# MODIFICATIONS IN THE STRUCTURE OF THE TRIACYLGLYCEROLS OF BOVINE MILK FAT

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at Massey University

Ian Malcolm Morrison

1976

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ABSTRACT

A pair of monozygous twin cows was used to investigate the influence of the increased availability of linoleic acid (18:2) to the mammary gland, on the structure and physical properties of the milk triacylglycerols (TGs). The cows were grazing fresh pasture, and in addition one of the pair was provided with a daily supplement of encapsulated sunflower oil. The milk fat of the cow given the supplement (18:2-rich milk fat) contained 15.5% 18:2 compared with 1.8% 18:2 in the milk fat of the other cow (control milk fat). This increase in the proportion of 18:2 in the milk fat was accompanied by decreases in the proportions of myristic acid (14:0) and palmitic acid (16:0). The effect of this altered fatty acid (FA) composition on the TG composition of the 18:2-rich milk fat was to increase the proportions of TGs with 40, 52 and 54 acyl carbons and to decrease the proportions of TGs with 34, 36, 38. 44, 46, 48 and 50 acyl carbons relative to the proportions in the control milk fat.

Both milk fat samples were separated, by column chromatography on silicic acid, into TG fractions of high, medium and low molecular weight. The relative proportions of the TG fractions of high, medium and low mol. wt. in the 18:2-rich milk fat were 43.0, 19.5 and 37.5% respectively compared with 36.1, 19.7 and 44.2% respectively in the control milk fat. Stereospecific analysis of these triacylglycerol fractions demonstrated that in fractions of similar mol. wt., the distribution of fatty acids within the TG molecule in the presence of high levels of 18:2, was not appreciably altered from that in the triacylglycerol fractions of the control milk fat. The positional distribution of fatty acids in the triacylglycerols of the total milk fats was also similar. In the milk fat, containing high levels of

linoleic acid, 18:2 showed a preference for positions 1 and 2 in the triacylglycerols of the low and medium mol. wt. fractions, and for positions 2 and 3 of the high mol. wt. fraction.

The three TG fractions of each milk fat were further resolved into TG classes of differing levels of unsaturation. The 18:2-rich milk fat contained higher levels of the unsaturated TGs, namely the diene, triene and tetraene TGs, and lower levels of the saturated TGs and to a lesser extent the monoene TGs. The diene TGs of the 18:2-rich milk fat included combinations of 18:2 with two saturated FAs, which are a minor constituent of normal milk fats. Likewise the triene TGs reflected the presence of 18:2 in combination with 18:1 and a saturated FA.

The thermal properties of the two milk fats were examined to investigate the influence of the altered TG composition and structure on the physical characteristics of the milk fat. The 18:2-rich milk fat melted at a lower temperature than the control milk fat with a large proportion of the sample melting over the narrow temperature range between 5 and 15$^{o}$C. In contrast the control milk fat melted over a much wider temperature range.

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ABBREVIATIONS

| | |
|---|---|
| ATP | adenosine-5′-triphosphate |
| B | butyric acid |
| BSA | bovine serum albumen |
| CoA | coenzyme A |
| cpm | counts per minute |
| DG | diacylglycerol |
| dpm | disintegrations per minute |
| D.S.C. | differential scanning calorimeter |
| D.T.A. | differential thermal analysis |
| DTT | dithiothreitol |
| EDTA | ethylenediaminetetraacetic acid |
| Eth. | ethanol |
| FA | fatty acid |
| Fw | weight response factor |
| g.l.c. | gas-liquid chromatography |
| HDL | high density lipoprotein |
| i.d. | internal diameter |
| LDL | low density lipoprotein |
| M | moles per litre |
| MG | monoacylglycerol |
| nmoles | nanomoles |
| N.M.R. | nuclear magnetic resonance |
| O | oleic acid |
| Orig. | original |
| P | palmitic acid |
| P-Ph | phosphoryl phenol |
| 2,3-PL | 2,3-diacyl-sn-glycerol-3-phosphoryl phenol |
| 1-PL | 1-acyl-sn-glycerol-3-phosphoryl phenol |
| $R_f$ | distance moved relative to solvent front |
| s | seconds |
| St | stearic acid |
| TG | triacylglycerol |
| t.l.c. | thin-layer chromatography |
| Tris | tris(hydroxymethyl)aminomethane |
| VLDL | very low density lipoprotein |

# ABBREVIATIONS

Triacylglycerols of milk fat are referred to by their total number of acyl carbon atoms e.g. Glycerol tristearate = $C_{54}$.

Pure stereospecific and racemic triacylglycerols are abbreviated as by Litchfield (1972) e.g. sn-glycerol-1-palmitate-2-oleate-3-stearate = sn - POSt.

Fatty acids are designated by the shorthand notation - number of carbon atoms : number of double bonds, e.g. octadecanoic acid (stearic acid) = 18:0.

Shorthand notation for triacylglycerols separated by degree of unsaturation is written as 000 = saturated TG, 001 = monounsaturated or monoene TG (where 0 and 1 refer to saturated and monoenoic fatty acids respectively). When the stereospecific distribution of fatty acids in the triacylglycerols is known, these triacylglycerols are referred to by double underlining e.g. 020 = diene TG containing a dienoic fatty acid in position 2.

Other abbreviations used in this thesis, unless otherwise designated in the text, follow the guide lines set down by the Biochemical Journal (1973) 131, 1 - 20.

# Chapter 1

# INTRODUCTION

## Section 1.1. General introduction

Polyunsaturated fatty acids in ruminant diets are rapidly hydrogenated in the rumen to give mainly saturated and monoenoic fatty acids (FAs) which then are available for incorporation into ruminant milk and tissue triacylglycerols (TGs). As a consequence of this process of biohydrogenation, the composition of ruminant milk and tissue fats, unlike that of non-ruminants, is fairly constant despite changes in the lipid composition of the diet. However the introduction of techniques for protecting dietary lipid from hydrogenation in the rumen, e.g. Scott et al (1970), permits the modification of ruminant tissue and milk fats by dietary means. This technique provides an opportunity to study the effect of altering the FAs available for absorption on the composition and structure of the milk TGs secreted by the mammary gland and to observe its effect on the physical properties of the milk fat.

## Section 1.2. Analysis of the triacylglycerols of milk fat

### 1.2.1. Studies of the composition and structure of milk fat

Milk fat contains a wide and complex range of triacylglycerol (TG) species (Litchfield, 1972), and therefore before determination of the composition and structure of the TGs of milk fat it is an advantage to fractionate the TGs into fractions of simpler composition. Techniques that have been used to achieve fractions of high and low mol. wt. and often an intermediate fraction of medium mol. wt., include: t.l.c. on silicic acid (Breckenridge and Kuksis, 1968b 1969), and column chromatography on silicic acid or florisil (Blank and Privett, 1964; Nutter and Privett, 1967; Shehata et al, 1971, 1972; Taylor and Hawke,

1975a). These TG fractions have been subjected to argentation t.l.c. to separate the TGs on the basis of their degree of unsaturation. Analysis of fractionated TGs was carried out by g.l.c. of the methyl esters of the constituent fatty acids (FAs) and of the intact TGs. The sizes of the TG fractions of low, medium and high mol. wt. and the proportions of the TG classes with differing levels of unsaturation are summarized in Table 1. The proportions of the TG classes in each fraction of low and medium mol. wt., obtained by the different workers were quite similar, with saturated TGs comprising 39 - 47% and the monoene TGs 34 - 41% of the two fractions. On the other hand in the fractions of high mol. wt. the results of Breckenridge and Kuksis 1969) and Blank and Privett (1964) differed from those of Taylor and Hawke (1975a) in that the New Zealand milk fat contained higher levels of the saturated TGs of high mol. wt. and consequently lower levels of the more unsaturated TGs of high mol. wt., than the North American milk fats.

The more abundant molecular species of TG, without regard to the intramolecular distribution of the FAs, have been determined for several milk fat fractions, with the additional application of preparative g.l.c. (Breckenridge and Kuksis, 1968b), and liquid - liquid partition chromatography (Nutter and Privett, 1967) (Table 2). Similar proportions of the major FAs were present in the TG fractions of low mol. wt. analysed by Nutter and Privett (1967), and Breckenridge and Kuksis (1968b). It was apparent that the molecular species consisted of 4:0 in combination with two of either 14:0, 16:0, 18:0 or 18:1. The major molecular species in two fractions of high mol. wt. (Shehata et al, 1972) consisted of combinations of the FAs 14:0, 16:0, 18:0 and 18:1 (Table 2).

