3 Milk lipids

3.1 Introduction

The milks of all mammals contain lipids but the concentration varies widely between species from *c.* 2% to greater than 50% (Table 3.1). The principal function of dietary lipids is to serve as a source of energy for the neonate and the fat content in milk largely reflects the energy requirements of the species, e.g. land animals indigenous to cold environments and marine mammals secrete high levels of lipids in their milks.

Milk lipids are also important:

1. as a source of essential fatty acids (i.e. fatty acids which cannot be synthesized by higher animals, especially linoleic acid, $C_{18:2}$) and fat-soluble vitamins (A, D, E, K); and
2. for the flavour and rheological properties of dairy products and foods in which they are used.

Because of its wide range of fatty acids, the flavour of milk fat is superior to that of other fats. In certain products and after certain processes, fatty acids serve as precursors of very flavourful compounds such as methyl ketones and lactones. Unfortunately, lipids also serve as precursors of compounds

Table 3.1 The fat content of milks from various species ($g\ l^{-1}$)

Species	Fat content	Species	Fat content
Cow	33–47	Marmoset	77
Buffalo	47	Rabbit	183
Sheep	40–99	Guinea-pig	39
Goat	41–45	Snowshoe hare	71
Musk-ox	109	Muskrat	110
Dall-sheep	32–206	Mink	134
Moose	39–105	Chinchilla	117
Antelope	93	Rat	103
Elephant	85–190	Red kangaroo	9–119
Human	38	Dolphin	62–330
Horse	19	Manatee	55–215
Monkeys	10–51	Pygmy sperm whale	153
Lemurs	8–33	Harp seal	502–532
Pig	68	Bear (four species)	108–331

From Christie (1995).

that cause off-flavour defects (hydrolytic and oxidative rancidity) and as solvents for compounds in the environment which may cause off-flavours.

For many years, the economic value of milk was based mainly or totally on its fat content, which is still true in some cases. This practice was satisfactory when milk was used mainly or solely for butter production. Possibly, the origin of paying for milk on the basis of its fat content, apart from its value for butter production, lies in the fact that relatively simple quantitative analytical methods were developed for fat earlier than for protein or lactose. Because of its economic value, there has long been commercial pressure to increase the yield of milk fat per cow by nutritional or genetic means.

To facilitate the reader, the nomenclature, structure and properties of the principal fatty acids and of the principal lipid classes are summarized in Appendices 3A, 3B and 3C. The structure and properties of the fat-soluble vitamins, A, D, E and K, are discussed in Chapter 6.

3.2 Factors that affect the fat content of bovine milk

Bovine milk typically contains *c.* 3.5% fat but the level varies widely, depending on several factors, including: breed, individuality of the animal, stage of lactation, season, nutritional status, type of feed, health and age of the animal, interval between milkings and the point during milking when the sample is taken.

Of the common European breeds, milk from Jersey cows contains the highest level of fat and that from Holstein/Friesians the lowest (Figure 3.1). The data in Figure 3.1 also show the very wide range of fat content in individual-cow samples.

The fat content of milk decreases during the first 4–6 weeks after parturition and then increases steadily throughout the remainder of lactation, especially toward the end (Figure 3.2). For any particular population, fat content is highest in winter and lowest in summer, due partly to the effect of environmental temperature. Production of creamery (manufacturing) milk in Ireland, New Zealand and parts of Australia is very seasonal; lactational, seasonal and possibly nutritional effects coincide, leading to large seasonal changes in the fat content of milk (Figure 3.3), and also in the levels of protein and lactose.

For any individual animal, fat content decreases slightly during successive lactations, by *c.* 0.2% over a typical productive lifetime (about five lactations). In practice, this factor usually has no overall effect on the fat content of a bulk milk supply because herds normally include cows of various ages. The concentration of fat (and of all other milk-specific constituents) decreases markedly on mastitic infection due to impaired

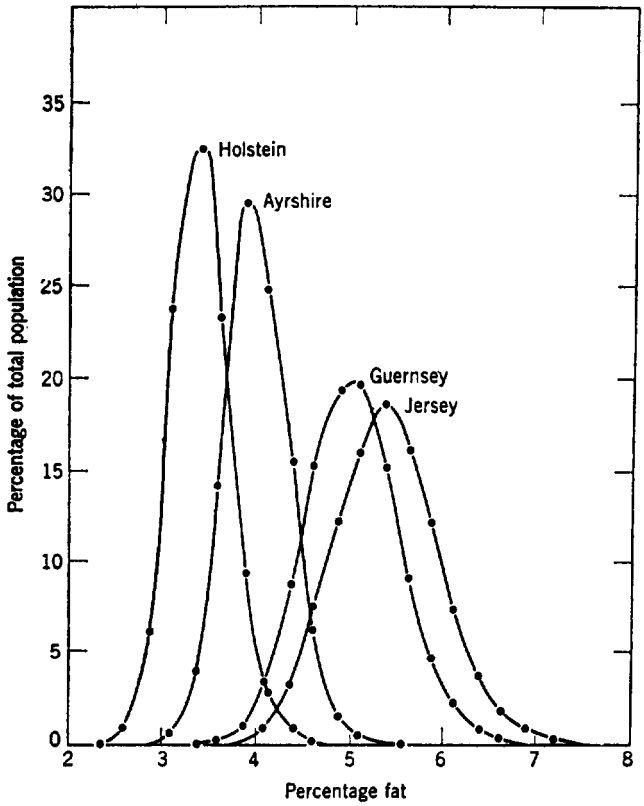


Figure 3.1 Range of fat content in the milk of individual cows of four breeds (from Jenness and Patton, 1959).

synthesizing ability of the mammary tissue; the effect is clear-cut in the case of clinical mastitis but is less so for subclinical infection.

Milk yield is reduced by underfeeding but the concentration of fat usually increases, with little effect on the amount of fat produced. Diets low in roughage have a marked depressing effect on the fat content of milk, with little effect on milk yield. Ruminants synthesize milk fat mainly from carbohydrate-derived precursors; addition of fat to the diet usually causes slight increases in the yield of both milk and fat, with little effect on fat content of milk. Feeding of some fish oils (e.g. cod liver oil, in an effort to increase the concentrations of vitamins A and D in milk) has a very marked (*c.* 25%) depressing effect on the fat content of milk, apparently due to the high level of polyunsaturated fatty acids (the effect is eliminated by hydrogenation), although oils from some fish species do not cause this effect.

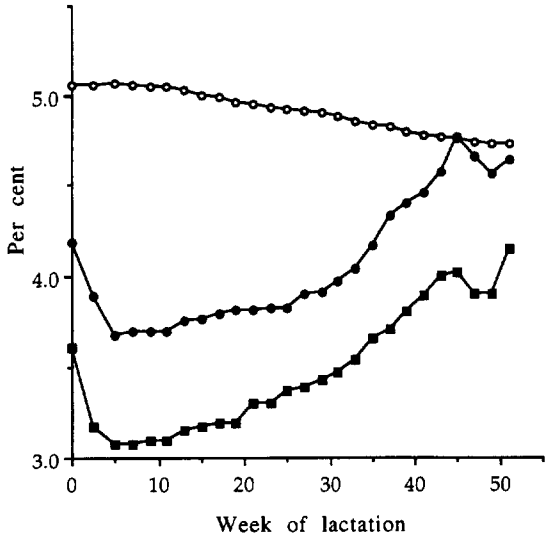


Figure 3.2 Typical changes in the concentrations of fat (●), protein (■) and lactose (○) in bovine milk during lactation.

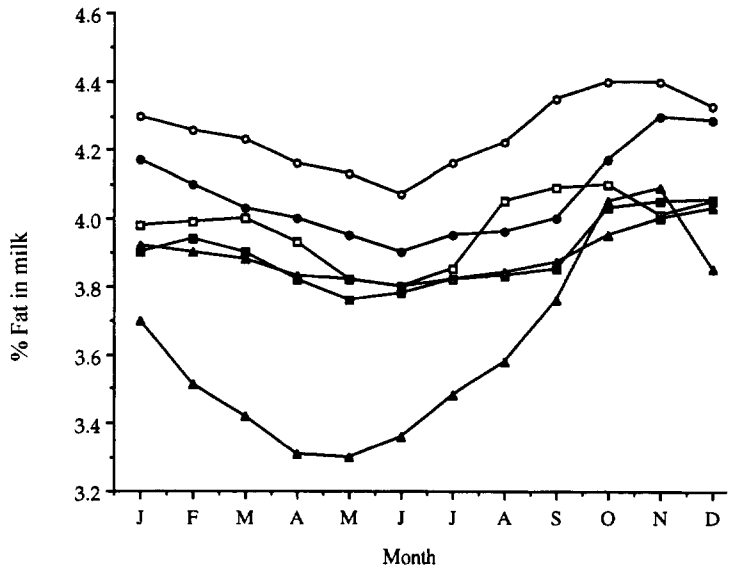


Figure 3.3 Seasonal changes in the fat content of bovine milk in some European countries: (Denmark (○), Netherlands (●), United Kingdom (□), France (■), Germany (△), Ireland (▲) (From An Foras Taluntais, 1981.)

The quarters of a cow's udder are anatomically separate and secrete milk of markedly different composition. The fat content of milk increases continuously throughout the milking process while the concentrations of the various non-fat constituents show no change; fat globules appear to be partially trapped in the alveoli and their passage is hindered. If a cow is incompletely milked, the fat content of the milk obtained at that milking will be reduced; the 'trapped' fat will be expressed at the subsequent milking, giving an artificially high value for fat content.

If the intervals between milkings are unequal (as they usually are in commercial farming), the yield of milk is higher and its fat content lower after the longer interval; the content of non-fat solids is not influenced by milking interval.

3.3 Classes of lipids in milk

Triacylglycerols (triglycerides) represent 97–98% of the total lipids in the milks of most species (Table 3.2). The diglycerides probably represent incompletely synthesized lipids in most cases, although the value for the rat probably also includes partially hydrolysed triglycerides, as indicated by the high concentration of free fatty acids, suggesting damage to the milk fat globule membrane (MFGM) during milking and storage. The very high level of phospholipids in mink milk probably indicates the presence of mammary cell membranes.

Although phospholipids represent less than 1% of total lipid, they play a particularly important role, being present mainly in the MFGM and other membraneous material in milk. The principal phospholipids are phosphatidylcholine, phosphatidylethanolamine and sphingomyelin (Table 3.3). Trace amounts of other polar lipids, including ceramides, cerobrosides and gangliosides, are also present. Phospholipids represent a considerable proportion of the total lipid of buttermilk and skim milk (Table 3.4), reflecting

Table 3.2 Composition of individual simple lipids and total phospholipids in milks of some species (weight % of the total lipids)

Lipid class	Cow	Buffalo	Human	Pig	Rat	Mink
Triacylglycerols	97.5	98.6	98.2	96.8	87.5	81.3
Diacylglycerols	0.36		0.7	0.7	2.9	1.7
Monoacylglycerols	0.027		T	0.1	0.4	T
Cholesteryl esters	T	0.1	T	0.06	–	T
Cholesterol	0.31	0.3	0.25	0.6	1.6	T
Free fatty acids	0.027	0.5	0.4	0.2	3.1	1.3
Phospholipids	0.6	0.5	0.26	1.6	0.7	15.3

From Christie (1995). T, Trace.

