Characterization and Modification of Milk Lipids

Young K. Yeo, Byung K. Choi and Ah Y. Lim
Lipid Chemistry Laboratory
Kyungpook National University
Taegu, Korea

I. INTRODUCTION

Milk lipids (ML) have been extensively studied for long time because of their nutritional importance. The composition, structure and chemistry of ML have been studied more intensively than those from any other natural products. The content and composition of lipids from milks from different species varies with such factors as diet, stage of lactation, number of lactations, breed and season. A very high proportion (about 98%) of the lipids consist of triacylglycerols (TG), with other componentsat low concentrations. However, the minor components may have appreciable nutritional biochemical importance (1). The milk fatty acids (FA) have a wider range of chain lengths in glyceride forms compared to other species. Table 1 shows general composition of cow milk (CM) fat globule membranes (2).

II. THE MILK LIPID CONTENTS

Milk fat (MF) globules mainly consist of neutral lipids like TG, whereas the globule membranes contain the complex lipids mostly. MF is secreted in the globule forms surrounded by membrane. Parts of the membranes release and are found together with the structural phospholipids (PL), with fragments of other mammary epithelial cell membranes during and after secretion. The skim-milk PL in cow milk are found to be identical in composition and structure to those of the MF globule membrane, confirming their same origin. The MF globules vary in their size, and the other species including human have the same aspects. The average fat content of milks from various animal species are shown in Table 2.

The content of fat in CM is dependent on the breed with 37 g litre^{-1} of average value. The other ruminant milk has a higher fat content comparatively. The highest values have been found for marine mammals, especially those from colder waters such as seals and whales. Presumably this is an evolutionary adaptation which ensures that the young of these species can

Table 1. Composition of cow 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 to 0.34
Phosphatidyl choline	34% of total lipid phosphorus
Phosphatidyl ethanolamine	28%
Sphingomyelin	22%
Phosphatidyl inositol	10%
Phosphatidyl serine	6%
Neutral lipid	56~80% of total lipid
Hydrocarbons	1.2%
Sterols	0.2~5.2%
Sterol esters	0.1~0.8%
Glycerides	53~74%
Free fatty acids	0.6~6.3%
Cerebrosides	3.5 nmoles per mg protein
Gangliosides	6 to 7,4 nmoles sialic acid per mg protein
Total sialic acids	63 nmoles per mg protein
Hexoses	0.6 mmoles
Hexosamines	0.3 mmoles
Cytochrome b_5 + P-420	30 pmoles
Uronic acids	99 ng
RNA	20 mg

T.W. Keenan et al., Developments in Dairy Chemistry (1983)

Table 2. Fat content of milks from various species

Species	Fat content (g litre^{-1})	Species	Fat Content (g litre^{-1})
Cow	33~47	Rabbit	183
Buffalo	47	Cottontail rabbit	336~352
Sheep	40~99	Bottle-nosed dolphin	62~330
Goat	41~45	Pygmy sperm whale	153
Elephant	85~190	Harp sela	502~532
Human	38	Polar bear	314~331
Horse	19		

W. Christie, Developments in Dairy Chemistry (1983)

rapidly build up their stores of fat for energy and insulation to protect against the harsh environment. It has been pointed out that differences in the calorific values of milks from various species are due almost entirely to differences in the fat content. The young of each species can vary markedly in their dependence on milk as a source of nutrients. Particularly, human infants are mainly dependent to their milk for nutritional requirements[1].

Ⅲ. THE COMPOSITION AND BIOSYNTHESIS OF MILK LIPID

1. The neutral lipid classes

The TG are so far the major lipid class, accounting for more than 95% of the milk total lipids (Table 3).

The TG are always accompanied by small amounts of di- and mono-acylglycerols, free cholesterol and cholesterol esters, free FA and PL. Also a number of minor simple lipids and glycolipids have been existed. The small proportion of diacylglycerols were found mainly in the sn-1,2-position and were therefore probably intermediates in the bio-synthesis of TG rather than degradation products. The data are sufficiently similar to suggest comparable mechanisms of synthesis and secretion without species difference.

2. The structures of TG

Stereospecific analysis procedures have been devised that have permitted the determination of the positional distributions of FA in the TG, and results for animal fats, including MF, have been reviewed. Although severe technical problems are encountered with milk TG because of the presence of short-chain FA, data for a number of species have been obtained in recent years and some representative results are listed in Table 4[3].

