., S. Samman, L. Gatto, and A. J. Brown. 1999. Rapid hepatic metabolism of 7-dehydrocholesterol in vivo: Implications for dietary oxysterols. J. Lipid Res. 40:1846–1857.

Ma, D. W. L., A. A. Wierzbecki, C. J. Field, and M. T. Clandinin. 1999. Conjugated linoleic acid in Canadian dairy and beef products. J. Agric. Food Chem. 47:1956–1960.

MacDonald, H. B. 2000. Conjugated linoleic acid and disease prevention: A review of current knowledge. J. Am. Coll. Nutr. 19:1115–1185.

Maijala, K. 2000. Cow milk and human development and well being. Livestock Prod. Sci. 65:1–18.

Manganiello, L., A. Rios, and M. Valcarel. 2000. Automatic microgravimetric determination of fats in milk products by use of supercritical fluid extraction with on-line piezoelectric detection. J. Chromatogr. A 874:265–274.

Manninen, P., P. Laakso, and H. Kallio. 1995. Method for characterization of triacylglycerols and fat-soluble vitamins in edible oils and fats by supercritical fluid chromatography. J. Am. Oil Chem. Soc. 72:1001–1008.

Mansbridge, R. J., and J. S. Blake. 1997. Nutritional factors affecting the fatty acid composition of bovine milk. Br. J. Nutr. 78(Suppl. 1):S37–S47.

Massart-Leen, A. M., H. De Pooter, M. Decloedt, and N. Schamp. 1981. Composition and variability of the branched-chain fatty acid fraction in the milk of goats and cows. Lipids 16:286–292.

Mather, I. H., and T. W. Keenan. 1998a. The cell biology of milk secretion: Historical notes. J. Mam. Gland Biol. Neoplasia 3:227–237.

Mather, I. H., and T. W. Keenan. 1998b. Origin and secretion of milk lipids. J. Mam. Gland Biol. Neoplasia 3:259–273.

Maxwell, R. J., D. Mondimore, and J. Tobias. 1986. Rapid method for the quantitative extraction and simultaneous class separation of milk lipid. J. Dairy Sci. 69:321–325.

McCrae, H., and A. Lepoetre. 1998. Characterization of dairy emulsions by forward lobe laser light scattering-Application to milk and cream. Int. Dairy J. 6:247–256.

McGlade, L. T., B. A. Milat, and J. Scales. 1996. Lipid terminology. Am. J. Clin. Nutr. 64:668.

McGuire, M. A., M. K. McGuire, M. A. Guy, W. K. Sanchez, T. D. Shultz, L. Y. Harrison, D. E. Bauman, and J. M. Griinari. 1996. Short-term effect of dietary lipid concentration on content of con-