Table 3.3 Composition of the phospholipids in milk from various species (expressed as mol % of total lipid phosphorus)

Species	Phosphatidyl-choline	Phosphatidyl-ethanolamine	Phosphatidyl-serine	Phosphatidyl-inositol	Sphingomyelin	Lysophospho-lipids[a]
Cow	34.5	31.8	3.1	4.7	25.2	0.8
Sheep	29.2	36.0	3.1	3.4	28.3	
Buffalo	27.8	29.6	3.9	4.2	32.1	2.4
Goat	25.7	33.2	6.9	5.6[b]	27.9	0.5
Camel	24.0	35.9	4.9	5.9	28.3	1.0
Ass	26.3	32.1	3.7	3.8	34.1	
Pig	21.6	36.8	3.4	3.3	34.9	
Human	27.9	25.9	5.8	4.2	31.1	5.1
Cat	25.8	22.0	2.7	7.8[b]	37.9	3.4
Rat	38.0	31.6	3.2	4.9	19.2	3.1
Guinea-pig	35.7	38.0	3.2	7.1[b]	11.0	2.0
Rabbit	32.6	30.0	5.2	5.8[b]	24.9	0.4
Mouse[c]	32.8	39.8	10.8	3.6	12.5	
Mink	52.8	10.0	3.6	6.6	15.3	8.3

[a]Mainly lysophosphatidylcholine but also lysophosphatidylethanolamine.
[b]Also contains lysophosphatidylethanolamine.
[c]Analysis of milk fat globule membrane phospholipids.
From Christie (1995).

Table 3.4 Total fat and phospholipid content of some milk products

Product	Total lipid (%, w/v)	Phospholipids (%, w/v)	Phospholipid as %, w/w, of total lipids
Whole milk	3–5	0.02–0.04	0.6–1.0
Cream	10–50	0.07–0.18	0.3–0.4
Butter	81–82	0.14–0.25	0.16–0.29
Butter oil	~100	0.02–0.08	0.02–0.08
Skim milk	0.03–0.1	0.01–0.06	17–30
Buttermilk	2	0.03–0.18	10

the presence of proportionately larger amounts of membrane material in these products.

Cholesterol (Appendix 3C) is the principal sterol in milk (>95% of total sterols); the level (~0.3%, w/w, of total lipids) is low compared with many other foods. Most of the cholesterol is in the free form, with less than 10% as cholesteryl esters. Several other sterols, including steroid hormones, occur at trace levels.

Several hydrocarbons occur in milk in trace amounts. Of these, carotenoids are the most significant. In quantitative terms, carotenes occur at only trace levels in milk (typically ~200 $\mu g\,l^{-1}$) but they contribute 10–50% of the vitamin A activity in milk (Table 3.5) and are responsible for the yellow colour of milk fat. The carotenoid content of milk varies with breed (milk from Channel Island breeds contains 2–3 times as much β-carotene as milk from other breeds) and very markedly with season (Figure 3.4). The latter reflects differences in the carotenoid content of the diet (since they are totally derived from the diet); fresh pasture, especially if it is rich in clover and alfalfa, is much richer in carotenoids than hay or silage (due to oxidation on conservation) or cereal-based concentrates. The higher the carotenoid content of the diet, the more yellow will be the colour of milk and milk fat, e.g. butter from cows on pasture is yellower than that

Table 3.5 Vitamin A activity and β-carotene in milk of different breeds of cows

	Channel Island breeds		Non-Channel Island breeds	
	Summer	Winter	Summer	Winter
Retinol ($\mu l\,l^{-1}$)	649	265	619	412
β-Carotene ($\mu l\,l^{-1}$)	1143	266	315	105
Retinol/β-carotene ratio	0.6	11.0	2.0	4.0
Contribution (%) of β-carotene to vitamin A activity	46.8	33.4	20.3	11.4

Modified from Cremin and Power (1985).

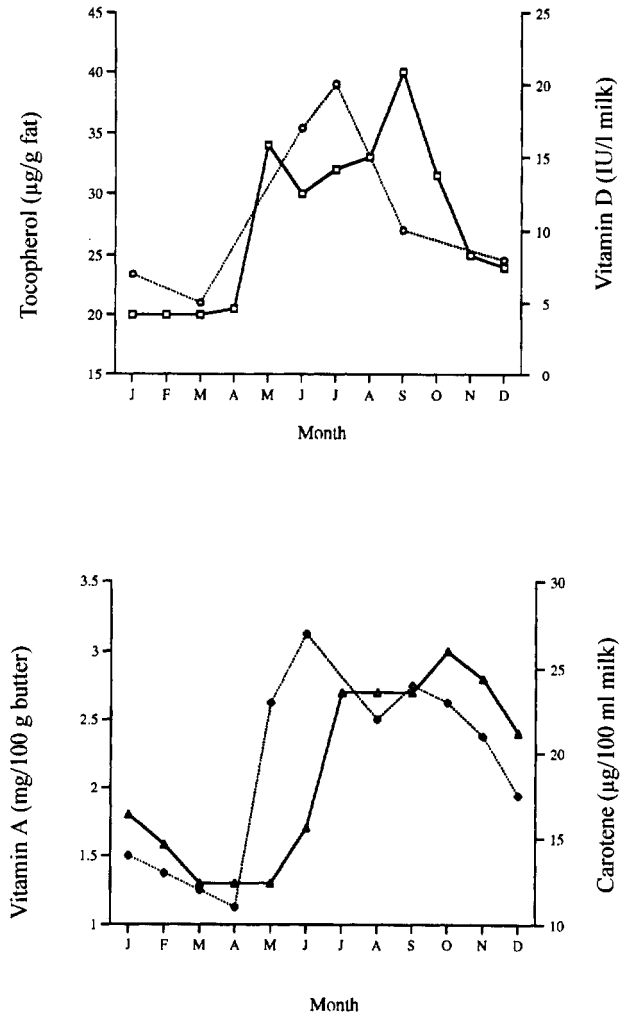


Figure 3.4 Seasonal variations in the concentration of β-carotene (◇) and of vitamins A (△), D (○) and E (□) in milk and milk products (from Cremin and Power, 1985).

from cows on winter feed, especially if the pasture is rich in clover (New Zealand butter is more yellow than Irish butter which in turn is more yellow than mainland European or US butter). Sheep and goats do not transfer carotenoids to their milks which are, consequently, much whiter than bovine milk. This may reduce the acceptability of dairy products (e.g. cheeses, butter, cream, ice-cream) made from bovine milk in regions where goats' or sheep's milk is traditional (the carotenoids may be bleached by using

peroxides, e.g. H_2O_2 or benzoyl peroxide, or masked, e.g. with chlorophyll or titanium oxide).

Milk contains significant concentrations of fat-soluble vitamins (Table 3.5, Figure 3.4) and milk and dairy products make a significant contribution to the dietary requirements for these vitamins in Western countries. The actual form of the fat-soluble vitamins in milk appears to be uncertain and their concentration varies widely with breed of animal, feed and stage of lactation, e.g. the vitamin A activity of colostrum is *c.* 30 times higher than that of mature milk.

Several prostaglandins occur in milk but it is not known whether they play a physiological role; they may not survive storage and processing in a biologically active form. Human milk contains prostaglandins E and F at concentrations 100-fold higher than human plasma and these may have a physiological function, e.g. gut motility.

3.4 Fatty acid profile of milk lipids

Milk fats, especially ruminant fats, contain a very wide range of fatty acids: more than 400 and 184 distinct acids have been detected in bovine and human milk fats, respectively (Christie, 1995). However, the vast majority of these occur at only trace concentrations. The concentrations of the principal fatty acids in milk fats from a range of species are shown in Table 3.6.

Notable features of the fatty acid profiles of milk lipids include:

1. Ruminant milk fats contain a high level of butanoic acid ($C_{4:0}$) and other short-chain fatty acids. The method of expressing the results in Table 3.6 (%, w/w) under-represents the proportion of short-chain acids – if expressed as mol %, butanoic acid represents *c.* 10% of all fatty acids (up to 15% in some samples), i.e. there could be a butyrate residue in *c.* 30% of all triglyceride molecules. The high concentration of butyric (butanoic) acid in ruminant milk fats arises from the direct incorporation of β-hydroxybutyrate (which is produced by micro-organisms in the rumen from carbohydrate and transported via the blood to the mammary gland where it is reduced to butanoic acid). Non-ruminant milk fats contain no butanoic or other short-chain acids; the low concentrations of butyrate in milk fats of some monkeys and the brown bear require confirmation.

   The concentration of butanoic acid in milk fat is the principle of the widely used criterion for the detection and quantitation of adulteration of butter with other fats, i.e. Reichert Meissl and Polenski numbers, which are measures of the volatile water-soluble and volatile water-insoluble fatty acids, respectively.

   Short-chain fatty acids have strong, characteristic flavours and aromas. When these acids are released by the action of lipases in milk or

Table 3.6 Principal fatty acids (wt % of total) in milk triacylglycerols or total lipids from various species

Species	4:0	6:0	8:0	10:0	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	C_{20}–C_{22}
Cow	3.3	1.6	1.3	3.0	3.1	9.5	26.3	2.3	14.6	29.8	2.4	0.8	T
Buffalo	3.6	1.6	1.1	1.9	2.0	8.7	30.4	3.4	10.1	28.7	2.5	2.5	T
Sheep	4.0	2.8	2.7	9.0	5.4	11.8	25.4	3.4	9.0	20.0	2.1	1.4	–
Goat	2.6	2.9	2.7	8.4	3.3	10.3	24.6	2.2	12.5	28.5	2.2	–	–
Musk-ox	T	0.9	1.9	4.7	2.3	6.2	19.5	1.7	23.0	27.2	2.7	3.0	0.4
Dall-sheep	0.6	0.3	0.2	4.9	1.8	10.6	23.0	2.4	15.5	23.1	4.0	4.1	2.6
Moose	0.4	T	8.4	5.5	0.6	2.0	28.4	4.3	4.5	21.2	20.2	3.7	–
Blackbuck antelope	6.7	6.0	2.7	6.5	3.5	11.5	39.3	5.7	5.5	19.2	3.3	–	–
Elephant	7.4	–	0.3	29.4	18.3	5.3	12.6	3.0	0.5	17.3	3.0	0.7	–
Human	–	T	T	1.3	3.1	5.1	20.2	5.7	5.9	46.4	13.0	1.4	T
Monkey (mean of six species	0.4	0.6	5.9	11.0	4.4	2.8	21.4	6.7	4.9	26.0	14.5	1.3	–
Baboon	–	0.4	5.1	7.9	2.3	1.3	16.5	1.2	4.2	22.7	37.6	0.6	–
Lemur macaco	–	–	0.2	1.9	10.5	15.0	27.1	9.6	1.0	25.7	6.6	0.5	–
Horse	–	T	1.8	5.1	6.2	5.7	23.8	7.8	2.3	20.9	14.9	12.6	–
Pig	–	–	–	0.7	0.5	4.0	32.9	11.3	3.5	35.2	11.9	0.7	–
Rat	–	–	1.1	7.0	7.5	8.2	22.6	1.9	6.5	26.7	16.3	0.8	1.1
Guinea-pig	–	T	–	–	–	2.6	31.3	2.4	2.9	33.6	18.4	5.7	T
Marmoset	–	–	–	8.0	8.5	7.7	18.1	5.5	3.4	29.6	10.9	0.9	7.0
Rabbit	–	T	22.4	20.1	2.9	1.7	14.2	2.0	3.8	13.6	14.0	4.4	T
Cottontail rabbit	–	–	9.6	14.3	3.8	2.0	18.7	1.0	3.0	12.7	24.7	9.8	0.4
European hare	–	T	10.9	17.7	5.5	5.3	24.8	5.0	2.9	14.4	10.6	1.7	T
Mink	–	–	–	–	0.5	3.3	26.1	5.2	10.9	36.1	14.9	1.5	–
Chinchilla	–	–	–	–	T	3.0	30.0	–	–	35.2	26.8	2.9	–
Red kangaroo	–	–	–	–	0.1	2.7	31.2	6.8	6.3	37.2	10.4	2.1	0.1
Platypus	–	–	–	–	–	1.6	19.8	13.9	3.9	22.7	5.4	7.6	12.2
Numbat	–	–	–	–	0.1	0.9	14.1	3.4	7.0	57.7	7.9	0.1	0.2
Bottle-nosed dolphin	–	–	–	–	0.3	3.2	21.1	13.3	3.3	23.1	1.2	0.2	17.3
Manatee	–	–	0.6	3.5	4.0	6.3	20.2	11.6	0.5	47.0	1.8	2.2	0.4
Pygmy sperm whale	–	–	–	–	–	3.6	27.6	9.1	7.4	46.6	0.6	0.6	4.5
Harp seal	–	–	–	–	–	5.3	13.6	17.4	4.9	21.5	1.2	0.9	31.2
Northern elephant seal	–	–	–	–	–	2.6	14.2	5.7	3.6	41.6	1.9	–	29.3
Polar bear	–	T	–	T	0.5	3.9	18.5	16.8	13.9	30.1	1.2	0.4	11.3
Grizzly bear	–	T	–	–	0.1	2.7	16.4	3.2	20.4	30.2	5.6	2.3	9.5

From Christie (1995).

dairy products, they impart strong flavours which are undesirable in milk or butter (they cause hydrolytic rancidity) but they contribute to the desirable flavour of some cheeses, e.g. Blue, Romano, Parmesan.