As the overall FA compositions of the TG are very different for each species, similarities be-

Table 3. Composition of milk lipid classes from various species

	Amount (wt% of the total lipids)			
Lipid class	Cow	Human	Pig	Mink
Triacylglycerols	97.5	98.2	96.8	81.3
Diacylglycerols	0.36	0.7	0.7	1.7
Monoacylglycerols	0.027	T	0.1	T
Cholesterol esters	T	T	0.06	T
Cholesterol	0.31	0.25	0.6	T
Free fatty acids	0.027	0.4	0.2	1.3
Phospholipids	0.6	0.26	1.6	15.3

W. Christie, Developments in Dairy Chemistry (1983)

Table 4. Composition of fatty acids esterified to each position of the triacy sn-glycerols in the milks of various species

Fatty acid	Fatty acid composition (mol% of the total)								
	Cow			Human			Seal		
	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						
10:0	1.9	3.0	6.2	0.2	0.2	1.1			
12:0	4.9	6.2	0.6	1.3	2.1	5.6	0.2	0.3	0.2
14:0	9.7	17.5	6.4	3.2	7.3	6.9	7.3	23.6	3.8
16:0	34.0	32.3	5.4	16.1	58.2	5.5	13.1	31.0	1.0
16:1	2.8	3.6	1.4	3.6	4.7	7.6	10.2	16.8	14.1
18:0	10.3	9.5	1.2	15.0	3.3	1.8	4.5	0.7	1.0
18:1	30.0	18.9	23.1	46.1	12.7	50.4	53.8	19.4	5.4
18:2	1.7	3.5	2.3	11.0	7.3	15.0	1.3	2.3	2.8
18:3				0.4	0.6	1.7	0.3	0.5	0.7
C_{20}-C_{22}							5.6	0.8	28.7

N. Mozes and S. Smith (1982), Unpublished Results.

tween them are not immediately apparent, but a close perusal can reveal certain common features, especially for the longer-chain FA. For example, for The most abundant source of TG, unsaturated FA tend to be concentrated in position sn-2 and the saturated components are found in positions sn-1 and sn-3. The main exception is the pig where a high proportion of the FA in position sn-2 of the TG from many tissues is palmitic acid, due to the presence of an unidentified factor in the cell that modifies the specificities of the acyltransferases involved in triacylglycerol biosynthesis. In the milk TG of most of the species examined, myristic acid (like palmitic acid) is found in the greatest concentration in position sn-2, but stearic acid is concentrated in the position sn-1. The seal has the very long-chain (C_{20}-C_{22}) FA in position sn-3. The other distinctive feature of milk fats is the unique distribution of the short-chain FA, which in ruminants are concentrated in position sn-3. It is now evident that butyric and hexanoic acids are esterified virtually entirely to position sn-3, octanoic acid is mainly found in position sn-3. A complete structural analysis of a natural triacylglycerol sample requires that it be fractionated into molecular species containing single specific FA residues in each position. The problem can be extremely complicated as, for example, TG with only five different FA constituents may consist of 75 different molecular species [1].

3. Biosynthesis of TG

Triacylglycerols are synthesized through the so-called glycerol-3-phosphate pathway with the possible alternative dihydroxyacetone phosphate and 2-mono-glyceride pathways in the mammary gland. The glycerol-3-phosphate pathway utilizes sn-glycerol-3-3phosphate and acyl-CoA's-as substrate, and the three transferases involved, namely acyl-CoA: sn-glycerol-3-phosphate acyl transferase, acyl-CoA:1-acyl sn-glycerol-3-phosphate acyl transferase and acyl-CoA:1,2-diglyceride acyl transferase, appear to be closely associated in the microsomal fraction and are referred to as the triglyceride synthetase. The remaining enzyme required for triglyceride synthesis, phosphatidate phosphatase, appears to be less firmly associated with the endoplasmic reticulum and is partly cytosolic. FA are utilized as their CoA-esters and, as discussed above, the short-and medium-chain FA seem to be released from the FA synthetase in this form via transacylase reactions [4].

4. The PL

PL are a small but important fraction of the ML and are found mainly in the MF globule membrane and other membranous material in the skim-milk phase. Their composition and biosynthesis have been reviewed. The composition of the milk PL of a number of species is given in Table 5.

The major PL are phosphatidylcholine (PC), phosphatidylethanolamine (PE) and sphingomyelin with minor phosphatidylserine (PS), phosphatidylinositol (PI) and lysophospholipids. Usually phosphatidic acid has not been found as a component of CM in a careful analysis. Cardiolipin could not be detected in the CM but was found in small amounts in sheep and goat milks. There are marked similarities in the relative proportions of each of the PL among species, because these may perform the same structural function in individual species. The mink milk

Table 5. Composition of the milk phospholipids from various species

	Amount (mol% of the total lipid phosphorus)					
Species	PC	PE	PS	PI	SM	LPC
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	27.9	0.5
Human	27.9	25.9	5.8	4.2	31.1	5.1
Cat	25.8	22.0	2.7	7.8	37.9	3.4
Mink	52.8	10.0	3.6	6.6	15.3	8.3

W. Christie, Deveolpments in Dairy Chemistry (1983)

has exceptional amounts of PL. The CM has alkyl- and alk-1-enyl-ether forms of PC and PE; 4% of the PE and 1.3% of the PC are in the alk-1-enyl-ether form [1].