Table 1. Proportions of triacylglycerol classes of differing levels of unsaturation prepared by absorption chromatography and argentation t.l.c. of milk fat

| TG class | Proportions in TG fractions of differing mol. wt. | | | | | | | | | | Proportions in total milk fat | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High mol. wt. | | | | Medium mol. wt. | | Low mol. wt. | | | | | | |
| | Ref.1[a] | Ref.3[c] | Ref.4[d] | Ref.5[e] | Ref.3[c] | Ref.5[e] | Ref.1[a] | Ref.2[b] | Ref.3[c] | Ref.5[e] | Ref.1[a] | Ref.3[c] | Ref.5[e] |
| | (wt.%) | (mole%) | (mole%) | (mole%) | (mole%) | (mole%) | (wt.%) | (wt.%) | (mole%) | (mole%) | (wt.%) | (mole%) | (mole%) |
| Saturated TGs | 19.6 | 16.5 | 16.4 | 28.5 | 38.7 | 45.4 | 43.7 | 47.2 | 45.0 | 46.6 | 28.1 | 32.9 | 39.2 |
| Monoene TGs | 58.3 | 36.7 | 38.5 | 39.3 | 38.3 | 37.5 | 40.8 | 33.5 | 38.1 | 37.2 | 50.4 | 37.6 | 38.0 |
| Diene TGs | 22.1 | 27.7 | 34.8 | 21.7 | 14.3 | 10.3 | 9.8 | 15.2 | 11.7 | 9.9 | 16.9 | 21.7 | 14.7 |
| Triene TGs | tr. | 12.9 | 10.8 | 10.5 | 8.7 | 6.8 | 5.6 | 4.1 | 5.2 | 6.3 | 2.1 | 5.4 | 8.1 |
| Polyene TGs | tr. | 6.2 | - | - | - | - | - | - | - | - | - | 2.4 | - |
| Proportion of total FAs (%) | 60.2 | 38-40 | 24.5 | 39.9 | 16-17 | 16.9 | 37.3 | 24.3 | 43-45 | 43.2 | 100 | 100 | 100 |

[a] Blank and Privett (1964)

[b] Nutter and Privett (1967)

[c] Breckenridge and Kuksis (1968b. 1969)

[d] Shehata et al (1971, 1972)

[e] Taylor and Hawke (1975a)

Table 2. The more abundant molecular species of triacylglycerol in triacylglycerol fractions of milk fat [a]

| TG class | High mol. wt. | Low mol. wt. | |
|---|---|---|---|
| | Ref.1[b] | Ref.3[d] | Ref.2[c] |
| Saturated TGs | 16:0 - 16:0 - 16:0 | 16:0 - 16:0 - 4:0 | 16:0 - 14:0 - 4:0 |
| | 18:0 - 16:0 - 14:0 | 16:0 - 14:0 - 4:0 | 18:0 - 16:0 - 4:0 |
| | 18:0 - 18:0 - 14:0 | 18:0 - 16:0 - 4:0 | 16:0 - 16:0 - 4:0 |
| | 18:0 - 16:0 - 16:0 | | 18:0 - 14:0 - 4:0 |
| Monoene TGs | 18:1 - 16:0 - 16:0 | 18:1 - 16:0 - 4:0 | 18:1 - 16:0 - 4:0 |
| | 18:1 - 18:0 - 14:0 | 18:1 - 18:0 - 4:0 | 18:1 - 14:0 - 4:0 |
| | 18:1 - 18:0 - 16:0 | | 18:1 - 18:0 - 4:0 |
| | 18:1 - 16:0 - 14:0 | | |
| Diene TGs | 18:1 - 16:0 - 18:1 | 18:1 - 18:1 - 4:0 | 18:1 - 18:1 - 4:0 |
| | 18:1 - 18:0 - 18:1 | | 18:2 - 16:0 - 4:0 |
| | 18:1 - 14:0 - 18:1 | | 18:2 - 14:0 - 4:0 |
| Triene TGs | 18:1 - 18:1 - 18:1 | | 18:2 - 18:1 - 4:0 |
| | | | 18:3 - 16:0 - 4:0 |

a TG species listed in order of decreasing abundance within each TG class

b Shehata et al (1972)

c Nutter and Privett (1967)

d Breckenridge and Kuksis (1968b)

1.2.2. Stereospecific analysis of the triacylglycerols of milk fat

Subsequent to the first stereospecific analysis of TGs carried out by Brockerhoff (1965), the positional distribution of FAs in numerous TGs have been determined, and various modifications made to the original procedure (Christie and Moore, 1969).

(a) Special features of the analysis of ruminant milk fats

The results obtained by Pitas et al (1967) would indicate that acceptable data for the distribution of FAs in the TGs of the total milk fat cannot be obtained when using pancreatic lipase to hydrolyse TGs from total milk fat. Comparison with the results of Jack et al (1963), Sampugna et al (1967) and Smith et al (1965) as reviewed by Taylor (1973) would suggest that pancreatic lipase will exhibit minimal chain specificity only when presented with TGs containing FAs of similar chain length and when using a short time of hydrolysis.

This difficulty and source of error has been overcome by carrying out stereospecific analyses on high and low mol. wt. fractions of milk fat. This has the added advantages of reducing the complexity of the TG mixture and also helping in the interpretation of stereospecific data.

(b) Stereospecific distribution of FAs in the triacylglycerols of ruminant milk fats

In the low mol. wt. TGs of sheep and goat milk, 4:0, 6:0, 8:0 and 10:0 were preferentially esterified in position 3, 14:0 in position 2, and, 16:0 and 18:0 in position 1. 18:1 was distributed equally between positions 1 and 2. (Marai et al, 1969). In the high mol. wt. TGs of sheep and goat milk, 10:0 and 18:1 were preferentially esterified in position 3, 14:0 in position 2 and 18:0 in position 1. 16:0 was distributed differently in the two milk fats, being concentrated in position 1 of the sheep milk fat, but distributed evenly

between positions 1 and 2 of goat milk fat.

Two molecular distillates of bovine butteroil, one a fraction of high mol. wt., the other a fraction of low mol. wt. were subjected to stereospecific analysis by Breckenridge and Kuksis (1968a, 1969). In both fractions. 4:0, 6:0 and 8:0 were esterified almost exclusively in position 3. with 14:0 preferentially esterified in position 2, 16:0 in positions 1 and 2, and 18:0 in position 1. 18:1 showed a difference in distribution in the two fractions, being predominantly in positions 1 and 3 of the high mol. wt. fraction and in position 1 of the low mol. wt. fraction. 18:2 although present only in small amounts tended to prefer position 2 of the TG.

Stereospecific analysis of several high mol. wt. fractions of bovine milk fat prepared by fractional crystallization from acetone showed the same trends in the lower mol. wt. TGs as observed by Breckenridge and Kuksis (1968a, 1969) (Barbano and Sherbon, 1974). As found by Dimick et al (1965), 16:0 appeared to increase its preference for position 2 with increasing mol. wt. of the TG fractions.

Taylor and Hawke (1975b) investigated the stereospecific distribution of FAs in the TGs of three samples of New Zealand milk fat, which had been obtained at different stages of the dairying season. The positional distribution of FAs in the TGs of milk fat, were found to be relatively constant throughout the season. The positional distribution in one of their milk fat samples, obtained by recalculation from the positional distributions in milk fat fractions of high, medium and low mol. wt. is given in Table 3. In the total milk fat 4:0 and 6:0 were esterified almost entirely in position 3 and 10:0, 12:0 and 14:0 were preferentially esterified at position 2. Palmitic acid was concentrated at positions 1 and 2, and the proportions of 18:0 were greatest at position 1 and least at position 2.

Although 18:1 tended to be esterified preferentially at position 1, considerable amounts were esterified at positions 2 and 3. Linoleic acid appeared to be relatively evenly distributed in the TG, even though it was present only in small quantities. The only significant differences in the distribution of FAs between the fractions of high, medium and low mol. wt. were changes in the distribution of 18:0 and 18:1, which were esterified equally at positions 1 and 3 in the high mol. wt. fraction but in the low and medium mol. wt. fraction, 18:0 and 18:1 were esterified mainly in position 1.

Table 3. Stereospecific distribution of fatty acids in the triacylglycerols of bovine milk fat (Taylor and Hawke, 1975b)

| | FA Composition (mole %) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FA[a] | 4:0 | 6:0 | 8:0 | 10:0 | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 |
| Position 1 | - | 1.3 | 0.8 | 1.3 | 2.1 | 8.1 | 29.4 | 0.9 | 22.9 | 30.0 | 0.4 |
| Position 2 | - | - | 1.9 | 7.2 | 7.2 | 21.9 | 31.4 | 1.7 | 6.6 | 17.4 | 0.7 |
| Position 3 | 32.4 | 13.1 | 3.5 | 3.5 | 2.2 | 3.3 | 8.3 | 1.0 | 11.3 | 18.6 | 0.9 |

[a] only major FAs shown (>1% of total)

## Section 1.3. Metabolism of dietary lipids in relation to milk fat synthesis in the ruminant

### 1.3.1. Digestion and absorption of dietary lipids

The diet of most New Zealand domestic ruminants is comprised of different types of pasture grasses supplemented at certain times of the year, mostly in winter, by various feed crops and hay. The average polyenoic fatty acid content of the lipids of pasture grasses is about 75% (Hawke, 1973), but the milk fat of the ruminant contains only 1 - 6% polyenoic FAs (Hilditch and Williams, 1964). This low

level of polyenoic FAs in ruminant milk fat arises from the enzymic processes in the rumen, namely the hydrolysis of dietary lipids by microbial and plant lipases, and the hydrogenation of the polyenoic FAs to yield more saturated FAs. (Hawke and Silcock, 1970; Viviani, 1970; Dawson and Kemp, 1970; Faruque et al, 1974).