jugated linoleic acid (CLA) in milk from dairy cattle. J. Anim. Sci. 74(Suppl. 1):266. (Abstr.)
McGuire, M. K., M. A. McGuire, K. Ritzenthaler, and T. D. Shultz. 1999. Dietary sources and intakes of conjugated linoleic acid intake in humans. Pages 369–377 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.
McNamara, J. P., and R. L. Baldwin. 2000. Estimation of parameters describing lipid metabolism in lactation: Challenge of existing knowledge described in a model of metabolism. J. Dairy Sci. 83:129–143.
Miller, G. D., J. K. Jarvis, and L. D. McBean. 1999. Dairy foods and cardiovascular health. Pages 65–115 in Handbook of Dairy Foods and Nutrition. 2nd ed. National Dairy Council. CRC Press, Boca Raton, FL.
Molimard, M., and H. E. Spinnler. 1996. Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origin and properties. J. Dairy Sci. 79:169–184.
Molkentin, J. 1999. Bioactive compounds naturally occurring in bovine milk. Nahrung 43:185–189.
Molkentin, J., and D. Precht. 1993. The influence of autoxidation on milk fat composition. Kieler Milchwirtschaft Forschg. Berichte 45:373–383.
Molkentin, J., and D. Precht. 1994. Comparison of packed and capillary columns for quantitative gas chromatography of triglycerides in milk fat. Chromatographia 39:265–270.
Molkentin, J., and D. Precht. 1995a. Development of a precise capillary method for rapid triglyceride analysis of milk fats. Fat Sci. Technol. 97:43–49.
Molkentin, J., and D. Precht. 1995b. Optimized analysis of trans octadecenoic acids in edible fats. Chromatographia 41:267–272.
Molkentin, J., and D. Precht. 1997a. Occurrence of *trans*-C16:1 acids in bovine milkfats and partially hydrogenated edible fats. Milchwissenschaft 52:380–385.
Molkentin, J., and D. Precht. 1997b. Representative determination of the butyric acid in European milk fats. Milchwissenschaft 52:22–25.
Molkentin, J., and D. Precht. 2000a. Equivalence of packed and capillary GC columns for detection of foreign fat in butter by use of the triglyceride formula method. Chromatographia 52:791–797.
Molkentin, J., and D. Precht. 2000b. Validation of a gas-chromatographic method for the determination of milk fat contents in mixed fats by butyric acid analysis. Eur. J. Lipid Sci. Technol. 2000:194–201.
Morales, M. Sol, D. L. Palmquist, and W. P. Weiss. 2000a. Effects of fat source and copper on unsaturation of blood and milk triacylglycerol fatty acids in Holstein and Jersey cows. J. Dairy Sci. 83:2105–2111.
Morales, M. Sol, D. L. Palmquist, and W. P. Weiss. 2000b. Milk fat composition of Holstein and Jersey cows with control or depleted copper status and fed whole soybeans or tallow. J. Dairy Sci. 83:2112–2119.
Morrison, W. R., and J. D. Hay. 1970. Polar lipids in bovine milk. II. Long-chain bases, normal and 2-hydroxy fatty acids and isomeric cis and trans monoenoic fatty acids in the sphingolipids. Biochim. Biophys. Acta 202:460–467.
Nagao, J., H. Watanabe, N. Goto, K. Orizawa, H. Taguchi, N. Matsuo, T. Yasukawa, R. Tsushima, H. Shimasaka, and H. Itakura. 2000. Dietary diacylglycerol suppresses accumulation of body fat compared to triacylglycerol in men in a double-blind controlled study. J. Nutr. 130:792–797.
National Dairy Council (NDC). 1993. Newer knowledge of milk and other fluid dairy products. NDC, Rosemont, IL.
Newberg, D. S., and P. Chatervedi. 1992. Neutral glycolipids of human and bovine milk. Lipids 27:923–927.
Nielsen, J. H., C. E. Olsen, C. Jensen, and L. H. Skibstead. 1996a. Cholesterol oxidation in butter and dairy spread during storage. J. Dairy Res. 63:159–167.
Nielsen, J. H., C. E. Olsen, J. Lyndon, J. Sorenson, and L. H. Skibstead. 1996b. Cholesterol oxidation in feta cheese produced from high-temperature bleached and from non-bleached butteroil from bovine milk. J. Dairy Res. 63:615–621.
Nikolova-Damyanova, B., S. Momchilova, and W. W. Christie. 2000. Silver-ion high-performance liquid chromatographic separation of conjugated linoleic acid isomers and other fatty acids after conversion to *para*-methoxyphenacyl derivatives. J. High Resol. Chromatogr. 21:348–352.
Noakes, M., P. J. Nestel, and P. M. Clifton. 1996. Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. Am. J. Clin. Nutr. 63:42–44.
Nyberg, L. 1995. Sphingomyelin from bovine milk. Pages 120–125 in Phospholipids: Characterization, Metabolism, and Novel Biological Applications. G. Cevc and F. Paltauf, F. Am. Oil Chem. Soc. Press, Champaign, IL.
Offer, N. W., M. Marsden, J. Dixon, B. K. Speake, and F. E. Thacker. 1999. Effect of dietary fat supplements on levels of *n*-3 polyunsaturated fatty acids, *trans* acids and conjugated linoleic acid in bovine milk. Anim. Sci. 69:613–625.
Ollson, N. U., P. Kaufmann, and S. Dzeletović. 1997. Preparation and gas chromatographic-mass spectrometric analysis of N-acetyl-O-trimethylsilyl derivatives of long-chain base residues of natural sphingomyelin. J. Chomatogr. B. 698:1–8.
O'Shea, M., R. Devery, F. Lawless, and C. Stanton. 2000. Enrichment of the conjugated linoleic acid content of bovine milk by dry fractionation. Int. Dairy J. 10:289–294.
Ostrowska, E., F. P. Dunshea, M. Murolitharan, and R. F. Cross. 2000. Comparison of silver-ion high-performance liquid chromatographic quantification of free and methylated conjugated linoleic acids. Lipids: 35:1147–1153.
Palmquist, D. L., A. D. Beaulieu, and D. M. Barbano. 1993. Feed and animal factors influencing milk fat composition. J. Dairy Sci. 76:1753–1771.
Palmquist, D. L., and J. E. Santora. 1999. Endogenous synthesis of rumenic acid in rodents and humans. Pages 201–208 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.
Pan, L. I., and T. Izumi. 2000. Variation of the ganglioside composition of human milk, cow's milk, and infant formulas. Early Hum. Develop. 57:25–31.
Papalois, M., F. W. Leach, S. Dungey, Y. L. Yep, and C. Versteeg. 1996. Australian milkfat survey-physical properties. Austral. J. Dairy Tech. 51:114–116.
Pariza, M. W. 1999. The biological activities of conjugated linoleic acid. Pages 12–20 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.
Pariza, M. W., Y. Park, and M. E. Cook. 1999. Conjugated linoleic acid and the control of cancer and obesity. Toxicol. Sci. 52(Suppl):107–110.
Pariza, M. W., Y. Park, and M. E. Cook. 2000. Mechanisms of action of conjugated linoleic acid: Evidence and speculation. Proc. Soc. Exp. Biol. Med. 223:8–13.
Parodi, P. W. 1975. Detection of acetodiacylglycerols in milkfat by thin-layer chromatography. J. Chromatogr. 111:223–226.
Parodi, P. W. 1979. Stereospecific distribution of fatty acids in bovine milk fat triglycerides. J. Dairy Res. 46:75–81.
Parodi, P. W. 1999a. Conjugated linoleic acid: The early years. Pages 1–11 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.
Parodi, P. W. 1999b. Conjugated linoleic acid and other anticarcinogenic agents of milk fat. J. Dairy Sci. 82:1339–1349.
Patton, S., and R. G. Jensen. 1975. Lipid metabolism and membrane functions of the mammary gland. Pages 243–277 in Progress in the Chemistry of Fats and Other Lipids. Vol. XIV, Part V. R. T. Holman, ed. Pergamon Press, New York, NY.
Pfalzgraf, A., M. Timm, and H. Steinhart. 1993. Gehalte von trans-Fettsäuren in Lebensmittel. Z. Ernährungwissenschaft 33:24–43.