5. The structures of PL

The specific distributions of FA in positions sn-1 and sn-2 of the glycerophosphatides from milks of a number of species have been determined by means of phospholipase A hydrolysis and some representative results are listed in Table 6 [5]. In bovine PC and PE, for example, saturated FA are found in somewhat greater concentrations in position sn-1 and unsaturated (especially those with two or more double bonds) in position sn-2. A comprehensive fractionation of bovine milk PC into molecular species has been achieved, and the molecular structure of ceramides derived from the sphingomyelin of humanMF globule membrane has also been analyzed [1].

6. Biosynthesis of PL

The small amounts of PL present in milk, mainly as fat globule membranes, appear to be synthesized de novo within the mammary gland. Although there may be a small uptake of serum PL by the mammary gland, these are hydrolyzed during absorption and the products, including FA, are utilized by mammary tissue. PC, quantitatively the most important PL in milk and mammary tissue, and PE are synthesized in the mammary gland, as in mammalian tissues generally, via phosphatidic acid and sn-1,2-diglyceride. The phosphorylcholine and phosphoryle-

Table 6. Distributions of fatty acids in positions sn-1 and sn-2 of the phosphatidylcholine and phosphatidylethanolamine from milks of the cow and human

Fatty acid	mol % of the total							
	Cow				Human			
	PC		PE		PC		PE	
	sn-1	sn-2	sn-1	sn-2	sn-1	sn-2	sn-1	sn-2
14:0	5.6	10.8	1.9	1.3	3.4	4.9	1.0	1.0
16:0	41.9	30.6	19.7	4.7	34.2	32.3	9.3	8.2
16:1	0.6	1.2	1.2	2.2	1.5	2.2	1.8	3.3
18:0	17.5	2.4	19.0	1.3	43.9	2.1	65.4	1.3
18:1	20.3	27.8	45.8	47.8	14.3	13.7	18.1	15.3
18:2	2.7	9.2	2.9	21.4	2.7	30.9	4.4	30.2
18:3	0.8	1.8	1.1	4.5	–	2.0	–	5.1
20:3	–	1.6	0.2	2.2	–	3.9	–	5.4
20:4	0.2	1.2	0.2	3.0	–	6.6	–	20.9
22:6					–	0.8	–	5.2

W. Morrison and L. Smith, Lipids (1967)

thanolamine moieties are transferred to diglycerides from CDP-choline and CDP-ethanolamine in the synthesis of PC and PE, respectively. Two soluble kinases which lead to the formation of phosphorylcholine and phosphorylethanolamine in mammary tissue have been detected and, by analogy with other tissues, these compounds are the precursors of the CDP derivatives. Utilization of selected 1,2-diglycerides and these CDP-derivatives in phosphorylcholine and phosphorylethanolamine transferase reactions in PL biosynthesis, followed by deacylation-reacylation, appears to be responsible for their characteristic FA composition. The specificities of a re-acylating enzyme, namely acyl-CoA:1-acyl-sn-glycerol-3-phosphorylcholine acyl transferase, associated with bovine mammary gland microsomes have been found to vary with the nature of the 1-acyl residue. When 18:1 was a constituent of lysophosphatidylcholine, oleoyl-CoA was preferred over stearoyl- and linoleoyl-CoA while palmitoyl- and myristoyl-CoA were favoured when 16:0 was the lysophospholipid constituent FA. In alternative minor transformations in the mammary gland, PS may be decarboxylated to PE, and PE methylated to PC. It has been suggested that about 20% of the PC in rat liver is synthesized via the methylation pathway. As in other tissues, PS and PI are synthesized in mammary gland by cytidinediphosphodiacyl-sn-glycerol :serine and :myoinositol transferases, respectively. Microsomal and soluble PI phosphohydrolases may lead to the rapid breakdown of the inositide [4].

It seems likely that most, if not all, of the ML containing the sphingosine moiety are synthesized in the mammary gland. Sphingosine is synthesized de novo from serine by dispersed bovine mammary cells and, together with fatty acyl groups and the phosphorycholine group from CDP-choline, is incorporated into sphingomyelins. The biosynthetic pathways outlined in Fig. 7 are based on extensive studies in other mammalian tissues.

7. Minor simple lipids

Although present in small amounts, a number of lipid-soluble compounds have been detected in milks including several components(hormones, vitamins, organoleptic substance, etc.)of potential physiological importance to the newborn. Various foreign lipid-soluble substances may be found in milk, such as plasticizers or halogenated biphenyls.

Mostly the minor lipid classes in milks are simply forms of the more common ones with distinctive FA or other alkyl constituents. Triacylglycerols containing hydroxy-FA represent 0.61% of the total lipids of CM. Alkyldiacylglycerols have been found in the neutral lipids of milk of the human (0.1%), cow (0.01%) and sheep (0.02%), together with trace amounts of 2-O-methoxy-substituted analogues and the corresponding PL forms. Alk-1-enyldiacylglycerols or neutral plasmalogens comprise 0.015% of the total lipids of CM fat, the aldehydes derived from these contain a relatively high proportion (30%) of branched-chain components.