2. Ruminant milk fats contain low levels of polyunsaturated fatty acids (PUFAs) in comparison with monogastric milk fats. This is because a high proportion of the fatty acids in monogastric milk fats are derived from dietary lipids (following digestion and absorption) via blood. Unsaturated fatty acids in the diet of ruminants (grass contains considerable levels of PUFAs) are hydrogenated by rumen micro-organisms unless protected by encapsulation (section 3.16.1). The low level of PUFAs in bovine milk fat is considered to be nutritionally undesirable.
3. The milk fats from marine mammals contain high levels of long-chain, highly unsaturated fatty acids, presumably reflecting the requirement that the lipids of these species remain liquid at the low temperatures of their environments.
4. Ruminant milk fats are also rich in medium-chain fatty acids. These are synthesized in the mammary gland via the usual malonyl CoA pathway (section 3.5) and are released from the synthesizing enzyme complex by thioacylases; presumably, the higher levels of medium chain acids in ruminant milk fats compared with those of monogastric animals reflect higher thioacylase activity in the mammary tissue of the former.
5. The fatty acid profile of bovine milk fat shows a marked seasonal pattern, especially when cows are fed on pasture in summer. Data for Irish milk fat are shown in Figure 3.5; the changes are particularly marked for $C_{4:0}$, $C_{16:0}$ and $C_{18:1}$. These changes affect the Reichert Meissl, Polenski and iodine (a measure of unsaturation) (Figure 3.6) numbers and the melting point and hardness (spreadability) of butter made from these milks: winter butter, with low levels of $C_{4:0}$ and $C_{18:1}$ and a high level of $C_{16:0}$ is much harder than summer butter (Figure 3.7).
6. Unsaturated fatty acids may occur as *cis* or *trans* isomers; *trans* isomers, which have higher melting points than the corresponding *cis* isomers, are considered to be nutritionally undesirable. Bovine milk fat contains a low level (5%) of *trans* fatty acids in comparison with chemically hydrogenated (hardened) vegetable oils, in which the value may be 50% due to non-stereospecific hydrogenation.

Bovine milk fat contains low concentrations of keto and hydroxy acids (each at *c.* 0.3% of total fatty acids). The keto acids may have the carbonyl group (C=O) at various positions. The 3-keto acids give rise to methyl ketones ($R-\overset{\overset{O}{\|}}{C}-CH_3$) on heating (high concentrations of methyl ketones are produced in blue cheeses through the oxidative activity of *Penicillium roqueforti*). The position of the hydroxy group on the hydroxy acids also

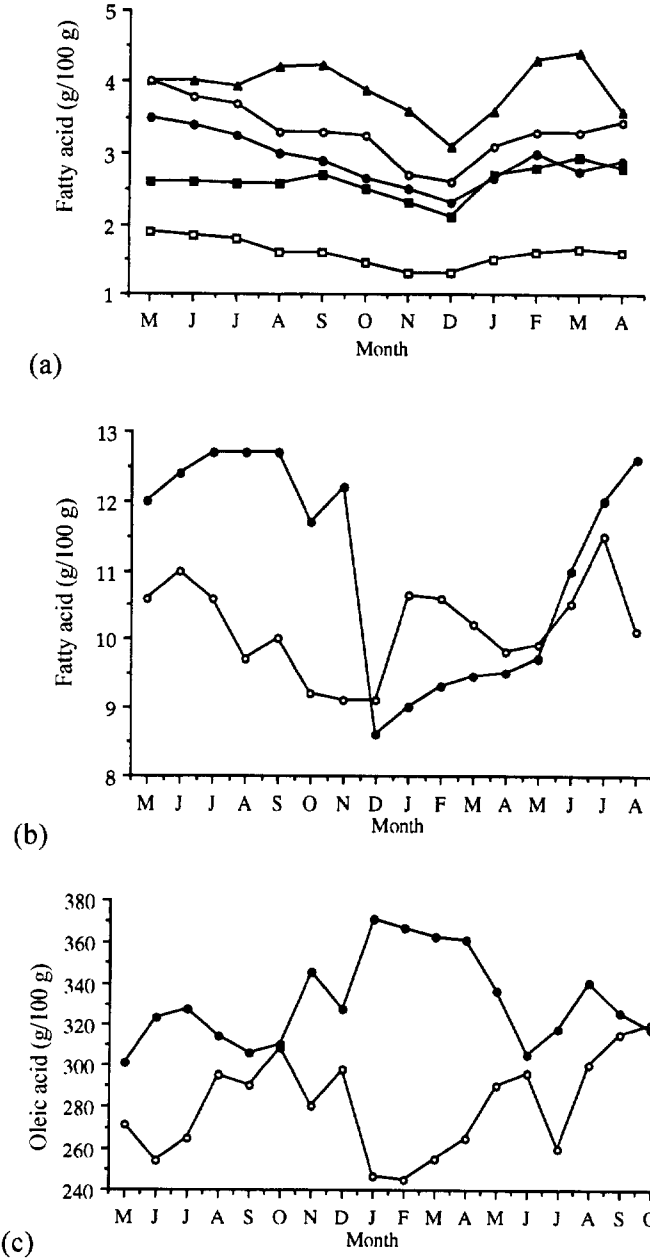


Figure 3.5 Seasonal changes in the concentration of individual fatty acids in Irish bovine milk fat. (a) $C_{4:0}$ (▲), $C_{6:0}$ (■), $C_{8:0}$ (□), $C_{10:0}$ (●), $C_{12:0}$ (○); (b) $C_{14:0}$ (○), $C_{18:0}$ (●); (c) $C_{16:0}$ (●), $C_{18:1}$ (○). From Cullinane *et al.*, 1984a.)

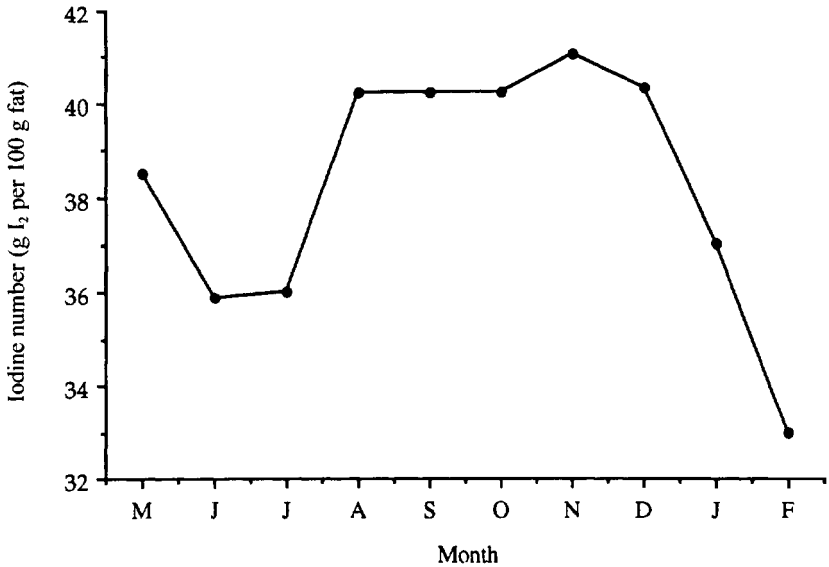


Figure 3.6 Seasonal changes in the iodine number of Irish bovine milk fat (from Cullinane *et al.*, 1984a).

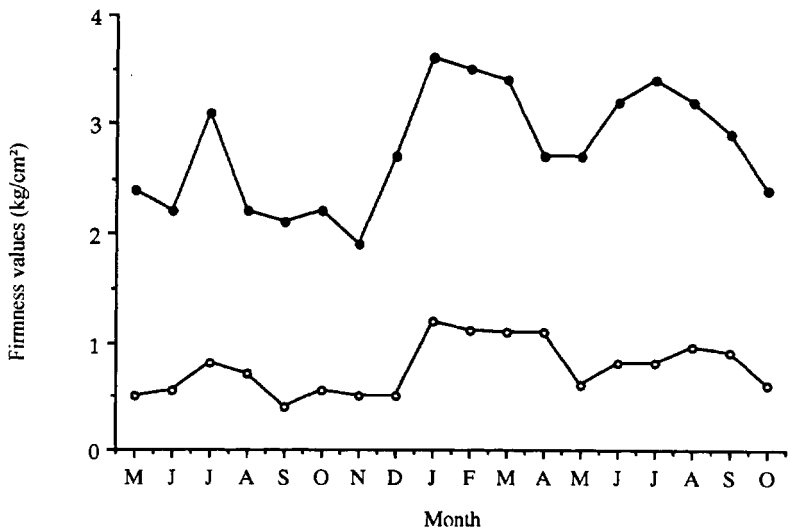


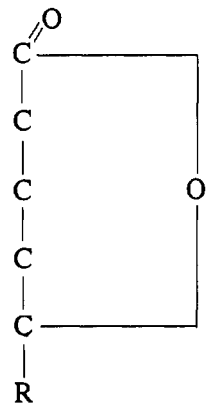
Figure 3.7 Seasonal variations in the mean firmness of Irish butter at 4°C (●) or 15°C (○) (from Cullinane *et al.*, 1984b).

Table 3.7 The fatty acid composition of cholesteryl esters, phosphatidylcholine and phosphatidylethanolamine in the milks of some species

	Fatty acid composition (wt% of the total)												
	Cow			Human			Pig		Mink			Mouse	
Fatty acid	CE	PC	PE	CE	PC	PE	PC	PE	CE	PC	PE	PC	PE
12:0	0.2	0.3	0.1	3.2	–	–			0.3	–	–	–	–
14:0	2.3	7.1	1.0	4.8	4.5	1.1	1.8	0.4	1.1	1.3	0.8	–	4.5
16:0	23.1	32.2	11.4	23.8	33.7	8.5	39.9	12.4	25.4	26.4	20.6	20.3	8.9
16:1	8.8	3.4	2.7	1.5	1.7	2.4	6.3	7.3	4.4	1.1	1.2	–	2.7
18:0	10.6	7.5	10.3	8.0	23.1	29.1	10.3	12.3	14.7	20.8	29.3	30.0	18.0
18:1	17.1	30.1	47.0	45.7	14.0	15.8	21.8	36.2	35.7	31.7	27.8	13.9	19.8
18:2	27.1	8.9	13.5	12.4	15.6	17.7	15.9	17.8	13.5	17.4	19.1	22.8	17.2
18:3	4.2	1.4	2.3	T	1.3	4.1	1.5	1.9	2.6	2.2	0.5	–	–
20:3	0.7	1.0	1.7	–	2.1	3.4	0.3	0.7	–	–	–	–	–
20:4	1.4	1.2	2.7	T	3.3	12.5	1.3	6.6	–	–	–	8.9	20.0
22:6	–	–	0.1	–	0.4	2.6	0.2	1.6	–	–	–	1.8	6.3

Abbreviations: CE, cholesteryl esters; PC, phosphatidylcholine; PE, phosphatidylethanolamine; T, trace amount. From Christie (1995).

varies; some can form lactones, e.g. the 4- and 5-hydroxy acids can form γ- and δ-lactones, respectively.