Piperova, L. S., B. B. Teter, I. Bruckental, J. Sampugna, S. E. Mills, M. P. Yurawecz, J. Fritsche, K. Ku, and R. A. Erdman. 2000. Mammary lipogenic enzyme activity, *trans* fatty acids and conjugated linoleic acids are altered in lactating dairy cows fed a milk fat-depressing diet. J. Nutr. 130:2568–2574.

Precht, D. 1994. Seasonal variation of butter fat parameters in different milk collection areas. Kieler Milchwirtschaftl. Forschungber. 46:65–79.

Precht, D., and J. Molkentin. 1996. Rapid analysis of the *trans*-octadecenoic acid in milk fat. Int. Dairy J. 6:791–809.

Precht, D., and J. Molkentin. 1997a. 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., and J. Molkentin. 1997b. Effect of feeding on *trans* positional isomers of octadecenoic acid in milk fats. Milchwissenschaft 52:564–568.

Precht, D., and J. Molkentin. 1997c. 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., and J. Molkentin. 1998. Correlations of anticarcinogenic conjugated linoleic acid with other C18 fatty acids in German bovine milk fat. Pages 150–158 in 3rd Karlsruhe Nutrition Symposium: European Research Towards Safer and Better Food, Review and Transfer Congress. Bundesforschunganstalt Ernährung. Karlsruhe, Germany. Druckerei.

Precht, D., and J. Molkentin. 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 Milchwirtsch. Forschungsber. 51:63–78.

Precht, D., and J. Molkentin. 2000a. Frequency of conjugated linoleic acid and *trans* fatty acid contents in European bovine milkfats. Milchwissenschaft 55:687–691.

Precht, D., and J. Molkentin. 2000b. *Trans* unsaturated fatty acids in bovine milk fat and dairy products. Eur. J. Lipid Sci. Technol. 102:635–639.

Precht, D., J. Molkentin, F. Destaillats, and R. L. Wolff. 2001a. Comparative studies on individual isomeric 18:1 acids in cow, goat, and ewe milk fats. Lipids 36:827–832.

Precht, D., J. Molkentin, and I. de Froidmont-Goertz. 1998b. Anhydrous butter fat reference material CRM 519: Certification of triglyceride composition and cholesterol content including homogeneity and stability tests. Fett/Lipid 100:546–554.