These products pass from the rumen and are absorbed into the mucosal cells of the small intestine where resynthesis of triacylglycerols (TGs) occurs (Senior, 1964; Johnston, 1970) by both the monoglyceride and glycerol-3-phosphate pathways (Cunningham and Leat, 1969). The newly synthesized TGs are assembled into chylomicrons which enter the blood stream via the thoracic lymph duct (Senior, 1964). In experiments with grazing cows Hartmann and Lascelles (1966) showed that over 400 g of esterified FAs were carried in the thoracic lymph duct daily. Chylomicron TG (50 - 70%) and phospholipid (20%) comprised most of the total lipid in the lymph.

The total lipid content of normal bovine plasma is about 350 - 560 mg per 100 ml, of which only 1 - 5% is TGs, the rest being cholesterol esters (40 - 50%), phospholipids (38 - 43%), cholesterol (6-9%) and unesterified FAs (0 - 2%)(Hartmann and Lascelles, 1965; Yamdagni and Schultz, 1970; Raphael et al, 1973). About 70 to 80% of the total lipid in bovine serum circulates as high density lipoprotein (HDL) and 24 - 30% as low density lipoprotein (LDL), whereas chylomicrons and very - low density lipoproteins (VLDL) comprise only 2 - 3% of the total serum lipid. (Dryden et al, 1971; Wendlandt and Davis, 1973).

### 1.3.2. Uptake of lipid from the blood by the mammary gland

The major components of the blood lipoproteins to contribute to milk are the TGs of the VLDL and to a lesser extent the TGs of the

chylomicrons (Barry et al, 1963; Glascock et al, 1966; Linzell, 1968; West et al, 1972; Gooden and Lascelles, 1973). At least 80% of these blood TGs are completely hydrolysed by lipoprotein lipase during uptake by the mammary gland (West et al, 1972).

Minor contributors to the uptake of lipid from the blood are non-esterified FAs (NEFA), which undergo an exchange with the FAs of the mammary gland (Annison et al, 1967; Massart-Leën et al, 1970), and 2-monoglycerides (Bickerstaffe et al, 1970). Acetate and β-hydroxybutyrate are also taken up into the mammary gland from the blood (Linzell, 1968).

### 1.3.3. Biosynthesis of milk fat in ruminants

The biosynthesis of milk fat in ruminants has been extensively reviewed in recent years (Dimick et al, 1970; Storry, 1970; Bickerstaffe, 1971; Storry, 1972; Emery, 1973; Moore, 1974; Bauman and Davis, 1974).

The major lipid component in milk is TG, comprising 97 - 98% of the total lipid constituents (Jensen, 1973). About 140 different FAs have been identified in milk fat, but most of these are present only in trace amounts. The major FAs (comprising more than 1% of the total FAs) number only about 13, and are comprised of FAs containing between 4 and 18 carbon atoms. A wide range of TG species exists, ranging in acyl carbon number from 26 to 54 for the quantitatively important TGs. The distribution of the FAs within these TGs has been found to be non-random.

#### 1.3.3.1. Biosynthesis of fatty acids in the mammary gland

The origin of the FAs of milk fat has been extensively studied and it is now generally accepted, that in ruminants, the short chain

FAs (4:0 to 10:0) are derived, by biosynthesis within the mammary gland, from acetate and β-hydroxybutyrate, the long chain FAs (18C) from the TGs of the VLDL and chylomicrons of the blood plasma and the intermediate chain FAs (12:0 - 16:0) from both of these sources (Storry, 1970). The total contributions of the various precursors to the FAs of milk fat in the goat, have been found to be acetate, 42%, β-hydroxybutyrate, 9.4%, and other plasma precursors (by difference), 48.6%. Acetate and β-hydroxybutyrate together accounted for all of the 4:0 - 12:0 FAs of milk fat, 75% of 14:0, 45% of 16:0 and 10% of 18:0 (Smith et al, 1974).

The biochemical pathways involved in the intramammary biosynthesis of FAs include:

(a) The malonyl CoA pathway operating in the cell cytosol, which involves the carboxylation of acetyl CoA to malonyl CoA and the subsequent addition of further malonyl CoAs as $C_2$ units to give FAs ranging from 4:0 to 16:0. This pathway was first elucidated in the mammary gland by Ganguly (1960) and later confirmed by Becker and Kumar (1965) and McCarthy and Smith (1972).

(b) The direct incorporation of β-hydroxybutyrate as a 4 carbon unit following reduction to butyrate, with subsequent additions of $C_2$ units to give FAs ranging from 4:0 to 16:0 (Smith and McCarthy, 1969; McCarthy and Smith, 1972; Smith et al, 1974). Some of the butyrate carbons were found in other regions of the FAs, indicating some breakdown of butyrate to 2 carbon units.

(c) An avidin-insensitive pathway, independent of malonyl CoA, associated with the mitochondria, that will incorporate acetate but very little β-hydroxybutyrate into short and medium chain FAs (4:0 to 10:0) by the postulated use of the reversal of the process of β-oxidation (Nandedkar and Kumar, 1969; McCarthy and Smith, 1972).

(d) The desaturation of stearate and palmitate to oleate and palmitoleate respectively, which occurs in the microsomal fraction and requires both NADPH, and CoA esters of 18:0 and 16:0 (Bickerstaffe and Annison, 1970; Storry, 1970; Kinsella, 1970a, 1972a). Oleic, linoleic and linolenic acids have been shown to inhibit the desaturase (Bickerstaffe and Annison, 1970).

Each ruminant species synthesizes a rather constant pattern of FAs in the mammary gland, thus implying a degree of cellular control. Results from the in vitro incubations of mammary tissue slices (Bauman et al, 1973) with acetate have demonstrated that the proportions of each FA synthesized are quite similar to those occurring in vivo, the major discrepancy being the lower levels of butyrate. In contrast, many workers, when using purified mammary FA synthetases from various animal sources have found that the major FA produced is 16:0 (Carey and Dils, 1970; Smith and Abraham, 1970, 1971). However, altering the concentration of the FA synthetase can bring about the synthesis of FAs very similar in composition to those found in vivo. (Kinsella et al, 1975). The ratios of acetyl CoA : malonyl CoA were also important since raising the ratio increased the proportions of short and medium chain FAs synthesized both by the particle-free supernatant (Smith and Dils, 1966), and a purified FA synthetase preparation (Carey and Dils, 1970), from the mammary gland of the lactating rabbit.

### 1.3.3.2. Biosynthesis of triacylglycerols in the mammary gland

In most mammalian tissues the biosynthesis of TGs occurs via two pathways i.e. the glycerol-3-phosphate pathway and the monoglyceride pathway (Hübscher, 1970). In mammary tissue the exact pathways have not been fully elucidated.

The glycerol required for TG biosynthesis is derived mainly from the plasma TGs with the remainder being synthesized from glucose in the mammary gland. Only small arteriovenous differences across the mammary gland have been observed for plasma glycerol, therefore the free glycerol of the blood is probably not an important precursor of TG - glycerol (Barry et al, 1963).

Incorporation of $[^{14}C]$ -glycerol into lipids by bovine mammary tissue (Kinsella, 1968) indicated the presence in mammary tissue of glycerokinase which activates glycerol to glycerol-3-phosphate. Glycerokinase has also been shown to be present in mammary tissue of the goat (Bickerstaffe and Annison, 1971b). The incorporation of $[^{14}C]$ -glycerol into glycerol-3-phosphate was found to be associated with a microsomal fraction, with the addition of the particle-free supernatant giving some stimulation.

Esterification of FAs by homogenates of bovine mammary tissue is associated with the particulate fraction of the cell (Askew et al, 1971a), but is stimulated by the addition of a particle-free fraction. The esterification was observed to be dependent on ATP, CoA, $Mg^{2+}$ and an acyl acceptor such as glycerol-3-phosphate. Similar results have been obtained using goat mammary tissue (Pynadath and Kumar, 1964; Bickerstaffe and Annison, 1971b).

Pynadath and Kumar (1964) demonstrated that D,L glycerol-3-phosphate, 1,2 - dipalmitin and diolein were good acyl acceptors for the in vitro synthesis of milk TGs in goats. Since 2-monoglycerides and monoolein were not used for the synthesis it was suggested that biosynthesis of TGs in goat mammary tissue occurs almost entirely via a glycerol-3-phosphate pathway. However evidence for the monoglyceride pathway follows from the incorporation of 2-glyceryl ethers into TGs by goat mammary tissue (Bickerstaffe and Annison, 1971b). Also the greater

proportions of 16:0 at position 2 with increasing mol. wt. of milk TGs, and the dilution of intravenously added $[^{14}C]$ - 16:0 in the high mol. wt. fractions of milk fat was interpreted by Dimick et al, (1965, 1966) as evidence for the incorporation of FAs, into position 2 of these TGs, that were not in equilibrium with other FAs e.g. a 2-monoglyceride precursor.