A δ-lactone

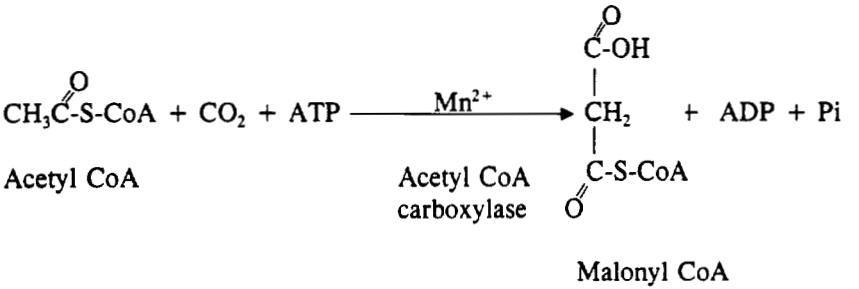
Lactones have strong flavours; traces of δ-lactones are found in fresh milk and contribute to the flavour of milk fat, but higher concentrations may occur in dried milk or butter oil as a result of heating or prolonged storage and may cause atypical flavours.

The fatty acids in the various polar lipids and cholesteryl esters are long-chain, saturated or unsaturated acids, with little or no acids of less than $C_{12:0}$ (Table 3.7; for further details see Christie, 1995).

3.5 Synthesis of fatty acids in milk fat

In non-ruminants, blood glucose is the principal precursor of fatty acids in milk fat; the glucose is converted to acetyl CoA in the mammary gland. In ruminants, acetate and β-hydroxybutyrate, produced by micro-organisms in the rumen and transported to the blood, are the principal precursors; in fact, ruminant mammary tissue has little 'ATP citrate lyase' activity which is required for fat synthesis from glucose. Blood glucose is low in ruminants and is conserved for lactose synthesis. The differences in fatty acid precursors are reflected in marked interspecies differences in milk fatty acid profiles. Restriction of roughage in the diet of ruminants leads to suppression of milk fat synthesis, possibly through a reduction in the available concentration of acetate and β-hydroxybutyrate.

In all species, the principal precursor for fatty acid synthesis is acetyl CoA, derived in non-ruminants from glucose and in ruminants from acetate or oxidation of β-hydroxybutyrate. Acetyl CoA is first converted, in the cytoplasm, to malonyl CoA:



Reduced bicarbonate supply (source of CO_2) depresses fatty acid synthesis.

Some β-hydroxybutyrate is reduced to butyrate and incorporated directly into milk fat; hence, the high level of this acid in ruminant milk fat.

In non-ruminants, the malonyl CoA is combined with an 'acyl carrier protein' (ACP) which is part of a six-enzyme complex (molecular weight *c.* 500 kDa) located in the cytoplasm. All subsequent steps in fatty acid synthesis occur attached to this complex; through a series of steps and repeated cycles, the fatty acid is elongated by two carbon units per cycle (Figure 3.8, see also Lehninger, Nelson and Cox, 1993).

The net equation for the synthesis of a fatty acid is:

$$n\ \text{Acetyl CoA} + 2(n-1)\text{NADPH} + 2(n-1)\text{H}^+ + (n-1)\text{ATP}$$
$$+ (n-1)\text{CO}_2 \rightarrow \text{CH}_3\text{CH}_2(\text{CH}_2\text{CH}_2)_{n-1}\text{CH}_2\text{C}(=\text{O})\text{—CoA} + (n-1)\text{CoA}$$
$$+ (n-1)\text{ADP} + (n-1)\text{P}_i + 2(n-1)\text{NADP} + (n-1)\text{CO}_2$$

The large supply of NADPH required for the above reactions is obtained through the metabolism of glucose-6-phosphate via the pentose pathway.

In ruminants, β-hydroxybutyrate is the preferred chain initiator (labelled β-hydroxybutyrate appears as the terminal four carbons of short- to medium-chain acids), i.e. the first cycle in fatty acid synthesis commences at β-hydroxybutyryl-S-ACP.

Synthesis of fatty acids via the malonyl CoA pathway does not proceed beyond palmitic acid ($C_{16:0}$) and mammary tissue contains an enzyme, thioacylase, capable of releasing the acyl fatty acid from the carrier protein at any stage between C_4 and C_{16}. Probable interspecies differences in the activity of thioacylase may account for some of the interspecies differences in milk fatty acid profiles.

The malonyl CoA pathway appears to account for 100% of the C_{10}, C_{12} and C_{14}, and *c.* 50% of the $C_{16:0}$ acids in ruminant milk fat, as indicated by labelling experiments (Figure 3.9). However, C_4, C_6 and C_8 are synthesized

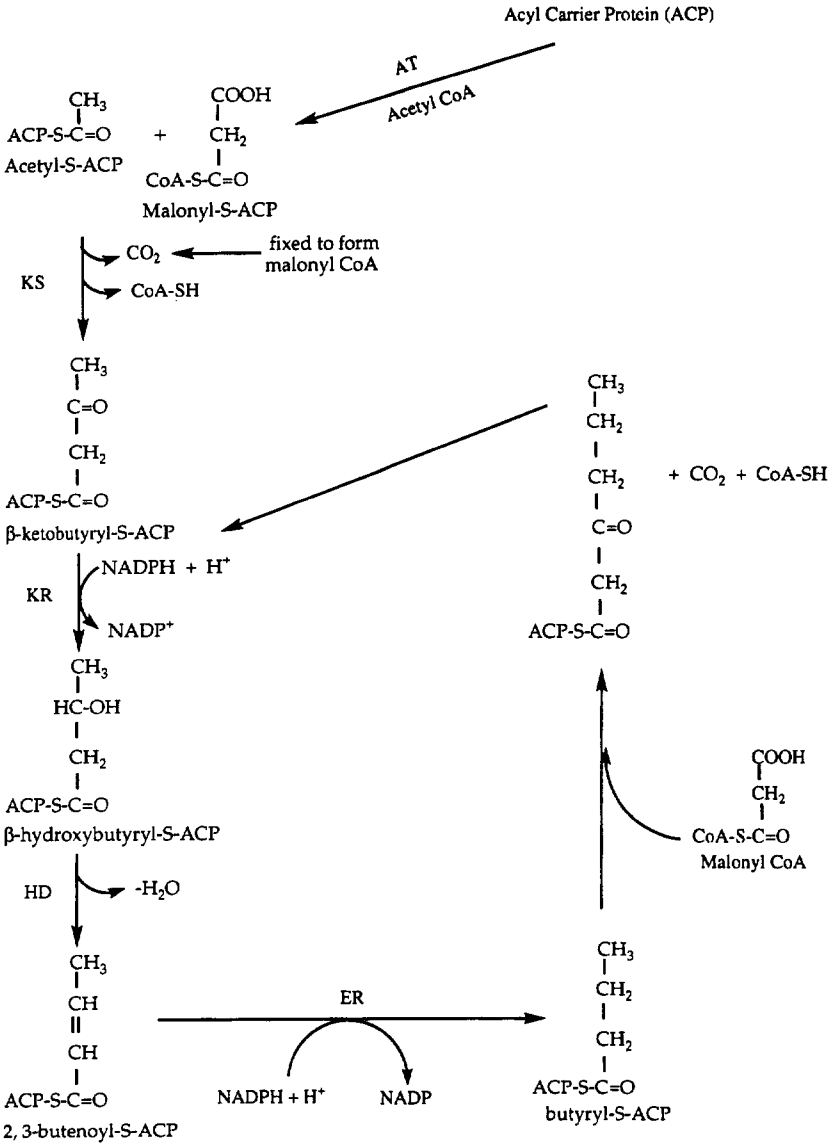


Figure 3.8 One complete cycle and the first step in the next cycle of the events during the synthesis of fatty acids. ACP = acyl carrier protein, a complex of six enzymes: i.e. acetyl CoA-ACP transacetylase (AT); malonyl CoA-ACP transferase (MT); β-keto-ACP synthase (KS); β-ketoacyl-ACP reductase (KR); β- hydroxyacyl-ACP-dehydrase (HD); enoyl-ACP reductase (ER).

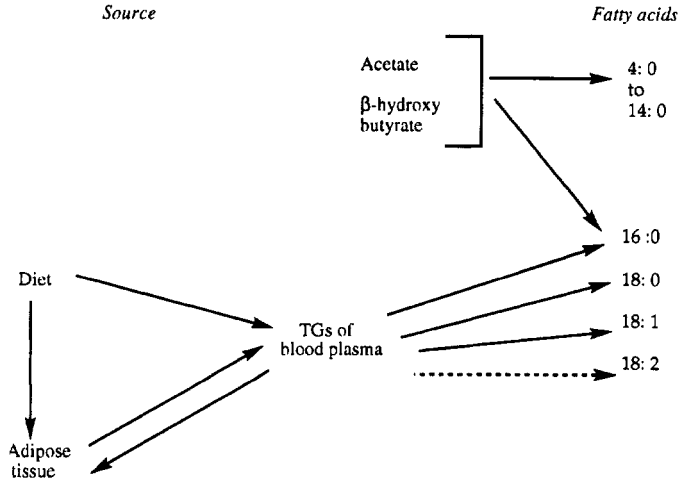


Figure 3.9 Sources of the fatty acids in bovine milk fat; TG, triglyceride (from Hawke and Taylor, 1995).

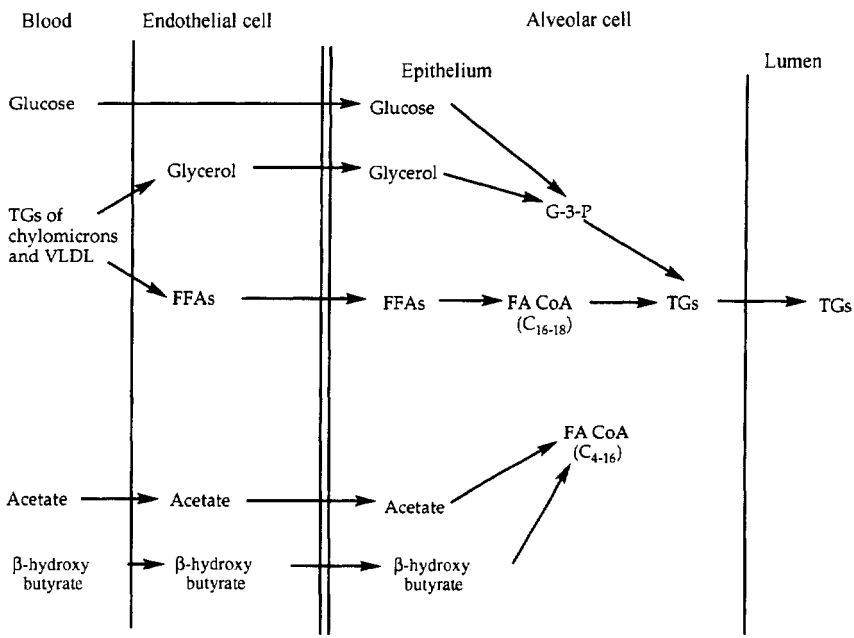


Figure 3.10 Uptake of blood constituents by the mammary gland; CoA, coenzyme A; G-3-P, glycerol-3-phosphate; FFA, free fatty acid; FA, fatty acid; TG, triglyceride, VLDL, very low density lipoprotein (from Hawke and Taylor, 1995).

from β-hydroxybutyrate and acetate mainly via two other pathways not involving malonyl CoA.