Precht, D., J. Molkentin, M. A. McGuire, M. K. McGuire, and R. G. Jensen. 2001b. Overestimates of 18:1 and 18:2 contents in materials containing *trans* fatty acids analyzed with short packed columns. Lipids 36:213–216.

Precht, D., J. Molkentin, and M. Vahlendieck. 1999. Influence of the heating temperature on the fat composition of milk fat with emphasis on *cis-trans* isomerization. Nahrung 43:25–33.

Przygonski, K., H. Jelen, and E. Wasowiez. 2000. Determination of cholesterol oxidation products in milk powder and infant formulas by gas chromatography and mass spectrometry. Nahrung 44:122–125.

Puente, R., L.-A. Garcia-Pardo, and P. Hueso. 1992. Gangliosides in bovine milk. Biol. Chem. Hoppe-Seyler 375:283–288.

Ratnayake, W. M. N., and J. L. Beare-Rogers. 1990. Problems of analyzing C_{18} *cis* and *trans* isomers of margarine on the SP-2340 capillary column. J. Chromatogr. Sci. 28:633–639.

Rattray, F. P., and P. F. Fox. 1999. Aspects of enzymology and biochemical properties of *Brevibacterium linens* relevant to cheese ripening: A review. J. Dairy Sci. 82:891–909.

Ren, S., J. N. Scarsdale, T. Ariga., Y. Zhang, R. A. Klein, R. Hartmann, Y. Kushi, H. Egge, and R. K. Ku. 1992. O-Acetylated gangliosides in bovine milk. J. Biol. Chem. 267:12632–12638.

Renner, E., and Y. C. Yoon. 1982. Untersuchungen über isomere Formen ungesättigter Fettsäuren in Nahrungsfetten. 2. Isomere der Octadecadiensäure. Milchwissenschaft 37:408–411.

Rickert, R., H. Steinhart, J. Fritsche, N. Sehat, M. P. Yurawecz, M. M. Mosssoba, J. A. G. Roach, K. Eulitz, Y. Ku, and J. K. G. Kramer. 1999. Enhanced resolution of conjugated linoleic acid isomers by tandem-column silver-ion high performance liquid chromatography. J. High Resol. Chromatogr. 72:144–148.

Ritter, G., E. Boosfeld, E. Markstein, R. K. Yu., S. Ren, W. B. Stallcup, H. F. Oettgen, L. J. Old, and P. O. Livingston. 1990. Biochemical and serological characteristics of natural 9-O-acetyl GD3 from human melanoma and bovine buttermilk and chemically O-acetylated GD3. Cancer Res. 50:1403–1410.

Rizvi, S. S. H., and A. R. Bhaskar. 1999. Supercritical fluid processing of milk fat: Fractionation, scale-up, and economics. Food Technol. 49:90–98.

Roach, J. A. G. 1999. Identification of CLA isomers in food and biological extracts by mass spectrometry. Pages 126–140 in Advances in Conjugated Linoleic Acid Research. Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.

Roach, J. A. G., M. P. Yurawecz, J. K. G. Kramer, M. M. Mossoba, K. Eulitz, and Y. Ku. 2000. Gas chromatography-high resolution selected-ion mass spectrometric identification of trace 21:0 and 20:2 fatty acids eluting with conjugated linoleic acid isomers. Lipids 35:797–802.

Robinson, N. P., and A. K. H. MacGibbon. 1998a. Separation of milk fat triacylglycerols by argentation thin-layer chromatography. J. Am. Oil Chem. Soc. 75:783–788.

Robinson, N. P., and A. K. H. MacGibbon. 1998b. The composition of New Zealand milk fat triacylglycerols by reversed-phase high-performance liquid chromatography. 1998. J. Am. Oil Chem. Soc. 75:993–999.

Robinson, N. P., and A. K. H. MacGibbon. 2000. Determination of the conjugated linoleic acid containing triacylglycerols in New Zealand milk fat. Lipids 35:789–796.

Romero K, P., S. S. H. Rizvi, M. L. Kelly, and D. E. Bauman. 2000. Short communication: Concentration of conjugated linoleic acid from milk fat with continuous supercritical fluid processing system. J. Dairy Sci. 83:20–22.

Romo, G. A., D. P. Casper, R. A. Erdman, and B. B. Teter. 1996. Abomasal infusion of *cis* or *trans* fatty acid isomers and energy metabolism of lactating dairy cows. J. Dairy Sci. 79:2005–2015.

Rowney, M., and M. Christian. 1996. Effect of cow diet and stage of lactation on the composition of milkfat for cheese manufacture. Austral. J. Dairy Technol. 51:118–122.