### 1.3.4. Fatty acid specificity of triacylglycerol biosynthesis in the mammary gland

Mammary preparations from both the bovine (Askew et al, 1971b) and the goat (Bickerstaffe and Annison, 1971b) esterified 16:0, with glycerol-3-phosphate as acceptor, at rates greater than other FAs. In vitro esterification rates in the bovine were 16:0 $\simeq$ 18:1 > 18:0 > 18:2, which is consistent with the FA composition found in milk fat. Linoleic acid as well as being poorly esterified was found to markedly inhibit the esterification of other FAs. Very little esterification of butyrate was achieved, and this was put forward as an explanation for the low levels of TG formation (2.8% of radioactivity incorporated into TGs), bearing in mind that in milk fat, butyrate and short chain FAs comprise about 90% of the FAs in position 3 of the TG. The in vitro esterification rates in the goat were 16:0 > 18:1 > 18:0 > 14:0 $\simeq$ 18:2 > 12:0 > 18:3. The inability of particulate fractions to esterify 8:0 and 10:0 was explained by the postulate that these FAs are activated in the cytosol and become esterified to 1,2 - diglycerides which have been released into the cytosol by the hydrolysis of phosphatidic acid synthesized on the microsomal particles. These studies with goat and bovine mammary tissue were carried out using free fatty acids as substrates, whereas Pynadath and Kumar (1964) had demonstrated that the synthesis of TGs in the particulate fraction of

goat mammary tissue using palmitoyl CoA as substrate was much greater than when using the free FA. *In vivo* the free FAs absorbed by mammary tissue are expected to be activated immediately, either in the cell membrane or in the cell, as demonstrated by Kinsella (1970b) using dispersed cells of bovine mammary tissue.

Using fatty acyl CoAs as substrates for bovine mammary microsomes, Kinsella and Gross (1973) demonstrated that palmitoyl CoA was by far the preferred substrate for the initial acylation of glycerol-3-phosphate but that all fatty acyl-CoAs were rapidly esterified to 1-palmitoyl-*sn*-glycerol-3-phosphate.

### 1.3.5. Biosynthesis of triacylglycerols in freshly secreted milk

An alternative means of investigating the esterification of FAs into TGs has been to use freshly secreted milk from the goat (McCarthy and Patton, 1964; McCarthy *et al*, 1965; Christie, 1974) and the bovine (McCarthy and Patton, 1964; Kinsella, 1972b) as an enzyme source. A major requirement for the activity of the microsomal enzymes (Patton *et al*, 1965) was the use of freshly secreted milk. A desaturase that converted stearate to oleate was also active (McCarthy *et al*, 1965).

The incorporation of glycerol into TGs, phosphatidyl choline and phosphatidic acid, by serum prepared from freshly secreted milk was further evidence for an active glycerokinase in mammary tissue (Kinsella, 1972b).

The rates of incorporation of individual FAs into TGs by freshly secreted goats milk were in the order 18:1 $\simeq$ 18:2 > 18:3 > 14:0 > 12:0 > 16:0 > 18:0 (Christie, 1974). The TGs, formed by incubation of milk with these isotopically labelled FAs, were subjected to stereospecific analysis to determine the distribution of the labelled FAs in the TGs.

The FAs entered all three positions of the TGs, with marked positional specificities. 16:0 was esterified preferentially in position 2 (53% of the 16:0), and 18:0 equally in positions 1 and 3 (87% of the 18:0). 18:2 was almost evenly distributed over all three positions. When 18:1 was added to the incubation it was esterified almost evenly in all three positions with slightly greater proportions in positions 1 and 3, whereas the 18:1 formed from 18:0 in the medium was preferentially esterified in position 3. The positional distribution of FAs differed somewhat from that reported for goat milk TGs (Kuksis et al, 1973), although some similarities, such as the low levels of 16:0 in position 3 were evident. This discrepancy was explained by the unknown effect of endogenous FAs present in such a poorly defined medium.

Freshly secreted milk was also found to contain some enzymes of the monoglyceride pathway, as indicated by the incorporation of FAs on to 2-O-hexadecylglycerol and to a small extent 1-O-hexadecylglycerol to give mostly monoalkylmonoacylglycerols (Christie, 1974).

## Section 1.4. Modification of the fatty acid composition of ruminant fats

### 1.4.1. Methods of avoiding biohydrogenation

Several different methods have been used to bypass the rumen in order to alter the FA composition of ruminant fats and to study the utilization of FAs by mammary tissue. These include: intravenous infusion (Tove and Mochrie, 1963; Storry et al, 1969), duodenal or abomasal infusions (Ogilvie et al, 1961; Bickerstaffe and Annison, 1971a; Rindsig and Schultz, 1974) and formaldehyde - protected lipid (Pan et al, 1972; Cook et al, 1972).

(a) Intravenous infusion of lipid into lactating ruminants

Whereas the feeding of soyabean oil resulted in no increase in polyunsaturated FAs in the milk fat, an intravenous infusion of cottonseed oil gave a dramatic increase in the linoleate content of milk (1.8% up to 21.6%). This was associated with decreased proportions of 14:0, 16:0, and 18:0 in the milk fat (Tove and Mochrie, 1963).

When monoacid TGs containing the FAs 3:0 to 18:1 were intravenously infused into lactating cows, the TGs, except those containing 3:0 and 4:0, caused increased milk fat yields and increased yields in the milk fat, of the particular infused FA. (Storry et al, 1969)

(b) Duodenal and abomasal infusions of lipid into lactating ruminants

Infusion of linseed oil emulsion into the duodenum of sheep increased the level of 18:2 and 18:3 in the depot fat (Ogilvie et al, 1961).

In checking for any adverse effects of infusion of polyunsaturated oils into the duodenum of lactating cows, Bickerstaffe and Annison (1971a) infused into the duodenum 650 g sunflower oil per day. Apart from having no adverse effects on the cow, the infusion of sunflower oil increased milk fat yields by about 300 g per day and increased the proportions of 18:2 in the milk fat from 3 to 27 mole %.

Abomasal infusions of 250 - 500 g safflower oil per day increased milk fat yields, increased the proportions of 18:2 and decreased the proportions of 14:0, 16:0, 18:0 and 18:1 in the milk fat (Rindsig and Schultz, 1974).

(c) Feeding oils protected from metabolism in the rumen

The possibility of avoiding the biohydrogenation of unsaturated lipids in the diet of ruminants arose from work by Ferguson et al

(1967) on the prevention of the microbial degradation of casein in the rumen after its treatment with formaldehyde. Scott et al (1970) devised a procedure for the protection of polyunsaturated oils with formaldehyde-treated casein, whereby polyunsaturated lipid-protein particles were prepared by spraying on homogenate, consisting of equal parts (by weight) of sunflower oil and sodium caseinate, with formalin vapour (37% formaldehyde). Feeding 1500 g per day of a protected safflower oil supplement to cows for two days, produced increases in the levels of 18:2 in milk fat from 2 to 35 wt. % and corresponding decreases in 16:0, 18:0 and 18:1. A supplement which had not been treated with formaldehyde caused only small increases in 18:2 and larger increases in 18:0 and 18:1 in the milk fat. Feeding the formaldehyde-treated safflower oil-protein also increased the 18:2 content in the depot fat of lambs (Cook et al, 1970), sheep (Scott et al, 1971) and young steers (Faichney et al, 1972), and in the milk fat of goats (Scott et al, 1970).

There followed a series of extensive experiments to determine the effects of feeding the protected polyunsaturated oils on the metabolism of ruminants as a foundation for its possible application in the commercial production of polyunsaturated ruminant products (Pan et al, 1972; Cook et al, 1972; Scott and Cook, 1975).

### 1.4.2. Composition of milk and milk fat with the feeding of diets supplemented with protected lipid

The effect of feeding cows diets supplemented with 1 kg formaldehyde-treated casein-safflower (1:1, w/w) per day was to increase fat yields in the milk by up to 100 g per day and to increase the proportions of 18:2 (by wt.) from 2% up to about 26%. The increased 18:2 was associated with decreases in the proportions of 14:0 and 16:0

in the milk fat. Protein yields in the milk were slightly increased and lactose yields tended to be depressed by feeding the protected supplement. The effect on the plasma TGs, the proposed major precursors of milk fat from the diet, was to increase the proportions of 18:2 and decrease the proportions of 16:0 (Pan *et al*, 1972; Cook *et al*, 1972)

Subsequent to this initial research, numerous studies have been carried out using the same and alternative protected supplements.

Plowman *et al* (1972) fed cows 1500 g per day of a formaldehyde-treated supplement of safflower oil - casein (55:40 w/w) and obtained milk fat containing up to 35% 18:2 (by wt.), thus confirming the results of the Australian research group. Chandler *et al* (1973), using a formaldehyde-treated supplement of sunflower seed, investigated the effect on milk fat yield and milk fat composition by using 14 cows, 7 of which were fed a control diet of lucerne chaff - crushed oats (1:1, w/w) and 7 of which were fed a mixture of the control diet and 2.7 kg per day of the protected supplement of sunflower seed, for a period of 72 days. The mean daily fat yields were increased, by up to 52%, by the protected supplement feeding whereas proportions of 10:0, 12:0, 14:0, 16:0 and 18:1 in the milk fat were decreased and proportions of 18:0 and 18:2 were increased. The milk fat from the cows fed the protected supplement contained 25.6% 18:2 (by wt.) compared to 1.3% 18:2 with the control cows. Gooden and Lascelles (1973), feeding 400 g - 1 kg per day of a protected supplement of safflower oil along with free grazing, achieved increases in milk yield from the control level of 11.3 kg/day up to 12.5 kg/day and increases in milk fat yield from 552 to 600 g per day. The proportion of 18:2 was increased from 2.6 to 24.6% (by wt.), and was compensated by a large decrease in 16:0 (30.4% to 15.6%) and smaller

decreases in all short and medium chain FAs (4:0 to 14:0). Only in 4:0 and 16:0 was there a decrease in total yield (by wt.), with the major changes in the proportions of FAs being caused by the greater yield (by wt.) of 18:2.