In the mammary gland, essentially 100% of $C_{18:0}$, $C_{18:2}$ and *c.* 50% of C_{16} are derived from blood lipids (chylomicrons, free triglycerides, free fatty acids, cholesteryl esters). The blood lipids are hydrolysed by lipoprotein lipase which is present in the alveolar blood capillaries, the activity of which increases eightfold on initiation of lactation. The resulting monoglycerides, free fatty acids and some glycerol are transported across the basal cell membrane and re-incorporated into triglycerides inside the mammary cell (Figure 3.10).

In blood, lipids exist as lipoprotein particles, the main function of which is to transport lipids to and from various tissues and organs of the body. There is considerable interest in blood lipoproteins from the viewpoint of human health, especially obesity and cardiovascular diseases. Lipoproteins are classified into four groups on the basis of density, which is essentially a function of their triglyceride content, i.e. chylomicrons, very low density lipoprotein particles (VLDL), low density lipoprotein (LDL) particles and high density lipoprotein (HDL) particles, containing *c.* 98, 90, 77 and 45% total lipid, respectively (Figure 3.11).

Lipoproteins, especially chylomicrons, are at an elevated level in the blood after eating, especially after high-fat meals, and give blood serum a milky appearance. They are also elevated during or after tension (so-called

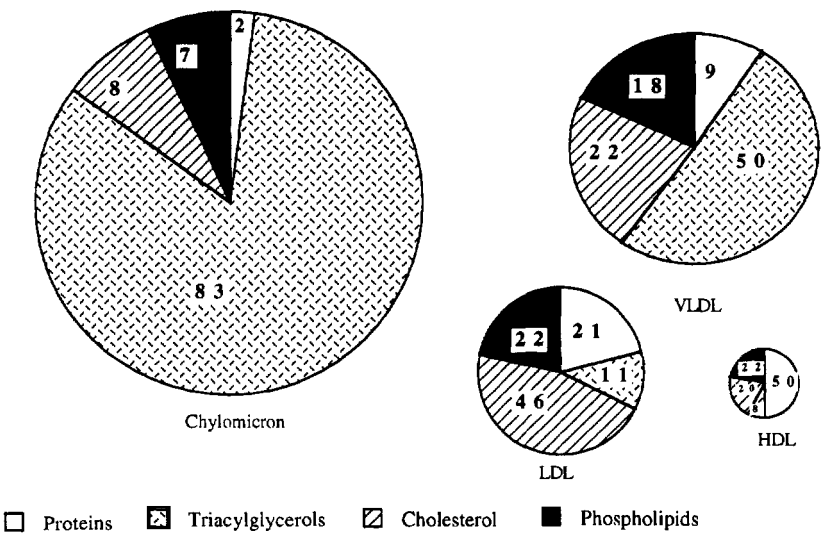
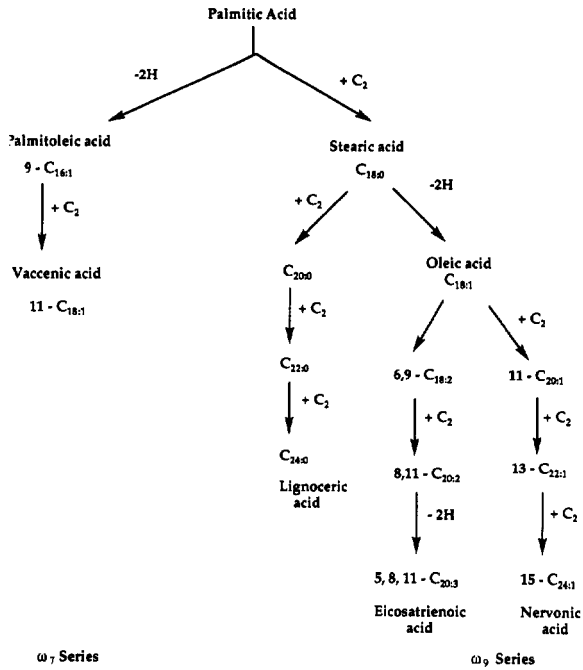


Figure 3.11 Composition (%) of human serum lipoproteins; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.



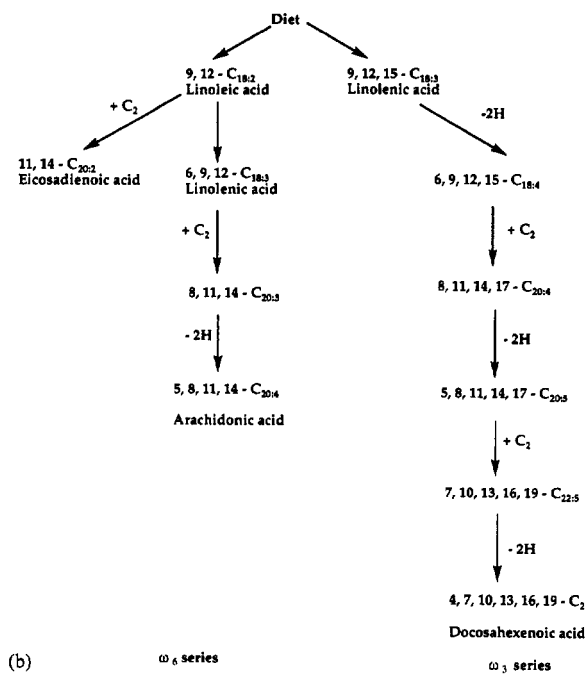


Figure 3.12 Elongation and/or desaturation of fatty acids in the mammary gland.

racing driver syndrome). Chylomicrons, which are formed in the intestinal mucosa, are secreted into the lymph and enter the blood via the thoracic duct. VLDL lipoproteins are synthesized in intestinal mucosa and liver. LDL lipoproteins are formed at various sites, including mammary gland, by removing of triglycerides from VLDL.

Since about 50% of $C_{16:0}$ and 100% of $C_{18:0}$, $C_{18:1}$ and $C_{18:2}$ are derived from blood lipids, about 50% of the total fatty acids in ruminant milk fat originate from the blood via diet or other organs.

In liver mitochondria, palmitic acid, as its CoA ester, is lengthened by successive additions of acetyl CoA. There is also a liver microsomal enzyme capable of elongating saturated and unsaturated fatty acids by addition of acetyl CoA or malonyl CoA.

The principal monoenoic acids, oleic ($C_{18:1}$) and palmitoleic ($C_{16:1}$), are derived from blood lipids but about 30% of these acids are produced by microsomal enzymes (in the endoplasmic reticulum) in the secretory cells by desaturation of stearic and palmitic acids, respectively:

$$\text{Stearyl CoA} + \text{NADPH} + O_2 \xrightarrow[\text{desaturase}]{} \text{oleoyl CoA} + \text{NADP}^+ + 2H_2O$$

Shorter chain unsaturated acids ($C_{10:1}$ to $C_{14:1}$) are probably also produced by the same enzyme.

Linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids cannot be synthesized by mammals and must be supplied in the diet, i.e. they are essential fatty acids (linoleic is the only true essential acid). These two polyenoic acids may then be elongated and/or further desaturated by mechanisms similar to stearic → oleic, to provide a full range of polyenoic acids. A summary of these reactions is given in Figure 3.12a, b.

δ-Hydroxy acids are produced by δ-oxidation of fatty acids and β-keto acids may arise from incomplete syntheses or via β-oxidation.

3.6 Structure of milk lipids

Glycerol for milk lipid synthesis is obtained in part from hydrolysed blood lipids (free glycerol and monoglycerides), partly from glucose and a little from free blood glycerol. Synthesis of triglycerides within the cell is catalysed by enzymes located on the endoplasmic reticulum, as shown in Figure 3.13.

Esterification of fatty acids is not random: C_{12}–C_{16} are esterified principally at the *sn*-2 position while C_4 and C_6 are esterified principally at the *sn*-3 position (Table 3.8). The concentrations of C_4 and C_{18} appear to be rate-limiting because of the need to keep the lipid liquid at body temperature. Some features of the structures are notable:

- Butanoic and hexanoic acids are esterified almost entirely, and octanoic and decanoic acids predominantly, at the *sn*-3 position.

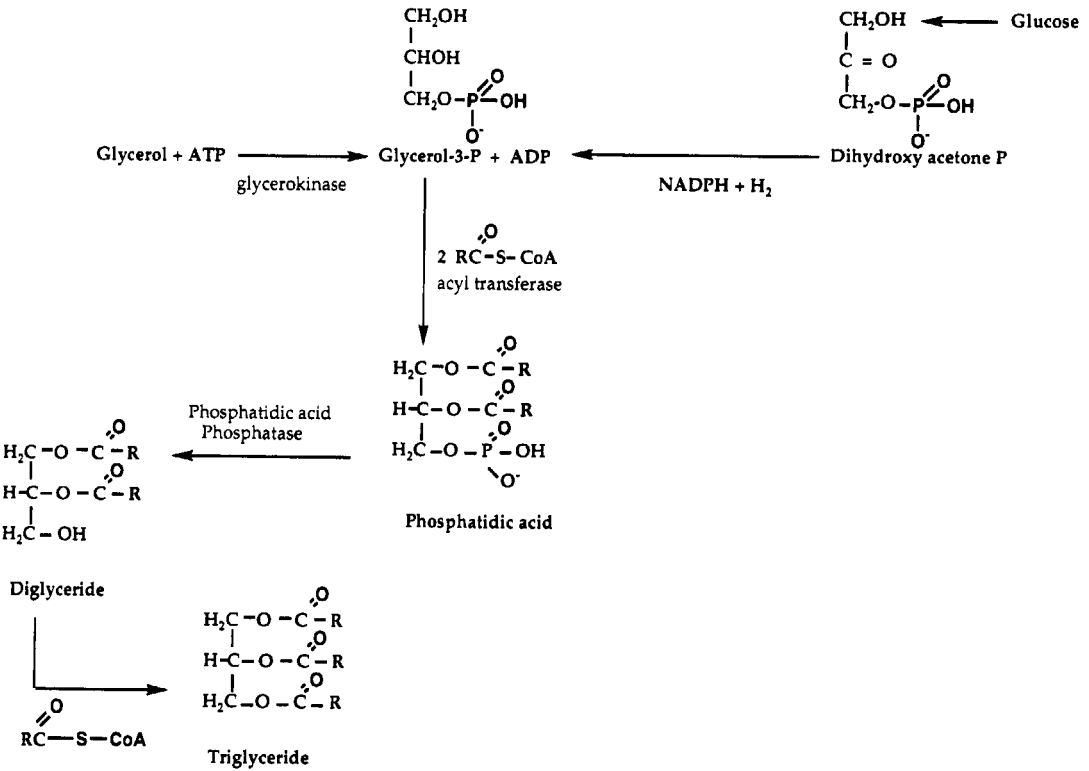


Figure 3.13 Biosynthesis of triglycerides in the mammary gland.