Rueda, R., J. Maldonaldo, E. Narbano, and A. Gil. 1998. Neonatal dietary gangliosides. Early Hum. Dev. 53(Suppl.):S135–S147.

Ruiz-Sala, P., M. T. G. Hierro, I. Martinez-Castro, and G. Santa-Maria. 1996. Triglyceride composition of ewe, cow, and goat milk fat. J. Am. Oil Chem. Soc. 73:283–293.

Sable, S., and G. Cottenceau. 1999. Current knowledge of soft cheeses flavor and development. J. Agric. Food Chem. 47:4825–4836.

Sampugna, J., L. A. Pallansch, M. G. Enig, and M. Keeney. 1982. Rapid analysis of *trans* fatty acids on SP-2340 glass columns. J. Chromatogr. 249:245–255.

Sauer, F. D., V. Fellner, R. Kinsman, J. K. G. Kramer, H. A. Jackson, A. J. Lee, and S. Chen. 1998. Methane output and lactation response in Holstein cattle with monensin or unsaturated fat added to the diet. J. Anim. Sci. 76:906–914.

Schmelz, E. M., M. C. Sullards, D. L. Dillehay, and A. H. Merrill, Jr. 2000. Colonic cell proliferation and aberrant crypt foci formation are inhibited by dairy glycosphingolipids in 1,2-dimethylhydrazine-treated CF-1 mice. J. Nutr. 130:522–527.

Schogt, J. C. M., and P. Havercamp Begemann. 1965. Isolation of 11-cyclohexylundecanoic acid from butter. J. Lipid Res. 6:466–470.

Schwartz, D. P. 1970. New micromethods for isolating and characterizing lipid constituents. Agric. Res. Rev. 8: 2 & 3 Second and Third quarter. 41–49. Published quarterly by Cooperative State Research Service, U. S. Department of Agriculture.

Schwartz, D. P. 1972. Methods for the isolation and characterization of trace components from milk fat. J. Am. Oil Chem. Soc. 49:312A. (Abstr.)

Sehat, N., J. K. G. Kramer, M. M. Mossoba, M. P. Yurawecz, J. A. G. Roach, K. Eulitz, K. M. Morehouse, and Y. Ku. 1998a. Identification of conjugated linoleic acid isomers in cheese by gas-chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. Lipids 33:963–971.

Sehat, N., R. Rickert, M. M. Mossoba, J. K. G. Kramer, M. P. Yurawecz, J. A. G. Roach, R. O. Adlof, K. M. Morehouse, J. Fritsche, H. Steinhart, and Y. Ku. 1999. Improved separation of conjugated fatty acid methyl esters by silver ion high-performance liquid chromatography. Lipids 34:407–413.

Sehat, N., M. P. Yurawecz, J. A. G. Roach, M. Mossoba, J. K. G. Kramer, and Y. Ku. 1998b. Silver ion high-performance liquid chromatographic separation and identification of conjugated linoleic acid isomers. Lipids 33:217–221.

Shantha, N. C., and E. A. Decker. 1993. Conjugated linoleic acid concentrations in processed cheese containing hydrogen donors, iron and dairy-based additives. Food Chem. 47:257–261.

Shantha, N. C., E. A. Decker, and Z. Ustonol. 1992. Conjugated linoleic acid concentration in processed cheese. J. Am. Oil Chem. Soc. 69:425–428.

Shantha, N. C., L. R. Ram, J. O'Leary, C. L. Hicks, and E. A. Decker. 1995. Conjugated linoleic acid concentrations in dairy products as affected by processing and storage. J. Food Sci. 60:695–697 and 720.

Sheppard, A. J. 1992. Lipid Manual. Methodology Appropriate for Fatty Acid-Cholesterol Analysis. W. C. Brown Publ., Dubuque, IA.

Shiratsuchi, H., M. Shimoda, K. Irnayoshi, K. Nada, and Y. Osajima. 1994. Volatile flavor compounds in spray-dried skim milk powder. J. Agric. Food Chem. 48:984–988.

Sieber, R. 1994. Cholesterol removal from animal food—can it be justified? Lebensmitt. Wissenschaft Technol. 26:375–387.

Smith, L. M., W. L. Dunkley, A. Franke, and T. Dairiki. 1978. Measurement of *trans* and other isomeric unsaturated fatty acids in butter or margarine. J. Am. Oil Chem. Soc. 55:257–261.