The major sterol component of most milks is a cholesterol (at least 95%), but small amounts of other sterols have also been found. The b-sitosterol, lanosterol, dihydrolanosterol, 4-cho-

lesten-3-one, 3,5-cholestadiene-7-one, and 7-dehydrocholesterol have been isolated and adequately characterized in CM, while campesterol, stigmasterol and 5-avenasterol are also present. Human milk contains phytosterols in addition to cholesterol but the relative concentrations are dependent on the nature of the diet and the stage of lactation. It is evident that the cholesterol in milk may be of dietary orgin, synthesized by other tissues in the animal, or synthesized in the mammary gland itself. The relative contribution of theses three sources appears to be subject to considerable interspecies variation and tofurther variations due to nutritional and other factors.

A number of steroidal hormones, especially progesterone, oestrogens and corticosteroids, have been found in milk. Although they may have some physiological importance for the newborn, most research interest has centred on monitoring progesterone levels for the prediction of oestrus and subsequently for the early diagnosis of pregnancy in cattle.

Squalene has long been recognized as a minor constituent ofMF. The distribution of the carotene between the fat globule and its membrane has been a matter of controversy, but the most recent work has suggested that carotene is located mainly in the fat droplet. Trace amounts of C17-C48 odd-and even-numbered, normal and branched-chain, and possibly 1-cyclohexyl series of hydrocarbons have been analyzed in bovine milk. Pristane and phytane have also been identified, as phyt-1-ene, phyt-2-ene, neophytadiene and many other related compounds [1].

The lipid-soluble vitamins(vitamins A, D and E) in milks are of great nutritional importance for the newborn, and the composition of the vitamins of various species milk are shown in Table 7 [6].

The nature of the biologically active vitamin D in human milk, in particular, has been a matter for debate. Recent evidence suggests that 25-hydroxy-vitamin D_3 accounts for 75% of the activity with vitamins D_2 and D_3 comprising most of the remainder. The sulphate form of vitamin D is not either present or possesses no biological activity. The α-tocopherol is the main component and γ-tocopherol is the only other isomer detectable in CM. However, human milk contains appreciable amounts of β-, γ- and δ-tocopherol and γ-tocotrienol in addition to a-tocopherol.

Table 7. Composition of lipid-soluble vitamins in various milks

Speices	Vitamin A (mg litre^{-1})	Vitamin D (IU ltire^{-1})	Vitamin E (mg litre^{-1})
Cow	410	25	1
Goat	700	23	<1
Human	750	50	3
Rat	1,440	5	3
Rabbit	2,080	–	–

R. Jenness, J. Dairy Sci.(1980)

The Prostaglandins E and F are found in human milk, at concentrations 100-fold greater than in adult plasma, which have a relatively long half-life. Biologically inactive forms of thromboxane A_2 and prostacyclin metabolites have been also detected. The significance of these compounds for the infant is uncertain, but they modulate some physiological function. It is known that prostaglandin $F_{2\alpha}$ passes into the milk of cows and goats, but it is doubtful whether it could retain its biological activity druing storage or processing.

Human milk also contains appreciable amounts of carnitine and some of this is apparently acylated by acetate and long-chain FA. In the human and rat, it has been established that a high proportion of the carnitine requirement of the newborn is met by milk as the enzymes required for synthesis have not developed.

Aliphatic compounds contribute greatly to the flavour and palatability of milk. Very different compounds arey involved and a high proportion are derived, chemically or biochemically, from ML. At the normal trace levels, they impart desirable flavours, but when the proportions are changed or specific components are increased in concentration they can give rise to off-flavours. It has recently been shown that oxidation of small amounts of (n-3) pentenoic FA in butter fat can give rise to the vinyl ketones, oct-1-en-3-one and octa-1, cis-5-dien-3-one, that impart a metallic flavour.

Milk fat contains relatively small amounts of γ-and δ-lactones in the free form. They are mainly C_{16} to C_{18} normal saturated compounds, although a small proportion of monoenoic and branched-chain constituents may also be present. The precursors are 4- and 5-hydroxy acids esterified to a primary position in TG (the other positions are occupied by normal FA); these hydroxy-FA form lactones spontaneously when the TG are hydrolysed. Trace amounts of b-ketoacids linked to TG, which are readily hydrolysed and the FA decarboxylated to form methyl ketones (alkan-2-ones) with one carbon atom fewer than the parent acid.

Like the PL, glycosphingolipids derived from ceramide are located primarily in theMF globule membrane. Mono- and di-hyxosylceramides are the main glycolipid constituents of bovine milk, and these have been shown to be glucosyl- and lactosyl-ceramides. On the other hand, galactosylceramide (88%) is the major monohexosylceramide in human MF globule membrane, but is also accompanied by lactosylceramide and more complex neutral glycosphingolipids. In addition, a number of gangliosides have been isolated and characterized from the MF globule membrane of the cow and mouse [1].