Table 3.8 Composition of fatty acids (mol% of the total) esterified to each position of the triacyl-*sn*-glycerols in the milks of various species

Fatty acid	Cow			Human			Rat			Pig			Rabbit			Seal			Echidna		
	sn-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3
4:0	–	–	35.4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6:0	–	0.9	12.9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8:0	1.4	0.7	3.6	–	–	–	3.7	5.7	10.0	–	–	–	–	19.2	33.7	38.9	–	–	–	–	–
10:0	1.9	3.0	6.2	0.2	0.2	1.1	10.1	20.0	26.0	–	–	–	–	22.5	22.5	26.1	–	–	–	–	–
12:0	4.9	6.2	0.6	1.3	2.1	5.6	10.4	15.9	15.1	–	–	–	–	3.5	2.8	1.8	0.3	0.2	–	–	–
14:0	9.7	17.5	6.4	3.2	7.3	6.9	9.6	17.8	8.9	2.4	6.8	3.7	2.7	2.1	2.6	0.7	23.6	3.8	1.7	0.9	0.4
16:0	34.0	32.3	5.4	16.1	58.2	5.5	20.2	28.7	12.6	21.8	57.6	15.4	24.1	12.7	23.8	6.1	31.0	1.0	31.5	9.0	27.9
16:1	2.8	3.6	1.4	3.6	4.7	7.6	1.8	2.1	1.8	6.6	11.2	10.4	4.1	1.3	1.5	1.1	16.8	14.1	–	–	–
18:0	10.3	9.5	1.2	15.0	3.3	1.8	4.9	0.8	1.5	6.9	1.1	5.5	6.9	3.5	0.9	1.9	0.7	1.0	16.8	2.1	14.3
18:1	30.0	18.9	23.1	46.1	12.7	50.4	24.2	3.3	11.8	49.6	13.9	51.7	40.8	16.6	3.8	11.4	19.4	45.4	33.1	57.6	39.8
18:2	1.7	3.5	2.3	11.0	7.3	15.0	14.1	5.2	11.6	11.3	8.4	11.5	15.6	15.1	6.4	9.7	2.3	2.8	4.1	18.3	4.9
18:3	–	–	–	0.4	0.6	1.7	1.2	0.5	0.7	1.4	1.0	1.8	3.4	3.5	2.0	2.3	0.5	0.7	1.0	2.9	2.0
C_{20}–C_{22}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.8	28.7	–	–	–

From Christie (1995).

- As the chain length increases up to $C_{16:0}$, an increasing proportion is esterified at the *sn*-2 position; this is more marked for human than for bovine milk fat, especially in the case of palmitic acid ($C_{16:0}$).
- Stearic acid ($C_{18:0}$) is esterified mainly at *sn*-1.
- Unsaturated fatty acids are esterified mainly at the *sn*-1 and *sn*-3 positions, in roughly equal proportions.

Fatty acid distribution is significant from two viewpoints:

- It affects the melting point and hardness of the fat, which can be reduced by randomizing the fatty acid distribution. Transesterification can be performed by treatment with $SnCl_2$ or enzymatically under certain conditions; increasing attention is being focused on the latter as an acceptable means of modifying the hardness of butter.
- Pancreatic lipase is specific for the fatty acids at the *sn*-1 and *sn*-3 positions. Therefore, $C_{4:0}$ to $C_{8:0}$ are released rapidly from milk fat; these are water-soluble and are readily absorbed from the intestine. Medium- and long-chain acids are absorbed more effectively as 2-monoglycerides than as free acids; this appears to be quite important for the digestion of lipids by human infants who have limited ability to digest lipids due to the absence of bile salts. Infants metabolize human milk fat more efficiently than bovine milk fat, apparently owing to the very high proportion of $C_{16:0}$ esterified at *sn*-2 in the former. The effect of transesterification on the digestibility of milk fat by infants merits investigation.

3.7 Milk fat as an emulsion

In 1674, Van Leeuwenhoek reported that the fat in milk exists as microscopic globules. Milk is an oil-in-water emulsion, the properties of which have a marked influence on many properties of milk, e.g. colour, mouthfeel, viscosity. The globules range in diameter from approximately 0.1 to 20 μm, with a mean of about 3.5 μm (the range and mean vary with breed and health of the cow, stage of lactation, etc.). The size and size distribution of fat globules in milk may be determined by light microscopy, light scattering (e.g. using the Malvern Mastersizer) or electronic counting devices (such as the Coulter counter). The frequency distribution of globule number and volume as a function of diameter for bovine milk are summarized in Figure 3.14. Although small globules are very numerous (*c.* 75% of all globules have diameters < 1 μm), they represent only a small proportion of total fat volume or mass. The number average diameter of the globules in milk is only *c.* 0.8 μm. The mean fat globule size in milk from Channel Island breeds (Jersey and Guernsey) is larger than that in milk from other breeds (the fat content of the former milks is also higher) and the mean globule diameter decreases throughout lactation (Figure 3.15).

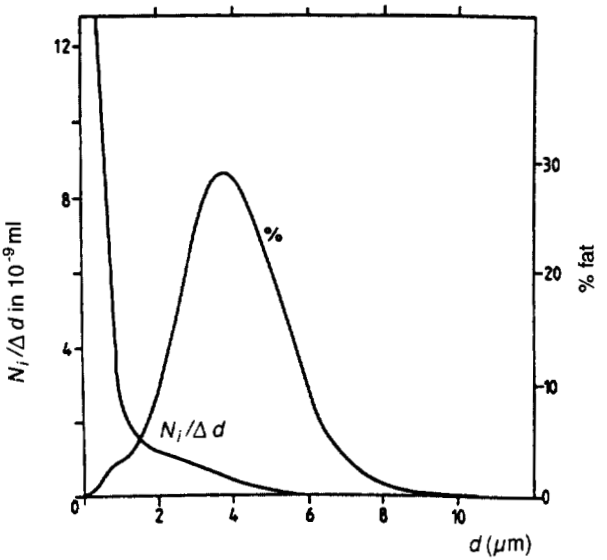


Figure 3.14 Number ($N_i/\Delta d$) and volume (% of fat) frequency of the fat globules in bovine milk (from Walstra and Jenness, 1984).

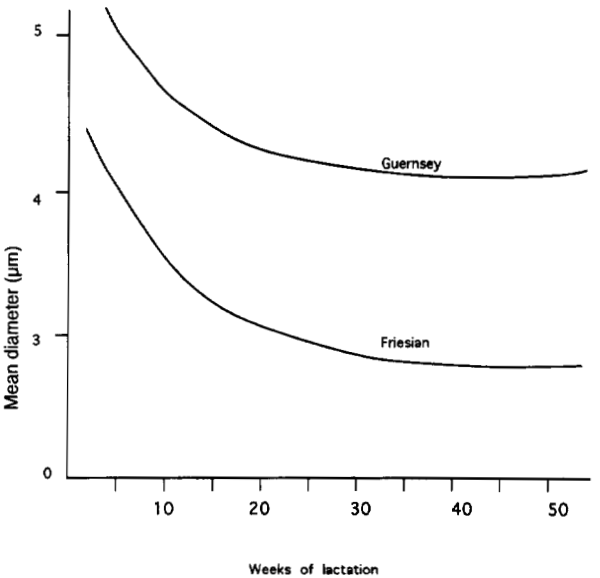


Figure 3.15 Average diameter of the fat globules in milk of Guernsey or Friesian cows throughout lactation (from Walstra and Jenness, 1984).

Milk contains $\sim 15 \times 10^9$ globules ml^{-1}, with a total interfacial area of 1.2–2.5 m^2 per g fat.

Example. Assume a fat content of 4.0%, w/v, with a mean globule diameter of 3 μm.

$$\text{Volume of typical globule} = \frac{4}{3}\pi r^3$$

$$= \frac{4}{3} \times \frac{22}{7} \times \frac{(3)^3}{2}\ \mu m^3$$

$$\sim 14\ \mu m^3.$$

$$\text{1 ml milk contains: } 0.04\text{ g fat}$$

$$= 4.4 \times 10^{10}\ \mu m^3.$$

$$\text{1 ml milk contains: } \frac{4.4 \times 10^{10}}{14} \sim 3.14 \times 10^9 \text{ globules.}$$

$$\text{Surface area of a typical globule} = 4\pi r^2$$

$$= 4 \times \frac{22}{7} \times \frac{9}{4}\ \mu m^2$$

$$= 28.3\ \mu m^2.$$

$$\text{Interfacial area per ml milk} = 28.3 \times (3.14 \times 10^9)\ \mu m^2$$

$$= 88.9 \times 10^9\ \mu m^2$$

$$= 889\ cm^2 \approx 0.09\ m^2.$$

$$\text{Interfacial area per g fat} = 88.9 \times 10^{-3} \times \frac{1}{0.04}\ m^2$$

$$= 2.22\ m^2.$$

3.8 Milk fat globule membrane

Lipids are insoluble in water and an interfacial tension therefore exists between the phases when lipids are dispersed (emulsified) in water (or vice versa). This tension *in toto* is very large, considering the very large interfacial area in a typical emulsion (section 3.7). Owing to the interfacial tension, the oil and water phases would quickly coalesce and separate. However, coalescence (but not creaming) is prevented by the use of emulsifiers (surface active agents) which form a film around each fat globule (or each water

droplet in the case of water-in-oil emulsions) and reduce interfacial tension. In the case of unprocessed milk, the emulsifying film is much more complex than that in 'artificial' emulsions, and is referred to as the milk fat globule membrane (MFGM).

In 1840, Ascherson observed an emulsion-stabilizing membrane surrounding the fat globules in milk and suggested that the membrane was 'condensed' albumin (from the skim-milk phase) aggregated at the fat/plasma interface. Babcock, in the 1880s, also felt that the milk fat emulsifier was adsorbed serum protein. Histological staining and light microscopy were employed around the turn of the century to identify the nature of the membrane material but it was early recognized that contamination of fat globules by skim-milk components presented a major problem. By analysing washed globules, it was shown that the MFGM contained phospholipids and protein which differed from the skim-milk proteins (see Brunner (1974) for historical review).

3.8.1 Isolation of the fat globule membrane

The definition of what precisely constitutes the membrane leads to considerable difficulty and uncertainty. The outer boundary is assumed to constitute everything that travels with the fat globule when it moves slowly through milk; however, the outer regions of the membrane are loosely attached and some or all may be lost, depending on the extent of mechanical damage the globule suffers. The inner boundary is ill-defined and depends on the method of preparation; there is considerable discussion as to whether a layer of high melting point triglyceride, immediately inside the membrane, is part of the membrane or not. Some hydrophobic constituents of the membrane probably diffuse into the core of the globules while components of the plasma may adsorb at the outer surface. Since the membrane contains numerous enzymes, enzymatic changes may occur.

Several methods are available for isolating all or part of the membrane. The usual initial step involves separating a cream from milk by mechanical centrifugation (which may cause some damage) or by gravity. The cream is washed repeatedly (3–6 times) with water or dilute buffer by dilution and gravity separation; soluble salts and other small molecules are probably lost into the serum. Mechanical damage may remove the loosely bound outer layers and may even cause some homogenization and adsorption of serum constituents; small globules are lost during each washing cycle.

The washed cream is destabilized by churning or freezing; then the fat (mainly triglycerides) is melted and separated from the membrane material by centrifugation. Cross-contamination of membrane with core material may be considerable, and methods must be carefully standardized. An elaborate scheme for the isolation and fractionation of the MFGM was developed by Brunner and co-workers (Brunner, 1974).

Treatment of washed cream with surfactants, usually sodium deoxycholate, releases part of the membrane, assumed to represent only the outer layer. Unless the treatment is carefully controlled, some inner material will be released also.

3.8.2 Gross chemical composition of MFGM

Yields of 0.5-1.5 g MFGM per 100 g fat have been reported; the range reflects variations in temperature history, washing technique, age, agitation, etc. The gross chemical composition of the membrane is reasonably well established and the relatively small differences reported are normally attributed to different methods used to isolate and fractionate the membrane material. The data in Table 3.9, from Mulder and Walstra (1974) and based on the investigations of many workers, give a reasonable estimate of the gross composition of the MFGM. A more detailed compositional analysis is provided by Keenan *et al.* (1983) (Table 3.10). Brunner (1965, 1974), Mulder and Walstra (1974), Patton and Keenan (1975), Keenan *et al.* (1983) and Keenan and Dylewski (1995) should be consulted for more detailed compositional data.