Snedmen, A. E. M., I.-B. Gustafsson, L. G. T. Berglund, and B. O. H. Vessby. 1999. Pentadecanoic acid in serum as a marker for intake of milkfat and metabolic risk factors. Am. J. Clin. Nutr. 69:22–29.

Solomon, R., L. E. Chase, B. Ben-Ghedalia, and D. E. Bauman. 2000. The effect of nonstructural carbohydrate and addition of full fat extruded soybeans on the concentration of conjugated linoleic acid in the milk fat of dairy cows. J. Dairy Sci. 83:1322–1329.

Spanos, G. A., S. J. Schwartz, R. B. van Breemen, and C-H. Huang. 1995. High-performance liquid chromatography with light-scattering detection and desorption chemical-ionization tandem mass spectrometry of milk fat triacylglycerols. Lipids 30:85–90.

Stanton, C., F. Lawless, G. Kjellmer, D. Herrington, R. Devery, J. F. Connolly, and J. Murphy. 1997. Dietary influences on bovine milk *cis*-9, *trans*-11 conjugated linoleic acid content. J. Food Sci. 62:1083–1086.

Strocchi, A., and R. T. Holman. 1971. Analysis of fatty acids of butter fat. Riv. Ital. Sostanze Grasse 48:617–622.

Svahn, J. C. E., F. Fedl, N. C. Raiha, B. Koletzko, and I. E. M. Axelsson. 2000. Fatty acid content of plasma lipid fractions, blood lipids, and apolipoproteins in children fed milk products containing different quantity and quality of fat. J. Pediatr. Gastroenterol. Nutr. 31:152–161.

Takamizawa, K., M. Iwamori, M. Mutai, and Y. Nagai. 1986. Gangliosides of buttermilk. J. Biol. Chem. 261:5625–5630.

Tesfa, A. T., M. Tuori, and L. Syrjäläquist. 1991. High rapeseed oil feeding to lactating dairy cows and its effect on milk yield and composition in ruminants. Finn. J. Dairy Sci. 74:65–81.

ten Grotenhuis, E., G. A. van Aken, K. F. van Malssen, and H. Schenck. 1999. Polymorphism of milk fat studied by differential scanning calorimetry and real-time X-ray powder diffraction. J. Am. Oil Chem. Soc. 76:1031–1039.

Teter, B. T., J. Sampugna, and M. Keeney. 1990. Milkfat depression in the C57BI/6J mice consuming partially hydrogenated fat. J. Nutr. 120:818–824.

Thivierge, M. C., P. Y. Chouinard, J. Lévesque, V. Girard, J. R. Seoane, and G. J. Brisson. 1998. Effects of buffers on milk fatty acids and mammary arteriovenous differences in dairy cows fed Ca salts of fatty acids. J. Dairy Sci. 81:2001–2010.

Tholstrup, T., B. Sandstrom, J. E. Hermansen, and G. Holmer. 1998. Effect of modified dairy fat on postprandial and fasting plasma lipids in lipoproteins in healthy young men. Lipids 33:11–21.

Thomas, L. H., S. O. Olpin, R. G. Scott, and M. P. Wilkins. 1987. Coronary heart disease and the composition of adipose tissue taken at biopsy. Hum. Nutr. Food Sci. Nutr. 41F:167–172.

Thomas, L., and M. Rowney. 1996. Australian milk fat survey-fatty acid composition. Austral. J. Dairy Technol. 51:112–114.

Timmen, H., and S. Patton. 1989. Milk fat globules: Fatty acid composition, size, and in vivo regulation of fat liquidity. Lipids 23:685–689.

Tuomala, T., and K. Kailio. 1996. Identification of free fatty acids and some other volatile flavour compounds from Swiss cheese using on-line supercritical fluid extraction-gas chromatography. Z. Lebensm. Unters. Forsch. 203:236–240.

Ulberth, F., R. G. Gabernig, and F. Schramme. 1999. Flame-ionization detector response to methyl, ethyl, propyl, and butyl esters of fatty acids. J. Am. Oil Chem. Soc. 76:263–266.

USDA Nutrient Database for Standard Reference, Release 13 (Nov 1999). www.nal.usda.gov/fnic.

Vaghela, M. N., and A. Kilara. 1995a. A rapid method for extraction of total lipids from whey protein concentrates and separation of lipid classes with solid phase extraction. J. Am. Oil Chem. Soc. 72:1117–1121.