Ⅳ. THE FATTY ACID COMPOSITION OF MILK LIPIDS

1. The main FA of the triacylglycerols and total lipids

A greater range of FA has been isolated or identified as components of milk fats than from any other natural source. The FA detected in bovine milk listed around 430 constituents, while

about 180 distinct FA had been recognized in human MF. In a single analysis of butter-fat by highly efficient capillary gas chromatography 80 components were resolved, while in a similar analysis on a packed column 53 components were detected. However, only a relative few of these are present in appreciable concentrations or are of particular nutritional significance. The FA in milk are derived from two sources, namely the plasma lipids and synthesis de novo in the mammary gland. The former may come from the diet, but also include FA released from body tissues, especially adipose tissue, by lipolysis. As a consequence, the FA composition of the milk of non-ruminants is highly dependent on the FA profile of the diet. In ruminant animals, changes in the level of unsaturated FA in the diet of under normal conditions have comparatively little effect on the composition of the milk FA because extensive biohydrogenation occurs in the rumen. Dietary linoleic acid (9c, 12c- 18:2), for example, is in part hydrogenated by the rumen microorganisms to stearic acid (18:0), but vaccenic acid (11t-18:1) and other monoenoic isomers and a conjugated dienoic acid (9c, 11t-18:2) are also found in smaller amounts. Within the tissues of the ruminant (and non-ruminant), further modification can take place, e.g. chain elongation, α-oxidation, β-oxidation and especially desaturation, and some of the stearic acid is desaturated to oleic acid (9c-18:1). Therefore, when either stearic acid or linoleic acid is added to ruminant diets, increased levels of stearic and oleic acids are found in MF. However, when palmitic acid is used as a supplement this is essentially the only FA to increase in concentration in the MF.

Those FA synthesized do novo within the mammary gland are generally the short- to medium-chain-lenght constituents (up to and including some of the C_{16}), the proportions of each being determined by the properties of acylthiol ester hydrolases associated with the FA synthase of each species; this and other facets of lipidbiosynthesis in the mammary gland have been reviewed. The mammary gland is often the only tissue within an animal in which short- and medium-chain-length FA are found in esterified form (1). The FA composition of the milk of a given species then represents a balance between the contributions of FA of dietary origin and those newly synthesized; it is dependent not only on the nature of each contribution but also on its relative size.

Some representative compositions of the main FA in the milks of a number of species are listed in Table 8.

The milk fats of ruminant animals are characterized by the presence of relatively high concentrations of short-chain FA, especially butyric and hexanoic acids, which are rarely found in milks of non-ruminants. One of the principal biosynthetic precursors of mammary butyric acid, β-hydroxybutyric acid, is available to ruminants in relatively large amounts following uptake of ruminal butyric acid and metabolism in the rumen wall. Appreciable amounts of medium-chain-length FA are also present in ruminant milk fats, but relatively low concentrations of polyunsaturated FA are found because of biohydrogenation in the rumen. Medium-chain length FA are often indicative of a marked degree of specificity during FA synthesis. Only C_8

Table 8. Fatty acids in milk triacylglgycerols from various species

	Fatty acid (wt% of the total)												
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
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		
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
Human		T	T	1.3	3.1	5.1	20.2	5.7	5.9	46.4	13.0	1.4	T
Snowshoe hare		0.1	3.3	11.1	6.8	5.9	15.5	1.9	13.9	17.8	4.5	9.5	3.0
Mink					0.5	3.3	26.1	5.2	10.9	36.1	14.9	1.5	
Bottle-nosed dolphin					0.3	3.2	21.1	13.3	3.3	23.1	1.2	0.2	17.3
Harp seal						5.3	13.6	17.4	4.9	21.5	0.9	0.9	31.2
Hooded seal						3.6	9.5	13.5	2.8	27.2	0.6	0.6	34.9
Polar bear		T		T	0.5	3.9	18.5	16.8	13.9	30.1	0.4	0.4	11.3

W. Christie, Developments in Dairy Chemistry (1983).

and C_{10} FA are synthesized in rabbit mammary gland, and mainly C_{10} and C_{12} FA are synthesized in elephant mammary gland. The rat mammary gland is capable of synthesizing the spectrum of FA from C_8 to C_{18}. Human MF contains relatively low concentrations of medium-chain-length FA, but appreciable amounts of C_{16} and C_{18} FA are found. Unlike ruminants absorb unchanged, appreciable amounts of polyunsaturated FA from the diet, so the linoleic acid and to some extent linolenic acid (18:3(n-3)) contents of their milk fats are reasonably high. The marine animals live on a diet of fish and krill which tend to contain high proportions of c20 and c22 FA and relatively low proportions of the C_{18} polyunsaturated FA. The high amount of a 16:1 FA in the pig milk is due to the activity of palmity-CoA desaturase in the mammary gland of this species. It should be recognized that the FA designated 18:1 in Table 9 is not necessarily oleic acid itself; it includes a variety of cis and trans isomers other than cis-9, and in marine animals, and to a lesser extent other species, it includes a number of cis positional isomers derived from the diet [7].