The yields and wt. percentages of the FAs of milk fat with 6 to 16 carbons were reduced, whereas butyrate and all 18 carbon FAs were increased by feeding 3.6 kg formaldehyde-treated full-fat soyflour per day. The proportion of 18:2 was increased from 7.2% to 16.2% (by wt.) (Mattos and Palmquist, 1974).

Protected tallow supplement was fed to investigate the problem of low milk fat yields in cows receiving low roughage diets (Storry et al, 1974). The feeding of the protected supplement increased the milk fat yields up to the level of cows on high roughage diets. This increased yield of milk fat when the supplement was fed, was due mostly to an increase in the 18:1 content of the milk.

The efficiency of transfer of 18:2 into milk fat with the feeding of a diet supplemented with different levels of formaldehyde-treated safflower - casein was found to range from 17 to 22% (Bitman et al, 1973). Similarly in another study a 22.6% efficiency of transfer of 18:2 from a protected supplement of full-fat soyflour into milk fat was achieved (Mattos and Palmquist, 1974).

### 1.4.3. Effect on plasma lipids of feeding supplements of protected lipid

Feeding cows protected lipid diets sharply increased the arterial concentration of TGs, cholesterol esters and phospholipids but only the TGs of the VLDL and chylomicrons were taken up by the mammary gland (Gooden and Lascelles, 1973). The VLDL and chylomicron fractions from cows on the protected lipid diet had greatly increased levels of

esterified FAs, with this increase being mainly attributable to higher levels of 18:2 and to a lesser extent 18:0 and 18:1. The major FA taken up from the blood by the mammary gland of cows on the protected lipid diet was 18:2 although 18:0 was also taken up in reasonable quantities. This was in contrast to the cows on a control diet where 16:0 and 18:0 were the major FAs taken up. The arterial concentration and uptake into the mammary gland of unesterified FAs were unchanged by feeding of the protected lipid diet, but there was a significant decrease in the arterial concentration and uptake of acetate. Gooden and Lascelles (1973) suspected that this decrease in acetate levels was caused by poor protection of the lipid supplement.

The 18:2 content, of the TGs, phospholipids and cholesterol esters of the plasma lipoproteins of sheep (Scott and Cook, 1975) and lactating cows (Cook et al, 1972) was also elevated by feeding supplements of protected lipid.

The average lipid level in the blood serum of steers fed a diet supplemented with safflower oil was 930 mg per 100 ml, which was about twice that of control animals (Dryden et al, 1975). On the diet of protected safflower oil the level of LDL was approximately fourfold higher than in control animals, while the levels of HDL remained unaffected. The levels of VLDL and chylomicrons were very low and did not vary over the trial period.

### 1.4.4. Positional distribution of fatty acids with the feeding of diets high in linoleate

Only a limited number of stereospecific analyses have been carried out to determine the distribution of FAs in TGs after the feeding of diets high in linoleate (Privett et al, 1965; Christie et al, 1974; Scott and Cook, 1975; Hawke et al, 1976).

The effects of corn oil (57% 18:2) on the positional distribution of FAs in epididymal fat pads, kidney and liver of rats, relative to either lard or fat-free diets, was determined by Privett et al (1965). The proportions of 16:0 and 18:1 in the tissue TGs were lowered with the increased 18:2. The distribution of 14:0, 16:0 and 18:0 between the primary and secondary positions in the TGs of the epididymal fat pads of all dietary groups was similar, but on the corn oil diet, 18:1 was displaced from position 2 to be replaced by 18:2.

Christie et al, (1974) found that feeding groups of rabbits linoleic acid along with one of a range of saturated FAs, caused an increase in the tissue TGs of each FA fed, and the presence of the saturated FA decreased the amount of 18:2 incorporated into TGs. Although the amounts of each FA in the TGs of each group of rabbits fed 18:2 and a saturated FA varied considerably the stereospecific distributions of these FAs were unchanged by the different diets.

When sheep were fed diets of protected safflower or sunflower oils, the 18:2 which was incorporated into adipose tissue was esterified mostly in positions 2 and 3 of the TGs with only low levels in position 1 (Scott and Cook, 1975; Hawke et al, 1976).

### 1.4.5. Effect of feeding high levels of 18:2 on the levels of the triacylglycerol classes of differing levels of unsaturation in depot fat

On feeding corn oil to rats (Privett et al, 1965), the replacement of 16:0 and 18:1, in the TGs of the epididymal fat pads, by 18:2, was accompanied by large increases in the proportions of triene TGs (021) i.e. 16:0 - 18:2 - 18:1, tetraene TGs (121) i.e. 18:1 - 18:2 - 18:1 and (022) 16:0 - 18:2 - 18:2, and pentaene TGs (122) i.e. 18:1 - 18:2 - 18:2. Corresponding decreases were evident in the levels of

monoene TGs (010) i.e. 16:0 - 18:1 - 16:0, diene TGs (011) i.e. 16:0 - 18:1 - 18:1 and triene TGs (111) i.e. 18:1 - 18:1 - 18:1. As a consequence of the low level of saturated FAs in the TGs of rat depot fats, diene TGs containing two saturated FAs and 18:2 were of minor importance and only showed a small increase with the feeding of corn oil.

Feeding a protected supplement of sunflower oil to lambs increased the proportions of the more unsaturated TGs such as 020, 021, 022, 121 and 122. There were corresponding decreases in the proportions of 000, 010 and 011. (Hawke et al, 1976)

## Section 1.5. Physical properties of milk fat

### 1.5.1. Factors influencing the physical characteristics of milk fat

The main physical processes affecting the melting characteristics of milk fat are polymorphism and solid solution formation. Polymorphism, defined as the ability of a compound to exist in more than one crystalline form, is a well known phenomenon associated with the crystallization of long chain compounds. Polymorphism is most evident in fats of relatively simple composition which contain only one TG species or TGs comprised of FAs of equal chain length. A good example of this type of fat is cocoa butter which consists predominantly of three monoene TG species comprised of the FAs 16:0, 18:0 and 18:1. (Sampugna and Jensen, 1969)

Polymorphism in milk fat was first described by Mulder, in 1953, who observed the classic demonstration of polymorphism, a double melting point after rapid cooling of milk fat. Since then the occurrence of various polymorphic crystal forms in milk fat has been established. Woodrow and de Man (1968) studied the occurrence of three polymorphic forms $\alpha$, $\beta'$, $\beta$ using x-ray and infrared spectroscopic

techniques. Slow cooling of milk fat was found to produce β´and β forms whereas rapid cooling produced the α form. If the rapidly cooled milk fat was held at 5°C it underwent a transformation to the β´and β forms. Van Beresteyn (1972) concluded from a range of experiments using differential scanning calorimetry (D.S.C.), x-ray diffraction, N.M.R. and infrared spectroscopy, that rapid cooling of milk fat to 11°C led to crystals in the α modification. After a period of time an additional crystallization took place, mainly in the β form. Upon heating polymorphic transitions took place, from α to β´ and from β´to β.

Solid solution formation was first formulated by Mulder (1953) who noted from available experimental data that certain characteristics of fats could only be explained by the formation of mixed crystals in the milk fat. Mulder (1953) pointed out that (a) The lower the temperature at which milk fat is solidified the lower the melting point of the solid formed. (b) Stepwise cooling results in the solidification of a smaller amount of fat than direct cooling. (c) Recrystallization can occur by tempering the solid milk fat, the rate of recrystallization being strongly dependent upon temperature. (d) If the fat is rapidly cooled to below the solidus line, the effect of the solidification temperature will be removed. Behaviour such as this would be expected to be typical of systems capable of forming mixed crystals or solid solutions. Evidence to support such a theory has been obtained by several workers. De Man and Wood (1959) noted an increase in solid fat content upon rapid cooling. Using x-ray diffraction, Knoop and Samhammer (1962) interpreted the patterns obtained as indicating the presence of two mixed crystal fractions, one consisting of saturated TGs and the other of monounsaturated TGs. Sherbon and Coulter (1966), using both x-ray diffraction and adiabatic

calorimetry, characterized the melting patterns of mixtures of tristearin and tributyrin and of mixtures of high and low melting fractions of milk fat. Rapidly cooled mixtures of milk fat fractions and mixtures of tributyrin and tristearin were all in the β form and slowly cooled mixtures of tristearin and tributyrin were also in the β form, but slowly cooled fractions of milk fat were in the β´form. Tristearin always cooled to the α form. Adding low melting TG fractions to high melting TG fractions increased the interplanar spacing of the crystal lattice. Both the chemical and thermal data seemed to indicate the presence of two solutions, the solid phase consisting of a solid solution of low melting fat in high melting fat and the liquid phase consisting of a solution of high melting fat in low melting fat. The solid solutions formed in the fat mixture were found to only slightly alter the liquid fat content, but to drastically increase the heat of fusion.