3.8.3 The protein fraction

Depending on the preparative method used, the membrane may or may not contain skim-milk proteins (i.e. caseins and whey proteins); if the membrane has been damaged prior to isolation, it may contain considerable amounts of these proteins. The membrane contains unique proteins which do not occur in the skim-milk phase. Many of the proteins are glycoproteins and contain a considerable amount of carbohydrate (hexose, 2.8–4.15%; hexosamine, 2.5–4.2%; and sialic acid, 1.3–1.8%).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE), with silver staining of the gels, resolves MFGM proteins into as many as 60 discrete bands, ranging in molecular mass from 11 to 250 kDa (Keenan and Dylewski, 1995). Most of these proteins are present at very low concentrations (many are detectable only when gels are stained with silver but not with Coomassie blue). Some of these proteins may be genetic variants and, since the MFGM contains a plasmin-like proteinase, some of the smaller polypeptides may be fragments of larger proteins. The three principal proteins, with molecular masses (by SDS–PAGE) of 155, 67 and 48 kDa, are xanthine oxidase, butyrophilin and glycoprotein B, respectively; five or six glycoproteins have been detected by staining with Schiff's reagent.

Xanthine oxidase, which requires Fe, Mo and flavin adenine dinucleotide (FAD) as co-factors, is capable of oxidizing lipids via the production of superoxide radicals. It represents about 20% of the MFGM protein and part is readily lost from the membrane, e.g. on cooling; isoelectric focusing

indicates at least four variants with isoelectric points (p*I*) in the range 7.0–7.5.

Butyrophilin, the principal MFGM protein and so named because of its high affinity for milk lipids, is a very hydrophobic, difficult to solubilize (insoluble or only sparingly soluble in most protein solvents, including detergents) glycoprotein. Isoelectric focusing indicates at least four variants (p*I*s 5.2–5.3). The amino acid sequence of butyrophilin has been determined and its gene has been cloned, which indicates that butyrophilin is synthesized with a leader sequence; it consists of 526 amino acids and has a molecular mass, without carbohydrate, of 56 460 Da. It binds phospholipids tenaciously and perhaps even contains covalently bound fatty acids. It is located only at the apical cell surface of the mammary epithelial cells, suggesting a role in membrane envelopment of fat globules.

Several of the minor proteins of the MFGM have been isolated and partially characterized (Keenan and Dylewski, 1995). A systematic nomenclature has not been developed for the MFGM proteins and most are referred to by their relative electrophoretic mobility on SDS–PAGE and whether or not they are glycoproteins. The proteins of the MFGM represent approximately 1% of the total proteins in milk.

3.8.4 The lipid fraction

The membrane contains 0.5–1.0% of the total lipid in milk and is composed principally of phospholipids and neutral lipids in the approximate ratio 2:1, with lesser amounts of other lipids (Tables 3.9 and 3.10); contamination with core lipid is a major problem. The phospholipids are principally phosphatidylcholine, phosphatidylethanolamine and sphingomyelin in the approximate ratio 2:2:1. The principal fatty acids and their approximate percentages in the phospholipids are $C_{14:0}$ (5%), $C_{16:0}$ (25%), $C_{18:0}$ (14%), $C_{18:1}$ (25%), $C_{18:2}$ (9%), $C_{22:0}$ (3%) and $C_{24:0}$ (3%). Thus, the membrane contains a significantly higher level of polyunsaturated fatty acids than milk

Table 3.9 Gross composition of the milk fat globule membrane

Component	mg 100 g^{-1} fat globule	mg m^{-2} fat globule surface	% (w/w) of total membrane
Protein	900	4.5	41
Phospholipid	600	3.0	27
Cerebrosides	80	0.4	3
Cholesterol	40	0.2	2
Neutral glycerides	300	1.5	14
Water	280	1.4	13
Total	2200	11.0	100

From Mulder and Walstra (1974).

Table 3.10 Composition of bovine milk fat globule membranes

Constituent class	Amount
Protein	25–60% of dry weight
Total lipid	0.5–1.2 mg per mg protein
Phospholipid	0.13–0.34 mg per mg protein
Phosphatidyl choline	34% of total lipid phosphorus
Phosphatidylethanolamine	28% of total lipid phosphorus
Sphingomyelin	22% of total lipid phosphorus
Phosphatidylinositol	10% of total lipid phosphorus
Phosphatidylserine	6% of total lipid phosphorus
Neutral lipid	56–80% of total lipid
Hydrocarbons	1.2% of total lipid
Sterols	0.2–5.2% of total lipid
Sterol esters	0.1–0.8% of total lipid
Glycerides	53–74% of total lipid
Free fatty acids	0.6–6.3% of total lipid
Cerebrosides	3.5 nmoles per mg protein
Gangliosides	6–7.4 nmoles sialic acid per mg protein
Total sialic acids	63 nmoles per mg protein
Hexoses	0.6 μmoles per mg protein
Hexosamines	0.3 μmoles per mg protein
Cytochrome b_5 + P420	30 pmoles per mg protein
Uronic acids	99 ng per mg protein
RNA	20 μg per mg protein

From Keenan *et al.* (1983).

Table 3.11 Structures of glycosphingolipids of bovine milk fat globule membrane

Glycosphingolipid	Structure
Glucosyl ceramide	β-Glucosyl-(1 → 1)-ceramide
Lactosyl ceramide	β-Glucosyl-(1 → 4)-β-glucosyl-(1 → 1)-ceramide
GM_3 (hematoside)	Neuraminosyl-(2 → 3)-galactosyl-glucosyl-ceramide
GM_2	*N*-Acetylgalactosaminyl-(neuraminosyl)-galactosyl-glucosyl-ceramide
GM_1	Galactosyl-*N*-acetylgalactosaminyl-(neuraminosyl)-galactosyl-glucosyl-ceramide
GD_3 (disialohematoside)	Neuraminosyl-(2 → 8)-neuraminosyl-(2 → 3)-galactosyl-glucosyl-ceramide
GD_2	*N*-Acetylgalactosaminyl-(neuraminosyl-neuraminosyl)-galactosyl-glucosyl-ceramide
GD_{1b}	Galactosyl-*N*-acetylgalactosaminyl-(neuraminosyl-neuraminosyl)-galactosyl-glucosyl-ceramide

From Keenan *et al.* (1983).

fat generally and is, therefore, more susceptible to oxidation. The cerebrosides are rich in very long chain fatty acids which possibly contribute to membrane stability. The membrane contains several glycolipids (Table 3.11).

The amount and nature of the neutral lipid present in the MFGM is uncertain because of the difficulty in defining precisely the inner limits of the membrane. It is generally considered to consist of 83–88% triglyceride, 5–14% diglyceride and 1–5% free fatty acids. The level of diglyceride is considerably higher than in milk fat as a whole; diglycerides are relatively polar and are, therefore, surface-active. The fatty acids of the neutral lipid fraction are longer-chained than in milk fat as a whole and in order of proportion present are palmitic, stearic, myristic, oleic and lauric.

Most of the sterols and sterol esters, vitamin A, carotenoids and squalene in milk are dissolved in the core of the fat globules but some are probably present in the membrane.

3.8.5 Other membrane components

Trace metals. The membrane contains 5–25% of the indigenous Cu and 30–60% of the indigenous Fe of milk as well as several other elements, e.g. Co, Ca, Na, K, Mg, Mn, Mo, Zn, at trace levels; Mo is a constituent of xanthine oxidase.

Enzymes. The MFGM contains many enzymes (Table 3.12). These enzymes originate from the cytoplasm and membranes of the secretory cell and are present in the MFGM due to the mechanism of globule excretion from the cells.

3.8.6 Membrane structure

Several early attempts to describe the structure of the MFGM included King (1955), Hayashi and Smith (1965), Peereboom (1969), Prentice (1969) and Wooding (1971). Although the structures proposed by these workers were inaccurate, they stimulated thinking on the subject. Keenan and Dylewski (1995) and Keenan and Patton (1995) should be consulted for recent reviews.

Understanding of the structure of the MFGM requires understanding three processes: the formation of lipid droplets from triglycerides synthesized in or on the endoplasmic reticulum at the base of the cell, movement of the droplets (globules) through the cell and excretion of the globules from the cell into the lumen of the alveolus.

The MFGM originates from regions of apical plasma membrane, and also from endoplasmic reticulum (ER) and perhaps other intracellular compartments. That portion of the MFGM derived from apical plasma

Table 3.12 Enzymatic activities detected in bovine milk fat globule membrane preparations

Enzyme	EC number
Lipoamide dehydrogenase	1.6.4.3
Xanthine oxidase	1.2.3.2
Thiol oxidase	1.8.3.2
NADH oxidase	1.6.99.3
NADPH oxidase	1.6.99.1
Catalase	1.11.1.6
γ-Glutamyl transpeptidase	2.3.2.1
Galactosyl transferase	2.4.1-
Alkaline phosphatase	3.1.3.1
Acid phosphatase	3.1.3.2
N^1-Nucleotidase	3.1.3.5
Phosphodiesterase I	3.1.4.1
Inorganic pyrophosphatase	3.6.1.1
Nucleotide pyrophosphatase	3.6.1.9
Phosphatidic acid phosphatase	3.1.3.4
Adenosine triphosphatase	3.6.1.15
Cholinesterase	3.1.1.8
UDP-glycosyl hydrolase	3.2.1-
Glucose-6-phosphatase	3.1.3.9
Plasmin	3.4.21.7
β-Glucosidase	3.2.1.21
β-Galactosidase	3.2.1.23
Ribonuclease I	3.1.4.22
Aldolase	4.1.2.13
Acetyl-CoA carboxylase	6.4.1.2

From Keenan and Dylewski (1995).

membrane, termed the primary membrane, has a typical bilayer membrane appearance, with electron-dense material on the inner membrane face. The components derived from ER appear to be a monolayer of proteins and polar lipids which covers the triacylglycerol-rich core lipids of the globule before its secretion. This monolayer or coat material compartmentalizes the core lipid within the cell and participates in intracellular fusions through which droplets grow in volume. Constituents of this coat also may be involved in interaction of droplets with the plasma membrane.

Milk lipid globules originate as small lipid droplets in the ER. Lipids, presumed to be primarily triacylglycerols, appear to accumulate at focal points on or in the ER membrane. This accumulation of lipids may be due to localized synthesis at these focal points, or to accretion from dispersed or uniformly distributed biosynthetic sites. It has been suggested that triacylglycerols accumulate between the halves of the bilayer membrane and are released from the ER into the cytoplasm as droplets coated with the outer or cytoplasmic half of the ER membrane. A cell-free system has been developed in which ER isolated from lactating mammary gland can be induced to release lipid droplets which resemble closely droplets formed *in situ* in both morphology and composition. In this cell-free system, lipid

droplets were formed only when a fraction of cytosol with a molecular mass greater than 10 kDa was included in the incubation mixture, suggesting that cytosolic factors are involved in droplet formation or release from ER.

By whatever mechanism they are formed, on or in, and released from the ER, milk lipid globule precursors first appear in the cytoplasm as droplets with diameters of less than 0.5 μm, with a triglyceride-rich core surrounded by a granular coat material that lacks bilayer membrane structure, but which appears to be thickened, with tripartite-like structure, in some regions. These small droplets, named microlipid droplets, appear to grow in volume by fusing with each other. Fusions give rise to larger droplets, called cytoplasmic lipid droplets, with diameters of greater than 1 μm.

Droplets of different density and lipid : protein ratios ranging from about 1.5 : 1 to 40 : 1 have been isolated from bovine mammary gland. Triglycerides are the major lipid class in droplets of all sizes and represent increasingly greater proportions of total droplet mass in increasingly less dense droplet preparations. Surface coat material of droplets contains cholesterol and the major phospholipid classes found in milk, i.e. sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine.

SDS–PAGE shows that micro- and cytoplasmic lipid droplets have complex and similar polypeptide patterns. Many polypeptides with electrophoretic mobilities in common with those of intracellular lipid droplets are present also in milk lipid globules. Some polypeptides of MFGM and intracellular lipid droplets share antigenic reactivity. Taken together, current information suggests that lipid droplet precursors of milk lipid globules originate in the ER and retain at least part of the surface material of droplets during their secretion as milk fat globules. The protein and polar lipid coat on the surface of lipid droplets stabilizes the triglyceride-rich droplet core, preventing coalescence in the cytoplasm. Beyond a stabilization role, constituents of the coat material may participate also in droplet fusions and in droplet–plasma membrane interactions. If elements of the cytoskeleton function in guiding lipid droplets from their sites of origin to their sites of secretion from the cell, coat constituents may participate in interaction with filamentous or tubular cytoskeletal elements.