The reason for the high concentration of short- and medium-chain-length FA in the MF of many species is not clear. In ruminants, the short-chain FA certainly help to maintain a degree of liquidity in the relatively saturated MF at body temperature that may be important for efficient secretion, but this cannot be a reason for the occurrence of medium-chain FA. Short- and medium-chain-length FA are absorbed directly via the portal blood stream rather than through the lymphatic system, so they can make a more rapid and direct contribution to the energy metabolism of the newborn. In addition, there is evidence that in some species the capacity to

Table 9. Positional and geometric isomers of bovine milk octadecenoic acid

Position of double bond	Lsomers (wt% of the total)	
	Cis isomers	Trans isomers
7		0.8
8	1.7	3.2
9	95.8	10.2
10	T	10.5
11	2.5	35.7
12		4.1
13		10.5
14		9.0
15		6.8
16		7.5

J. Hay, Biochim, Biophys. Acta(1970)

oxidize long-chain FA has not developed at birth. It is possible that this has provided the evolutional 'push' in such species for the biosynthesis of medium-chain-length FA. Certainly, short- and medium-chain-length FA are entirely catabolized by the newborn and are not laid down in the storage lipids or utilized for membrane lipid synthesis.

2. Minor FA of ML

It is important to recognize that part of the component designated '8:2'(or linoleic acid) on analysis by gas chromatography may consist of isomers other than cis-9, cis-12, and so may lack biological potency as an essential FA. A proportion of the monoenoic FA can consist of trans isomers-which may have some unwanted biological effects-the nutritional value of which has been the subject of some debate in recent years.

Those FA incow milk listed in a comprehensive compilation include all the odd- and even-numbered normal saturated FA from C_2 to C_{28}, monomethyl-branched FA from C_{11} to C_{28}, multimethyl-branched FA from C_{16} to C_{26}, a number of di- and poly-enoic FA, keto- and hydroxy-FA, and cyclohexylfatty acids.

Fewer FA have been isolated from human than from bovine milk but if the minor lipid classes inhumanr were investigated in greater detail. The list is still extensive and includes 17 normal saturated components from C_4 to C_{23}, 54 branched-chain, 62 monoenoic and 33 polyunsaturated FA. The content of trans FA in human milk is known to vary markedly with the diet and can range from 0 to 10% (1). FA compositions of the cholesterol ester and the main glycerophosphatide components of milks from some representative species are listed in Table 10.

The cholesterol ester fraction of ML is very small but may be important in the biochemistry

Table 10. Fatty acid compositions of the cholesterol esters, phosphatidycholines and phosphatidylethanolamines in various milks

Fatty acid	wt % of the total								
	Cow			Human			Mink		
	CE	PC	PE	CE	PC	PE	CE	PC	PE
12:0	3.4			3.2			0.3		
14:0	11.5	8.4	1.5	4.8	4.5	1.1	1.1	1.3	0.8
16:0	27.6	34.4	11.7	23.8	33.7	8.5	25.4	26.4	20.6
16:1	6.0	0.6	2.1	1.5	1.7	3.4	4.4	1.1	1.2
18:0	13.6	11.1	10.5	8.0	23.1	29.1	14.7	20.8	29.3
18:1	28.0	25.7	46.7	45.7	14.0	15.8	35.7	31.7	27.8
18:2	0.6	5.3	12.4	12.4	15.6	17.7	13.5	17.4	19.1
18:3		1.1	3.4	T	1.3	4.1	2.6	2.2	0.5
20:3		1.0	1.4		2.1	3.4			
20:4		0.7	0.9	T	3.3	12.5			
22:6					0.4	2.6			

CE, cholesterol ester; PC, phosphatidylcholin; PE, phosphatidylethanolamine W. Christie, Developments in dairy Chemistry(1983)

of the mammary gland. In ruminants, the milk cholesterol esters differ markedly from those of all other tissues and in plasma, for example, the cholesterol esters are relatively rich in polyunsaturated FA. In bovine milk, the cholesterol esters of the skim-milk fraction are very different in composition and contain more than 70% linoleic acid. PC and PE are the main glycerophosphatides of milks where they are located in the membranes. It appears that the biochemical strategy of the mother is to conserve these essential nutrients and export the minimum that will meet the requirements of the newborn. The same may well be true of non-ruminants, but the effects is not as obvious as they have much larger tissue stores of these components. No short- or medium-chain FA (<C14 essentially) have been found in milk PL.

3. The FA and Long-Chain Base Components of the Sphingolipids of Milk

The sphingomyelin and glycosphingol-ipids are minor components of milk, but they have important biochemical roles in the membranes of the mammary gland and in the MF globule membrane. They mainly contain very-long-chain-length FA, and the compositions of the principal constituents of bovine and human milk are listed in Table 11.