Other factors influencing the thermal properties of milk fat would be the FA composition, and the TG composition and structure of the milk fat. Yoncoskie *et al* (1969) showed that hydrogenation of milk fat altered the proportions of lower melting glycerides. If hydrogenation times were increased, further decreases occurred in the low melting glycerides. The totally hydrogenated milk fat melted over the range 0 to 60°C compared to -30 to 40°C for the original milk fat. Seasonal variations in the FA composition of New Zealand milk fats have been shown to correlate with variations in the thermal properties of the milk fats. (Gray, 1973; Norris *et al*, 1973). The seasonal variation in liquid fat contents was found to correlate, at most temperatures with seasonal variations in the sum of the short chain FAs and either the total or the *cis* unsaturated FAs. Taylor (1973) concluded from studies of both the positional specificities of

FAs and the levels of saturated and unsaturated TGs in milk fats from different stages of the dairying season that the variations in FA compositions over the dairying season affect only the relative proportions of constituent TG species not the nature of these TG species.

Interesterification of the TGs of milk fat caused an increase in the hardness of the fat, an increase in high melting glycerides, an increase in the solid fat content and an increase in the softening point (de Man, 1961). The randomized fat had, with slow cooling, an unusually large plastic range.

### 1.5.2. Influence of elevated levels of polyunsaturated fatty acids on the physical properties of milk fat

Alterations to the spreadability of butter may be obtained by the thermal treatment of cream or the mechanical treatment of butter (Taylor *et al*, 1971) but these procedures are reversible upon storage. Altering the chemical composition of butter, which would be expected to cause a permanent change in the physical properties of the butter may be achieved by fractional crystallization and recombination of desirable fractions of milk fat (Norris *et al*, 1971) or by feeding protected supplements of polyunsaturated oils to cows to produce milk fat high in linoleate and/or linolenate (Cook *et al*, 1972; Scott and Cook, 1975).

Butter manufactured from milk fat containing 33.2%, 19.0% and 14.9% 18:2 had softening points that were lower than for normal butter, and the butter containing 33.2% 18:2 was spreadable at 2 - 5°C (Buchanan *et al*, 1970).

For satisfactory spreadability at a specified temperature the % solid fat should be between 25 and 45%. At 10°C, butter containing

19.5% 18:2 and polyunsaturated margarine both contained about 30% solid fat indicating satisfactory spreadability at this temperature (Wood et al, 1975). Margarines have an even rate of melting indicating a good plastic range, whereas butter high in 18:2 had peaked melting curves, indicating a narrow plastic range (Wood et al, 1975). The butter high in linoleate had a tendency to "oil off" at 20°C indicating that at this temperature there was perhaps insufficient surface area of fat crystals to retain the higher proportions of liquid fat present. A blend of milk fat high in linoleate and normal milk fat yielded butter with a wider plastic range.

Milk fat containing 30% 18:2 had a lowered inception of melting and termination of melting when compared to normal milk fat (Edmondson et al, 1974). The main melting peak was shifted from 19°C towards 14°C with increasing unsaturation of the milk fat. The liquid fat contents at temperatures between -21°C and 30°C were increased with increasing unsaturation of the milk fat.

## Section 1.6. The use of monozygous twin cows in studies of milk composition

One set of monozygous twin cows may replace, without loss of statistical efficiency, two groups of 5 or 6 randomly selected cows for measurements of butterfat yield or butterfat percentage (Patchell, 1956). The saponification values, iodine values and softening points of milk fat from several sets of monozygous twin cows showed throughout the lactation period, little variation between the individuals of each twin pair, but significant variation between different sets of twin pairs (McDowall and Patchell, 1958). No similar measurements, for the FA composition of milk, have been published using twin cows, but measurements of FA composition available, (Wilson, G.F., personal

communication), indicated, that similar to other measurements of milk composition, the FA composition showed little variation between individuals of each twin pair, either when fed normal rations or when fed protected sunflower/soyabean supplements.

Section 1.7. The aim of the present work

In the work described here, feeding a "protected" lipid supplement high in linoleic acid to a monozygous twin cow has been used to study the effect of the availability of large amounts of 18:2 for milk fat synthesis on the composition, structure and physical properties of ruminant milk fat. It had been planned to carry out a comparison of FA esterification in freshly secreted milk from a cow which was fed "protected" supplement, and in freshly secreted milk from its twin on a normal diet. The low and variable level of incorporation of FAs into TGs recorded here, precluded the successful conclusion of this experiment.

# Chapter 2

# METHODS

## Section 2.1. Reagents

Protected sunflower seed supplement (Alta Lipids (N.Z.) Ltd.).

Phenyldichlorophosphate (Aldrich Chemical Co., Milwaukee, U.S.A.).

3% (w/w) JXR on Gas-Chrom Q, (100 - 120 mesh) (Applied Science Laboratories, California, U.S.A.).

14% (w/w) boron trifluoride in methanol (BDH Chemicals Ltd., Poole, England).

Diethylene glycol succinate polyester, (DEGS) (Analabs, Connecticut, U.S.A.).

Chromosorb W, (60 - 80 mesh) (Varian - Aerograph, California, U.S.A.).

Silica gel G (E. Merck, Darmstadt, Germany).

Silicic acid (100 mesh) (Mallinckrodt, St. Louis, U.S.A.).

Ophiophagus harnah snake venom (Sigma Chemical Co., St. Louis, U.S.A.).

$[1\text{-}^{14}C]$ palmitic acid; $[1\text{-}^{14}C]$ myristic acid; $[1\text{-}^{14}C]$ stearic acid; $[1\text{-}^{14}C]$ linoleic acid; $[^{14}C]$-hexadecane (Radiochemical Centre, Amersham, England).

Tricaproylglycerol; trilauroylglycerol; trimyristoylglycerol; tripalmitoylglycerol; tristearoylglycerol (Sigma Chemical Co., St. Louis, U.S.A.).

Bovine serum albumen (fraction V) (Sigma Chemical Co., St. Louis, U.S.A.).

Pancreatic lipase, Steapsin (Sigma Chemical Co., St. Louis, U.S.A.).

Nitrogen; hydrogen (Industrial Gases (N.Z.) Ltd.).

Standard triacylglycerols, diacylglycerols, monoacylglycerols (R. Norris, Dairy Research Institute, N.Z.).

ATP; CoA; glycerol-3-phosphate (Sigma Chemical Co., St. Louis, U.S.A.).

1,4-di-(2-(5-phenyloxazoyl))-benzene (Koch-Light Laboratories,

Colnbrook, England).

Methyl heptadecanoate (Analabs, Connecticut, U.S.A.).

All other chemicals were supplied by either BDH (Poole, England ) or May and Baker Ltd. (Dagenham, England).

## Section 2.2. Milk samples

A pair of monozygous twin cows was grazed on typical New Zealand ryegrass/clover pasture which was supplemented with concentrate feeding at milking times as follows: (a) One cow was fed 1.5 kg dried grass every milking, morning and night, throughout the experimental period. (b) During a preliminary experimental period the other cow was fed 1.5 kg dried grass every milking, and after one week a supplement of formaldehyde-treated sunflower seed was fed in amounts increasing from 0.5 to 3.0 kg/day over a 4 day period. The level of supplement was then held at 3 kg/day for a further eight days.

Milk was collected from both cows, on a selection of days during the feeding period. Milk from the last day of the experimental period was used for studies of TG composition.

Milk fat was obtained from milk samples using three extractions with diethyl ether and was stored in hexane under nitrogen at -10°C.

## Section 2.3. Analytical methods

### 2.3.1. Thin-layer chromatography

#### 2.3.1.1. Preparation and development of thin-layer chromatography plates

Silica gel G was slurried in either distilled water, 3% boric acid solution or 10% silver nitrate solution in the approximate ratio 1:2 w/v). T.l.c. plates (20 x 20 cm) were spread, using a commercial spreader (Desaga Co., U.S.A.), to a thickness of 0.25 mm or 0.5 mm, for

analytical or preparative separations respectively.

After spreading, plates were left to dry ($AgNO_3$/silica gel plates in the dark), then activated for 2 h at 120°C.

The appropriate solvent, to a depth of 0.5 cm, was added to chromatography tanks lined with filter paper 30 min before chromatography, to allow saturation of tanks with solvent vapours.

Samples were applied in hexane, as short bands or as one continuous band, 2 cm from one edge of the plate. Chromatograms were developed by the ascending method, at room temperature, to a distance of 15 cm from the origin.

2.3.1.2. Detection of lipids on thin-layer chromatography plates

(a) 2',7'-dichlorofluorescein

A 0.05% (w/v) solution of 2',7'-dichlorofluorescein in methanol was used as a general purpose lipid spray. Plates were sprayed then viewed under u.v. light.

(b) Rhodamine 6G

A 0.01% (w/v) aqueous solution of Rhodamine 6G was used to detect diacylglycerols during the preliminary steps of stereospecific analysis. Plates were sprayed then viewed under u.v. light.

(c) Phosphate ester spray

A specific spray for phosphate esters (Vaskovsky and Kostetsky, 1968) was used to detect phospholipids. After spraying, the phospholipids showed up as blue spots on a white background.

2.3.2. Gas - liquid chromatography

2.3.2.1. Gas - liquid chromatography of fatty acids

(a) Analysis of fatty acids

Fatty acids were analysed as their methyl esters using a Packard

Gas Chromatograph fitted with a hydrogen flame ionisation detector and a model 845 digital temperature programmer. The glass column (183 cm x 0.3 cm i.d.) was packed with 13% diethylene glycol succinate polyester (DEGS) on Chromosorb W (60 - 80 mesh). The "funnel coating method" (McNair and Bonelli, 1968) was used for preparation of the column packing. Column conditioning was carried out at 200°C for approximately 12 h with $N_2$ flow of 10 ml/min.