Vaghela, M. N., and A. Kilara. 1995b. Quantitative analyses of phospholipids from whey protein concentrates by high-performance liquid chromatography with a narrow-bore column and an evaporative light-scattering detector. J. Am. Oil Chem. Soc. 72:729–733.

Vaghela, M. N., and A. Kilara. 1996a. Foaming and emulsifying properties of whey protein concentrates as affected by lipid composition. J. Food Sci. 61:275–280.

Vaghela, M., and A. Kilara. 1996b. Lipid composition of whey protein concentrates manufactured commercially and in the laboratory. J. Dairy Sci. 79:1172–1183.

Valeur, A, N. U. Olsson, P. Kaufmann, S. Wada, C-G. Kroon, G. Westerdahl, and G. Odham. 1994. Quantification and comparison of some natural sphingomyelins by on-line high-performance liquid chromatography/discharge-assisted thermospray mass spectrometry. Biol. Mass Spectrom. 23:313–319.

Van Aken, G. A., and E. ten Grotenhuis, A. J. van Langevelde, and H. Schenk. 1999. Composition and crystallization of milk fat fractions. J. Am. Oil Chem. Soc. 76:1331–1323.

Van Aken, G. A., and K. A. Visser. 2000. Firmness and crystallization of milk fat in relation to processing conditions. J. Dairy Sci. 83:1919–1932.

Van Nevel, C. L., and D. I. Demeyer, 1994. Effect of pH on biohydrogenation of polyunsaturated fatty acids and their Ca-salts by microorganisms in vitro. Reprod. Nutr. Develop. 36:53–63.

Vesper, H., E.-M. Schmeitz, M. N. Nikolova-Karakashian, D. L. Dillehay, D. V. Lynch, and A. H. Merill, Jr. 1999. Sphingolipids in food and the emerging importance of sphingolipids to nutrition. J. Nutr. 129:1239–1250.

Visioli, F., P. Rise, E. Plasmati, F. Pazzucconi, C. R. Sirtori, and C. Galli. 2000. Very low intakes of N-3 fatty acids incorporated into bovine milk reduce plasma triacylglycerol and increase HDL concentrations in healthy subjects. Pharmacol. Res. 41:571–576.

Wagner, R., K. Aulrich, P. Lebzien, and G. Flachowsky. 1998. Research note: Effect of duodenal-infused unsaturated fatty acids on dairy milk composition. Arch. Anim. Nutr. 51:349–354.

Walstra, P. 1995. Physical chemistry of milk fat globules. Pages 131–178 in Advanced Dairy Chemistry. 2. Lipids. 2nd ed. P. F. Fox, ed. Chapman and Hall, New York, NY.

Walstra, P., T. von Vliet, and W. Klock. 1995, Crystallization and rheological properties of milk fat spreads. Pages 179–212 in Advanced Dairy Chemistry. 2. Lipids. 2nd ed., P. F. Fox, ed. Chapman and Hall, New York, NY.

Wang, W., J. C. Allen, and H. E. Swaisgood. 1997. Binding of vitamin D and cholesterol to β-lactoglobulin. J. Dairy Sci. 80:1054–1059.

Watkins, B. A., Y. Li, and M. S. Seifert. 1999. Bone metabolism and dietary conjugated linoleic acid. Pages 253–275 in Advances in Conjugated Linoleic Acid Research, Vol. 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press, Champaign, IL.

Weidong, M. A., B. A. Clement, and W. R. Klemm. 2000. Volatile compounds of bovine milk as related to the stage of the estrous cycle. J. Dairy Sci. 80:3227–3233.

Weihrauch, J. L. 1974. Trace constituents in milk fat. Lipids 9:883–890.

Weihrauch, J. L. 1988. Lipids of milk: Deterioration. Pages 215–278 in Fundamentals of Dairy Chemistry, 3rd. ed. N. P. Wong, R. Jenness, M. Keeney, and A. H. Marth, eds. Van Nostrand Reinhold Co., New York, NY.

Werner, S. A., L. O. Luedecke, and T. D. Shultz. 1992. Determination of conjugated linoleic acid content and isomer distribution in three Cheddar-type cheeses: Effects of cheese cultures, processing, and aging. J. Agric. Food Chem. 40:1817–1821.

Wolff, R. L. 1994. Contribution of *trans*-18:1 acids from dairy fat to European diets. J. Am. Oil. Chem. Soc. 71:277–283.

Wolff, R. L. 1995. Content and distribution of *trans*-18:1 acids in ruminant milk and meat fats. Their importance in European diets and their effect on human milk. J. Am. Oil Chem. Soc. 72: 259–272.