The simplest natural sphingolipid component is ceramide, which in the cow contains mainly saturated C_{22}, C_{23} and C_{24} FA, in proportions similar to those of the sphingomyelin and ceramide dihexoside fractions. In addition, the sphingomyelin, ceramide monohexoside and ceramide dih-

Table 11. Composition of the non-hydroxy long-chain fatty acids in the sphingolipids of bovine and human milk

	wt % of the total					
Fatty	Cow			Human		
acid	Sphingomyelin	CMH	CDH	Sphingomyelin	CMH	CDH
14:0	0.4	1.0	0.3	2.5		
16:0	7.8	9.3	7.7	22.5	13.6	16.2
16:1		1.4		1.0	1.3	0.9
18:0	1.6	13.7	3.3	8.1	6.9	8.7
18:1	0.2	12.2	1.3	6.2	5.2	7.8
18:2	0.2	2.0	0.2	0.5		
20:0	0.6	0.9	1.1	0.5	3.8	2.8
22:0	20.7	17.0	24.9	7.5	13.3	12.5
23:0	30.4	22.0	29.5	27.2	3.9	3.4
23:1	5.0	3.4	6.6	1.2	0.4	1.1
24:0	22.8	9.9	16.5	17.0	31.9	20.1
24:1	4.0	2.1	3.7	2.0	16.8	20.1
25:0	1.6		0.7		0.3	1.7
25:1	1.6		1.4		0.8	2.6

CMH: ceramide monohexoside; CDH: ceramide dihexosid W. Christie, Developments in Dairy Chemistry (1983)

exoside fractions contain small amounts of 2-hydroxy FA. Only the ceramide monohexoside contained 2-hydroxy FA.

Human milk sphingomyelin and monoglycosyl ceramide have relatively simple long-chain base compositions, in which more than 60% is a C_{18}-sphingosine.. Similar FA and long-chain base components to these are deposited in brain tissue during myelination, so it is possible that they have some nutritional importance for the newborn.

4. Biosynthesis of FA

The inclusion of fats and oils in the diet of ruminants may have the following effects, thereby influencing, either directly or indirectly, lipogenic pathways leading to MF secretion. 1.The synthesis de novo of FA, such as 16:0 and 18:0, by micro-organisma in the rumen may be reduced, leading to a reduction in blood lipids and mammary gland uptake and to a reduction in the yield of long-chain FA in milk. 2. The amounts of long-chain FA released in the rumen by lipolysis may be increased, leading to increases in the blood lipids, mammary gland uptake and the yield of long-chain FA in milk. 3. The intramammary synthesis of FA may be reduced thus decreasing the yield of short- and medium-chain FA in milk. Two mechanisms have been suggested for this

reduction: firstly, a reduction in the amounts of acetic and butyric acids produced in the rumen, leading to decreased supplies of acetate and b-hydroxobutyrate to the mammary gland; and secondly, an inhibition of mammary gland acetyl-CoA carboxylase. These effects appear to be influenced by factors such as : stage of lactation; roughage-to-concentrate ratio and fat content of the basal diet; and amount, composition and physical form of fat added to the basal diet [2].

V. MODIFICATION OF MILK LIPIDS

Consumer demand for food products of superior health quality has renewed interest in modifying the lipid composition of milk. While work involving the reduction of the saturated and n-6 FA as well as cholesterol content of milk has met with little success, dietary FA modification has proved to be a viable method of adding value to milks for the health conscious consume [8,9]. Because of the association with a decreased risk of coronary heart disease, recent dietary fat studies have centred on the manipulation of specific FA, i.e. 20-carbon omega-3 FA (eicosapentaenoic acid 20:5n3, decosahexaenoic acid 22:6n3) found in marine sources [10,11]. Seasonal availability, affordability and consumer preference often limit fish consumption, thereby excluding the primary source of 20-carbon omega-3 FA. Enrichment of milk with these FA might provide an excellent alternative source. The omega-3 FA content of milk can bereadily increased by manipulation of desaturase activity and the inclusion of specific FA in the diet [12, 13]. Off-flavours associated with milk enriched in this way have prompted investigations into the use of terrestrial sources of omega-3 FA. While effective in enriching milk with linoleic acid (18:3n3), plant sources result in only minor changes in the content of 20-carbon omega-3 FA. Continued investigation in the areas of sensory evaluation and product stability are needed if significant improvements in the health quality of foods available to the consumer are to be made.

Differences in specific FA ratios in milk reflected rarely the differences in these ratios in the dietary oils. The findings of Yeo [14] that FA composition of milk may be readily altered by FA desaturase activity and liposomes of dietary FA are very recent development. Table 12 shows modification of FA in milk by controlling either desaturase activity or dietary fats. Different ways of modifying the rheological properties of cream and butter are including homogenization and rebodying of cream, and where butter is concerned, temperature treatment of cream prior to churning, work softening and alteration of the composition of the fat either by fractionation of fat into portions with different melting points or by direct admixture of other fats, usually vegetable fats with low melting points.