Samples were injected on to the column at an initial column temperature of 50°C, then the oven temperature was programmed to increase at a rate of 4°C/min to a final temperature of 170°C, at which temperature the oven was held until the last of the sample had passed through the column. The mobile gas phase was $N_2$, flowing at 40 ml/min. The detector was operated at 240°C using $H_2$ and air flows of 25 and 200 ml/min respectively. The injector was held at 200°C.

(b) Preparation of fatty acid methyl esters

Three procedures were used to prepare methyl esters of fatty acids.

(1) 1 ml of 0.5 M - methanolic NaOH was added to 2 - 5 mg TG in a 25 ml round-bottom flask. The flask was attached to a water condenser and heated on a sand bath, to gently reflux the contents, for 2 min. One ml 14% (w/v) boron trifluoride in methanol was added through the condenser and the refluxing continued for a further 2 min. After which, 2 ml hexane was added, followed by refluxing for a further 30 s. After cooling the flask in iced-water, sufficient water was added to bring the hexane layer into the neck of the flask. The hexane layer was transferred to a glass-stoppered centrifuge tube using a Pasteur pipette, and evaporated to dryness. After dissolving in an appropriate amount of hexane, 2 - 5 µl of the sample was gas-chromatographed. (Adapted from the method of Van Wijngaarden, 1967).

(2) 25µl of transesterifying reagent (1.5 ml 0.5 M - sodium methoxide

in methanol, 6 ml hexane and 2.5 ml diethyl ether) was added to a 1 - 4 mg sample of lipid in a 0.3 ml reaction vial (Kontes Glass Co., U.S.A.) and immediately closed with a vapour-tight cap. The vial was rotated gently for 2 min to mix the contents, the cap was removed, 25 µl hexane was added, the vial immediately recapped, and rotated gently for a further 2 min (Shehata et al, 1970). 1 - 8 µl of the solvent mixture was used for analysis by g.l.c.

The transesterifying reagent was prepared fresh every 2 or 3 days from a stock solution of 0.5 M sodium methoxide in methanol (prepared by dissolving sodium in methanol), which was stored at 5°C in tubes fitted with teflon-lined screw caps.

(3) A 1 - 4 mg sample of lipid was weighed into a Kontes reaction vial, dissolved in 60 µl light petroleum (b.p. 40 - 60°C) and 4 µl 2 M - sodium methoxide added. The vial was capped securely and gently rotated for 5 min to facilitate mixing. After standing for a further 5 min, 1 - 8 µl of the light petroleum layer was injected into the g.l.c. (Adapted from Christopherson and Glass, 1969)

Procedure 1 was used for the methylation of free fatty acids, procedure 2 for the methylation of TGs, DGs and MGs, and procedure 3, which uses a higher concentration of sodium methoxide, for the methylation of phospholipids because procedure 2 appeared in some instances to not give complete methylation of these compounds.

(c) Quantitative measurement of fatty acids by g.l.c.

Methyl esters of fatty acids were identified by comparison with standard methyl esters chromatographed under identical conditions.

Proportions of the individual fatty acids were obtained from a measurement of peak areas using the product of peak height and width at half peak height. The peak areas were corrected for detector response using weight response factors (Fw) determined by the

injection of a mixture of pure FA methyl ethers into the gas chromatograph. The weight response factors were then calculated, from the ratio $Fw = \frac{\text{weight}}{\text{area}}$, for each FA methyl ester, from each of five injections, and a mean Fw value for each fatty acid methyl ester obtained. For the calculation of Fw values methyl palmitate was assigned a Fw value of 1.00.

Table 4. Weight response factors (Fw) for the methyl esters of fatty acids (methyl palmitate assigned a value of 1.00)

| Fatty acid methyl esters | Fw value | | |
|---|---|---|---|
| | Experimental | | Theoretical[b] |
| | Range[a] | Mean | |
| methyl butyrate | 1.34 - 1.45 | 1.41 | 1.50 |
| methyl hexanoate | 1.12 - 1.17 | 1.15 | 1.28 |
| methyl octanoate | 1.06 - 1.07 | 1.07 | 1.17 |
| methyl decanoate | 0.97 - 1.00 | 0.99 | 1.09 |
| methyl laurate | 0.99 - 1.02 | 1.01 | 1.05 |
| methyl myristate | 0.99 - 1.02 | 1.00 | 1.02 |
| methyl palmitate | 1.00 - 1.00 | 1.00 | 1.00 |
| methyl stearate | 0.99 - 1.02 | 1.01 | 0.98 |
| methyl oleate | 0.97 - 1.03 | 1.00 | 0.97 |
| methyl linoleate | 0.97 - 1.05 | 1.01 | 0.96 |

(1)[a] Determined from five injections

(2)[b] Calculation considering active carbons only as reported by Ackman and Sipos (1964)

The weight response factors determined in Table 4 were in reasonable agreement with the theoretical Fw values calculated according to Ackman and Sipos (1964). Molar proportions of FA

methyl esters in milk fat samples were determined using these Fw values. The values relative to palmitate were used in the following calculation

$$\text{moles } \%_i = \frac{\left[\frac{h_i\, b_i\, Fw_i}{M_i}\right]}{\sum\left[\frac{h_i\, h_i\, Fw_i}{M_i}\right]} \times \frac{100}{1}$$

where h = peak height.

b = peak width at half peak height.

M = molecular weight of FA methyl ester.

Fw = experimentally determined weight response factor.

2.3.2.2. Gas - liquid chromatography of triacylglycerols

TGs were analysed by g.l.c. using a Varian Aerograph, Series 1520 chromatograph fitted with a flame ionization detector and a linear temperature programmer. A glass column (0.58 m x 2.5 mm i.d.), fitted with glass to metal Kovar seals and packed with 3% (w/w) JXR on 100 - 120 mesh Gas Chrom Q (Applied Science Laboratories) to permit on column injection (Kuksis and Breckenridge, 1966), was used for separation of TGs. Prior to use, the column was conditioned overnight at 350°C with 50 ml/min $N_2$ flow. For normal operation the injector and detector temperatures were maintained at 325 and 350°C respectively. Temperature programming was commenced at 220°C and increased to 350°C at 4°C/min. $N_2$, air and $H_2$ flows were 150, 300 and 15 ml per min respectively. The column effluent was split in the ratio 1:5 between detector and collector. The column was calibrated by injecting mixtures of tricaproylglycerol, trilauroylglycerol, trimyristoylglycerol, tripalmitoylglycerol and tristearoylglycerol.

The calculated weight response factors (Fw) were: tricaproylglycerol (0.99), trilauroylglycerol (0.98), trimyristoylglycerol (1.00), tripalmitoylglycerol (1.02) and tristearoylglycerol (1.04), with trimyristoylglycerol being assigned a value of 1.00.

The relative areas of the peaks obtained for individual TGs were measured using a Varian Aerograph model 485 electronic integrator. The integrator was checked for accuracy against manual methods of measurement and was found to vary by less than 1 - 2% for major components.

## Section 2.4. Fractionation of the triacylglycerols of milk fat

### 2.4.1. Silicic acid column chromatography of triacylglycerols

Milk samples were fractionated into three fractions of different molecular weight, using silicic acid column chromatography, by a modification of the method of Taylor and Hawke (1975a).

(a) Chromatographic procedures

Fine particles were removed from the silicic acid (100 mesh, Mallinckrodt) by five separate washings with five volumes of distilled water, allowing the slurry to settle for 30 min and then decanting the supernatant between washings. The silicic acid was dried at 100°C and before use was activated at 120°C overnight.

For packing of the column, 45 g silicic acid was slurried in hexane, air bubbles were removed by placing the slurry under the vacuum of a water pump, and then the slurry was poured slowly into a glass column (40 cm x 1.8 cm i.d.) to produce a silicic acid column, after washing hexane through the column, approximately 35 cm high.

The column was loaded by carefully layering 600 - 900 mg milk fat dissolved in 5 ml hexane, on to the surface of the silicic acid.

Lipids were eluted from the column using a solvent system of

hexane in which the content of diethyl ether was linearly increased from 0 to 9% over 1500 ml. The eluted TGs were collected in 120 fractions each of 12 ml. Flow rates of 120 - 150 ml/h were maintained by positioning the solvent reservoirs 1 metre above the column. The tip of the column was washed periodically to prevent accumulation of TGs through solvent evaporation.

(b) Analysis of fractions

Selected eluant fractions were concentrated and spotted on thin layers of silica gel G (0.25 mm thick) which were developed in hexane-diethyl ether (4:1, v/v). Samples were detected by spraying with 2',7'dichlorofluorescein in methanol (0.05%, w/v). The $R_f$ values of these selected fractions, after reference to the $R_f$'s of standard TGs and the $R_f$ of the original milk fat, were used to determine the division of the eluant fractions into three fractions of low, medium and high mol. wt.. In addition, the g.l.c. of eluant fractions, to analyse the constituent TGs according to their mol. wt., was frequently used as an aid in bulking fractions.