Wolff, R. L. 1999. Simple methods for the identification and quantification by GLC of most individual *trans*-18:1 isomers present in foods and human tissues. Lipid Technol. 11:16–18.

Wolff, R. L., and C. C. Bayard. 1995. Improvement in the resolution of individual *trans*-18:1 isomers by capillary gas liquid chromatography: Use of a 100 m CP-Si1 88 column. J. Am. Oil Chem. Soc. 72:1197–1201.

Wolff, R. L., C. C. Bayard, and R. J. Fabien. 1995. Evaluation of sequential methods for the determination of butterfat composition with emphasis on *trans*-18:1 acids: Application to the study of seasonal variations in French butters. J. Am. Oil Chem. Soc. 72:1471–1483.

Wolff, R. L., N. A. Combs, D. Precht, J. Molkentin and W. M. Nimal Ratnayake. 1998a. Accurate determination of *trans*-18:1 isomers by capillary gas-liquid chromatography on cyanoalkyl polysiloxane stationary phases. Oleagineaux, Corps Gras. Lipides 5:295–300.

Wolff, R. L., D. Precht, and J. Molkentin. 1998b. *Trans*-18:1 acid content and profile in human milk lipids. Critical survey of data in connection with analytical methods. J. Am. Oil Chem. Soc. 75:661–671.

Wonsil, B. J., J. J. Herbein, and B. A. Watkins. 1994. Dietary and ruminally derived *trans*-18:1 fatty acids alter bovine milk lipids. J. Nutr. 124:556–565.

Wright, A. J., S. S. Narind, and A. G. Marangoni. 2000. Comparison of experimental techniques used in lipid crystallization studies. J. Am. Oil Chem. Soc. 77:1239–1242.

Wright, T. C., B. McBride, and B. J. Holub. 1998a. Method for enriching docosahexaenoic acid in expressed milk of cattle. University of Guelph, Canada, assignee. U. S. Pat. Appl. U. S. 879774.

Wright, T. C., B. McBride, and B. J Holub. 1998b. Docosahexaenoic acid enriched milk. World Review Nutrition and Dietetics, Pages 160–165 in The Return of ω 3 Fatty Acids into the Food Supply. 1. Land Based Animal Food Products and Their Health Effects. Vol. 83. A. P. Simopoulos, ed. Karger, Basel, Switzerland.

Yamasaki, M., K. Kishihara, I. Ineda, M. Sugano, and K. Yamada. 1999. A recommended esterification method for gas chromatographic measurement of conjugated linoleic acid. J. Am. Oil Chem. Soc. 76:933–938.

Yurawecz, M. P., J. K. Hood, M. M. Mossoba, J. A. G. Roach, and Y. Ku. 1995. Furan fatty acids determined as oxidation products of conjugated octadecadienoic acid. Lipids 30:595–598.

Yurawecz, M. P., J. K. G. Kramer, and Y. Ku. 1999. Methylation procedures for conjugated linoleic acid. Pages 64–82 in Advances in Conjugated Linoleic Acid Research. Vol 1. M. P. Yurawecz, M. M. Mossoba, J. K. G. Kramer, M. W. Pariza, and G. J. Nelson, eds. Am. Oil Chem. Soc. Press. Champaign, IL.

Yurawecz, M. P., J. A. G. Roach, N. Sehat, M. M. Mossoba, J. K. G. Kramer, J. Fritsche, H. Steinhart, and Y. Ku. 1998. A new conjugated linoleic acid isomer, 7 *trans*, 9 *cis*-octadecadienoic acid in cow, cheese, beef, and human milk and adipose tissue. Lipids 33:803–809.

Yurawecz, M. P., N. Sehat, M. M. Mossoba, J. A. G. Roach, and Y. Ku. 1997. Oxidation products of conjugated linoleic acid and furan fatty acids. Pages 183–215 in New Techniques and Application in Lipid Analysis. R. E. McDonald and M. M. Mossoba, eds. Am. Oil Chem. Soc. Press, Champaign, IL.

Zegarska, Z., B. Paszcyk, and Z. Borejszo. 1996. *Trans* fatty acids in milk fat. Pol. J. Food Nutr. Sci. 5/46:89–96.

Zhou, Q., E. Wasowicz, B. Handler, L. Fleischer, and F. A. Kummerow. 2000. An excess concentration of oxysterols in the plasma is cytotoxic to cultured endothelial cells. Atherosclerosis 149:191–197.