As given in Table 13 , the platelet aggregation was remarkably reduced in the subjects consumed n-3 PUFA-enriched milk relative to regularmilk. Similar trends were shown in human subjects fed eggs,chicken and pork high in n-3 FA, particularly DHA. The results support the

Table 12. Fatty acid composition of regular milk (RM) and n-3 PUFA enriched milk (NPEM) after modifying fatty acids in milk by biotechnology

Fatty acid	RM	NPEM
	..... mol %	
18:2n-6	2.7	3.5
18:3n-3	0.2	0.3
20:4n-6	0.7	0.1
20:5n-3	tr	0.2
22:6n-3	tr	0.2
n-6/n-3	8.2	2.7

Yeo, Health Effects of Animal Products Enriched with n-3 Fatty Acids, 1996.

Table 13. Degree of human (mail aged 14) platelet aggregation affected by either dietary regular milk (RM) or n-3 PUFA enriched milk (NPEM)[a]

Agonist	Dietary gorup	
	RM	NPEN
	..... mol %	
Collagen (5ml)	22.4 ± 2.1	17.1 ±1.8[b]

[a] Values represent means ± S.E. for 250 subjects (male aged 14) of the RM group and 280 subjects (male aged 14) of the NPEM group. Each subject was taken 585ml of milk daily for 4 weeks.

[b] Indicates significant defference from the RM group, $P<0.05$

Yeo, Health Effects of Animal Products Enriched with n-3 Fatty Acids, 1996.

effects of animal products rich in n-3 FA on pathophysiological states related to platelet activation (14).

Ⅵ. FUTURE DEVELOPMENT TRENDS OF MILK

The essential features ofMF composition, especially the FA composition, of a wide range of mammalian species have been obtained, sufficient indeed to give a reasonably clear picture of interspecies differences. The lipids of cow and human milks, in particular, have been investigated in such detail that it is not at all easy to foresee the direction of the future research effort. However, it seems likely that the minor components of potential physiological importance to the newborn, such as n-3 PUFA, vitamins, hormones and prostaglandins, will be a focus for further attention. As given in Table 14, most of the monor lipid conponents in CM are needed to be increased up to the levels of mother's milk. With other species relatively little is known of the functions of the various lipid classes or of their structures, and further work in this area

Table 14. Comparison of minor components in cow milk to human milk

	Human milk	Cow milk
	..... Ratio	
Vitamin A	2	1
Vitamin D	2	1
Vitamin E	3	1
Prostaglandins E	10	1
Prostaglandins F	10	1
AA	2.5	1
EPA	0.1	tr
DHA	0.2	tr
n-6/n-3 FA	1	2

would have both biochemical and nutritional relevance.

Ⅶ. ACKNOWLEDGEMENTS

This review paper is mainly extracted from Dr. W.W. Christie's chapter, the composition and structure of ML *in* Developments in Dairy Chemistry (P.F. Fox, eds),1983. The authors sincerely wish Applied Science Publishers Ltd to have a proper understanding for this matter.

Ⅷ. REFERENCES

1. Christie, W.W. 1983. The composition and structure of milk lipids. Developments in Dairy Chemistry (P.F. Fox ed), Applied Science Publishers, New York.
2. Keenan, T.W., Dylewski, D.P. and Woodford, T.A. 1983. Origin of milk fat globules and the nature of the milk fat globule membrane. Developments in Dairy Chemistry (P.F. Fox ed), Applied Science Publishers, New York.
3. Mozes, N. and Smith, S. 1982. Unpublished Results.
4. Hawke, J.C and Taylor, M.W. 1983. Influence of nutritional factors on the yield, composition and physical properties of milk fat. Developments in Dairy Chemistry (P.F. Fox ed), Applied Science Publishers, New York.
5. Morrison, W. R. and Smith, L. M. 1967. Lipids, 2: 178.
6. Jenness, R. 1980. J. Dairy Sci. 63: 1605.
7. Hay, J. D. and Morrison, W. R. 1970. Biochim. Biophys. Acta,202: 237.
8. Hargis, P.S. and Van Elswyk, M.E. 1993. World's Poultry Sci. J. 49:251.
9. Noakes, M., Nestel, P. and Clifton, P.M. 1996. Am. J. Clin. Nutr. 63:42.

10. Yeo, Y.K., Philbrick, D.J. and Holub, B.J. 1989. Biochem. Biophys. Res. Commun. 160: 1238.
11. Yeo, Y.K., Philbrick, D.J. and Holub, B.J. 1989. Biochim. Biophys. Acta, 1006.
12. Yeo, Y.K. and Holub, B.J. 1990. Lipids, 25: 811.
13. Park, J.H., Park, E.J., Kim, K.S. and Yeo, Y.K. 1995. Lipids, 30: 541.
14. Yeo, Y.K. 1996. Health effects of animal products high in n-3 fatty acids. Presented at Conference "Health Effects of Animal Products Enriched with n-3 Fatty Acids" Korea.