Within mammary epithelial cells, one mechanism by which lipid droplets can grow is by fusion of microlipid droplets. Microlipid droplets can also fuse with cytoplasmic lipid droplets, providing triacylglycerols for continued growth of larger droplets. The size range of lipid globules in milk can be accounted for, at least in part, by a droplet fusion-based growth process. Small milk fat globules probably arise from secretion of microlipid droplets which have undergone no or a few fusions while larger droplets can be formed by continued fusions with microlipid droplets.

While accumulated evidence favours the view that lipid droplets grow by fusion, there is no evidence as to how this process is regulated to control the ultimate size distribution of milk lipid globules. The possibility that fusion

is purely a random event, regulated only by probability of droplet–droplet contact before secretion, cannot be ruled out. Insufficient evidence is available to conclude that fusion of droplets is the sole or major mechanism by which droplets grow. Other possible mechanisms for growth, e.g. lipid transfer proteins which convey triglycerides from their site of synthesis to growing lipid droplets, cannot be excluded.

Available evidence indicates that lipid droplets migrate from their sites of origin, primarily in basal regions of the cell, through the cytoplasm to apical cell regions. This process appears to be unique to the mammary gland and in distinct contrast to lipid transit in other cell types, where triacylglycerols are sequestered within ER and the Golgi apparatus and are secreted as lipoproteins or chylomicrons that are conveyed to the cell surface via secretory vesicles.

Mechanisms which guide unidirectional transport of lipid droplets are not yet understood. Evidence for possible involvement of microtubules and microfilaments, elements of the cytoskeletal system, in guiding this transit has been obtained, but this evidence is weak and is contradictory in some cases. Cytoplasmic microtubules are numerous in milk-secreting cells and the tubulin content of mammary gland increases substantially prior to milk secretion. A general role for microtubules in the cytoplasm, and the association of proteins with force-producing properties with microtubules, provide a plausible basis for assuming the microtubules may be involved in lipid droplet translocation. Microfilaments, which are abundant in milk-secreting cells, appear to be concentrated in apical regions.

3.8.7 Secretion of milk lipid globules

The mechanism by which lipid droplets are secreted from the mammocyte was first described in 1959 by Bargmann and Knoop and has been confirmed by several investigators since (Keenan and Dylewski, 1995). The lipid droplets are pushed through and become enveloped progressively by

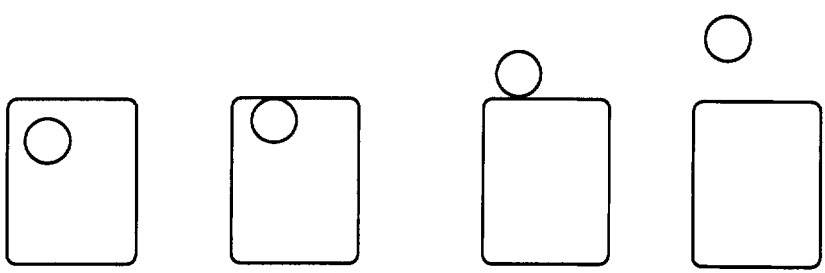


Figure 3.16 Schematic representation of the excretion of a fat globule through the apical membrane of the mammary cell.

the apical membrane up to the point where they are dissociated from the cell, surrounded entirely by apical membrane (Figure 3.16). Current concepts of the pathway by which lipid droplets originate, grow and are secreted are summarized diagrammatically in Figure 3.17.

Lipid droplets associate with regions of the plasma membrane that are characterized by the appearance of electron-dense material on the cytoplasmic face of the membrane. Droplet surfaces do not contact the plasma

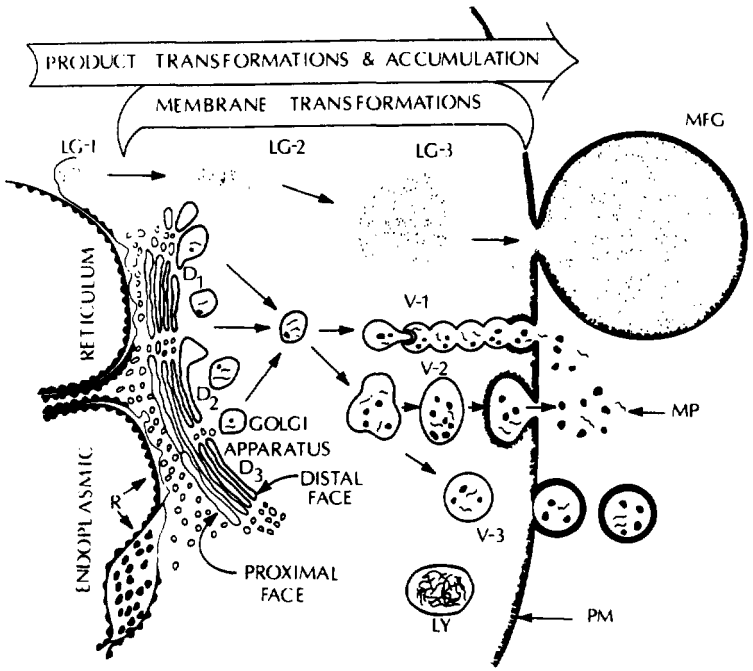


Figure 3.17 The roles of components of the endo-membrane system of mammary epithelial cells in the synthesis and secretion of the constituents of milk. Intracellular lipid globules (LG-1, LG-2, LG-3) are discharged from the cell by progressive envelopment in regions of apical plasma membrane. MFG denotes a lipid globule being enveloped in plasma membrane. Milk proteins (MP) are synthesized on polysomes of endoplasmic reticulum and are transported, perhaps in small vesicles which bleb from endoplasmic reticulum, to dictyosomes (D_1, D_2, D_3) of the Golgi apparatus. These small vesicles may fuse to form the proximal cisterna of Golgi apparatus dictyosomes. Milk proteins are incorporated into secretory vesicles formed from cisternal membranes on the distal face of dictyosomes. Lactose is synthesized within cisternal luminae of the Golgi apparatus and is incorporated into secretory vesicles. Certain ions of milk are also present in secretory vesicles. Three different mechanisms for exocytotic interaction of secretory vesicle with apical plasma membrane have been described: (1) through the formation of a chain of fused vesicles (V-1); (2) by fusion of individual vesicles with apical plasma membrane (V-2), with integration of vesicle membrane into plasma membrane; (3) by direct envelopment of secretory vesicles in apical plasma membrane (V-3). Lysosomes (LY) may function in the degradation of excess secretory vesicle membrane (from Keenan, Mather and Dylewski, 1988).

membrane directly but rather the electron-dense cytoplasmic face material; which constituents of the latter recognize and interact with constituents on the droplet surface are not known. Immunological and biochemical studies have shown that butyrophilin and xanthine oxidase, two of the principal proteins in the MFGM, are major constituents of the electron-dense material on the cytoplasmic face of apical plasma membrane. Butyrophilin, a hydrophobic, transmembrane glycoprotein that is characteristic of milk-secreting cells, is concentrated highly at the apical surface of these cells; it binds phospholipids tightly, and is believed to be involved in mediating interaction between lipid droplets and apical plasma membrane. Xanthine oxidase is distributed throughout the cytoplasm, but appears to be enriched at the apical cell surface.

In the secretion process, milk fat globules usually are enveloped compactly by apical plasma membrane, but closure of the membrane behind the projecting fat droplet occasionally entrains some cytoplasm as a so-called crescent or signet between the membrane and the droplet surface. These crescents can vary from thin slivers of cellular material to situations in which the crescent represents a greater volume than does the globule core lipid. Except for nuclei, cytoplasmic crescents contain nearly all membranes and organelles of the milk-secreting cell. Globule populations with a high proportion of crescents exhibit a more complex pattern of proteins by SDS–PAGE than low-crescent populations. Presumably, the many additional minor bands arise from cytoplasmic components in crescents. Crescents have been identified in association with the milk fat globules of all species examined to date, but the proportion of globules with crescents varies between and within species; about 1% of globules in bovine milk contain crescents.

Thus, the fat globules are surrounded, at least initially, by a membrane typical of eukaryotic cells. Membranes are a conspicuous feature of all cells and may represent 80% of the dry weight of some cells. They serve as barriers separating aqueous compartments with different solute composition and as the structural base on which many enzymes and transport systems are located. Although there is considerable variation, the typical composition of membranes is about 40% lipid and 60% protein. The lipids are mostly polar (nearly all the polar lipids in cells are located in the membranes), principally phospholipids and cholesterol in varying proportions. Membranes contain several proteins, perhaps up to 100 in complex membranes. Some of the proteins, referred to as extrinsic or peripheral, are loosely attached to the membrane surface and are easily removed by mild extraction procedures. The intrinsic or integral proteins, about 70% of the total protein, are tightly bound to the lipid portion and are removed only by severe treatment, e.g. by SDS or urea.

Electron microscopy shows that membranes are 79 nm thick, with a trilaminar structure (a light, electron-sparse layer, sandwiched between two

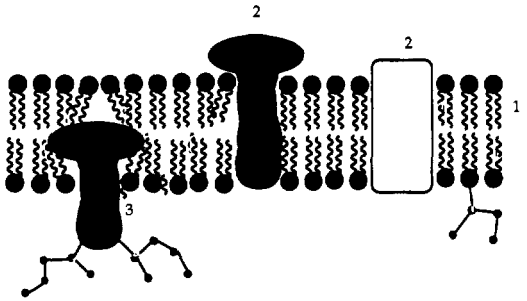


Figure 3.18 Schematic representation of a trilaminar cell membrane which is derived from the apical membrane of the mammary cell and forms the outer layer of the milk fat globule membrane following expression from the mammary cell, but which is more or less extensively lost on ageing. 1, phospholipid/glycolipid; 2, protein; 3, glycoprotein.

dark, electron-dense layers). The phospholipid molecules are arranged in a bilayer structure (Figure 3.18); the non-polar hydrocarbon chains are orientated inward where they 'wriggle' freely and form a continuous hydrocarbon base; the hydrophilic regions are orientated outward and are relatively rigid. In this bilayer, individual lipid molecules can move laterally, endowing the bilayer with fluidity, flexibility, high electrical resistance and low permeability to polar molecules. Some of the globular membrane proteins are partially embedded in the membrane, penetrating into the lipid phase from either side, others are completely buried within it, while others transverse the membrane. The extent to which a protein penetrates into the lipid phase is determined by its amino acid composition, sequence, secondary and tertiary structure. Thus, membrane proteins form a mosaic-like structure in an otherwise fluid phospholipid bilayer, i.e. the **fluid-mosaic** model (Figure 3.18).

Thus, the milk fat globules are surrounded and stabilized by a structure which includes the trilaminar apical membrane (which is replaced by Golgi membranes on secretion of proteins and lactose). The inner face of the membrane has a dense proteinaceous layer, 10–50 nm thick, probably acquired within the secretory cell during movement of the globule from the rough endoplasmic reticulum at the base of the cell, where the triglycerides are synthesized, to the apex of the cell. A layer of high melting triglycerides may be present inside this proteinaceous layer. Much of the trilaminar membrane is lost on ageing of the milk, especially if it is agitated; the membrane thus shed is present in the skim milk as vesicles (or microsomes), which explains the high proportion of phospholipids in skim milk.

McPherson and Kitchen (1983) proposed a detailed structural model of the MFGM, which appears rather speculative. Keenan *et al.* (1983), Keenan and Dylewski (1995) and Keenan and Patton (1995) describe the current

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