## 2.4.2. Argentation thin-layer chromatography of triacylglycerols

TG fractions of high, medium and low mol. wt. were resolved, on the basis of unsaturation, using t.l.c. on silica gel G (0.5 mm thick) impregnated with 20% (w/w) $AgNO_3$. 5 - 10 mg of sample, dissolved in chloroform, was applied as a band to the $AgNO_3$-impregnated silica gel G plate (20 x 20 cm). The developing solvent was chloroform-methanol (99.4 : 0.6, v/v), or (99.0 : 1.0, v/v) for the separation of the more unsaturated TG bands. Bands were identified, after spraying with 2',7'-dichlorofluorescein, by reference to standard TGs (tripalmitoyl-glycerol, rac-1-palmitoyl-2-oleoyl-3-stearoyl-sn-glycerol and trioleoyl-glycerol) used as markers. After removing the

fluorescent bands from the plate into centrifuge tubes, the lipid was eluted from the adsorbent by the addition of about 0.5 ml 1% NaCl in methanol-water (9:1, v/v) until the red colour of the silver - dichlorofluorescein complex disappeared, then 5 ml diethyl ether - methanol (9:1, v/v) was added, followed by slurrying on a Vortex mixer. After brief centrifugation and decantation of the solvent, the residue was extracted twice more with 4 ml portions of diethyl ether - methanol (9:1, v/v). (Hill et al, 1968). Solvent was removed under a stream of $N_2$ and the residue redissolved in hexane and washed with small volumes of water to remove traces of dichlorofluorescein and $AgNO_3$ prior to g.l.c. analysis.

The relative amounts of the TG bands were determined by adding known amounts of methyl heptadecanoate to all samples before transesterification. The number of moles of FA present in each TG band was calculated using the following formula

$$\text{moles of FAs in TG band} = \text{moles of internal standard} \times \frac{\sum \frac{h_s\, b_s\, Fw_s}{M_s}}{\frac{h_i\, b_i\, Fw_i}{M_i}}$$

h = peak height

b = peak width at half peak height

Fw = experimentally determined weight response factor

M = mol. wt. of FA methyl ester

s = Fatty acid methyl ester

i = internal standard

2.4.3. Argentation thin-layer chromatography of the triacylglycerols as fatty acid methyl esters

The trans 18:1 contents of the two milk fats were determined by

argentation t.l.c. of the FA methyl esters prepared from the TGs of the control and 18:2-rich milk fats. Trans 18:1 was separated from other FAs on t.l.c. plates of silica gel G impregnated with 20% (w/w) $AgNO_3$. The developing solvent was chloroform-methanol (99.4 : 0.6, v/v). The FA methyl esters were identified and removed from the adsorbent layer as for the TGs (Section 2.4.2.). The relative proportions of cis and trans 18:1 were determined by g.l.c. of the isolated FA methyl esters with appropriate known amounts of methyl heptadecanoate as an internal g.l.c. standard.

Section 2.5. Stereospecific analysis of triacylglycerols

Stereospecific analysis of the TG fractions of milk fat was carried out using a procedure similar to that of Taylor and Hawke (1975b) as modified from Brockerhoff (1965), Christie and Moore (1969) and Christie (1973). According to this procedure, the FA composition of position 1 on the TG is obtained from the 1-acyl-sn-glycerol-3-phosphoryl phenol (1-PL), position 2 from the 2-acyl-sn-glycerol (2-MG) obtained by pancreatic lipase hydrolysis and position 3 from the following calculations

$$\text{position 3} = 2 \times (\text{2,3-PLs}) - (\text{2-MGs})$$

$$\text{position 3} = 3 \times (\text{TGs}) - (\text{1-PLs}) - (\text{2-MGs})$$

Comparison of the results for position 3 obtained using these calculations allows a check on the accuracy of the method.

A check on whether the 1,2(2,3)-DGs used in subsequent analyses are representative of the FAs in the original TG is possible using the following calculation

$$\text{1,2(2,3)-DGs} = \frac{3 \times (\text{TGs}) + (\text{2-MGs})}{4}$$

The FA composition of the 1,3-DGs formed during deacylation of the

original TGs with Grignard reagent can be checked against the FA composition of 1,3-DGs calculated using the following formula

$$1,3\text{-DGs} = \frac{3 \times (\text{TGs}) - (2\text{-MGs})}{2}$$

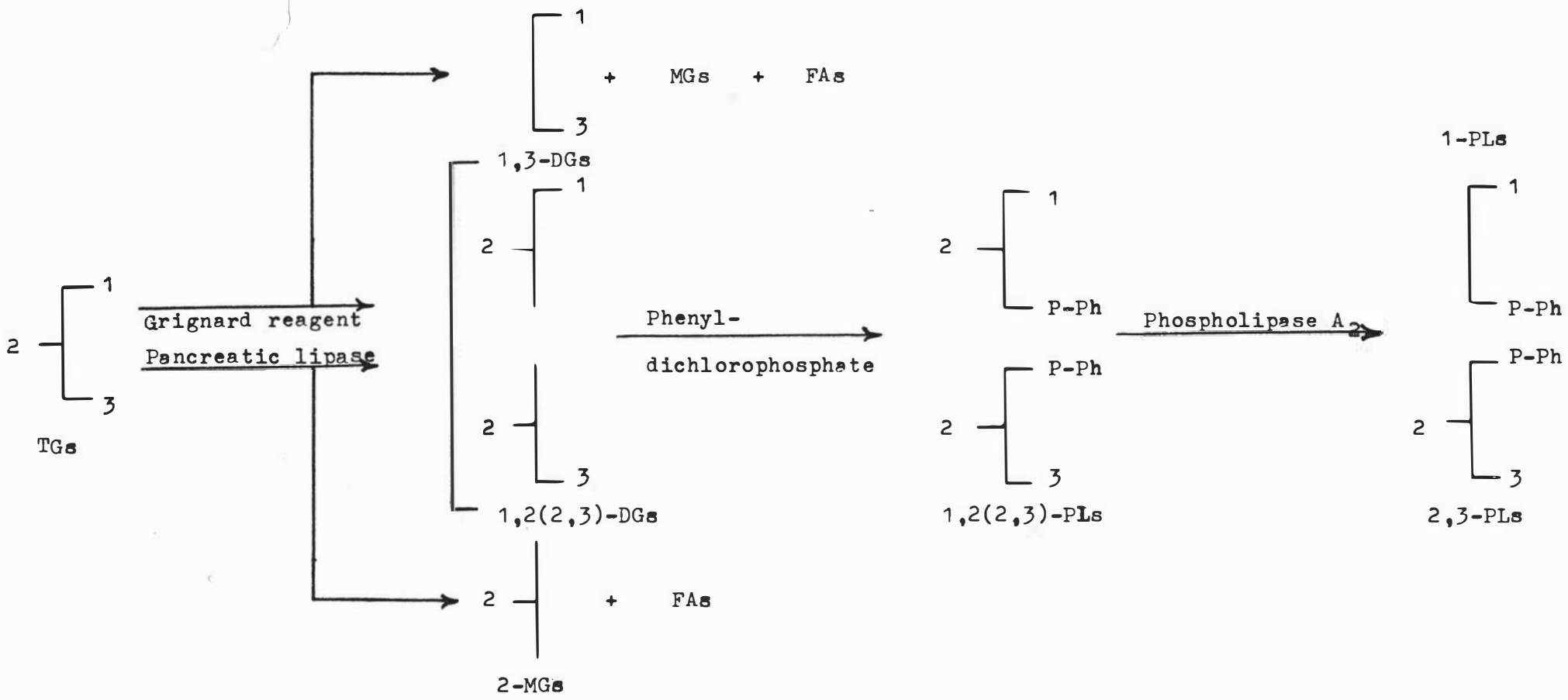
An outline of the procedure used for stereospecific analysis of the TGs is given in Figure 1.

2.5.1. Preparation of diacylglycerols

(a) High molecular weight triacylglycerols

About 40 mg TG dissolved in 2 ml dry diethyl ether was reacted, with shaking, with freshly prepared 1M-ethylmagnesium bromide in 0.5 ml dry diethyl ether. The deacylation of TGs was stopped after 50 s by the addition of 0.05 ml glacial acetic acid followed by 2 ml water. The reaction mixture was extracted three times with 20 ml portions of diethyl ether. The diethyl ether extract was washed with 5 ml aqueous 2% potassium bicarbonate, then with 5 ml water. The solvent was evaporated to dryness at room temperature under $N_2$, then the samples were dissolved in chloroform and applied to t.l.c. plates of silica gel G impregnated with 3% (w/w) boric acid and developed in hexane-diethyl ether (1:1, v/v). Lipid bands were identified under u.v. light, after spraying with aqueous Rhodamine 6G (0.01%, w/v), with reference to the appropriate DG standards. The 1,2(2,3)-DGs and 1,3-DGs were scraped into small chromatographic columns and eluted with 100 ml diethyl ether. The FA composition of the 1,3-DGs and 20% of the 1,2(2,3)-DGs were determined by g.l.c. The remaining 1,2(2,3)-DGs were used for stereospecific analysis.

The freshly prepared 1 M - ethyl magnesium bromide was prepared by placing 180 mg Mg turnings, 5 ml dry diethyl ether and a small iodine crystal in a 25 ml flask fitted with a condenser and drying tube, adding




1,2,3 = FAs in positions relative to sn-glycerol-3-phosphate.
P-Ph == Phosphoryl phenol

FIGURE 1. Stereospecific analysis of triacylglycerols.

0.4 ml bromoethane and quickly replacing the condenser and drying tube. After approximately 30 s, the red iodine colour disappeared and a spontaneous reaction occurred, to yield 1 M - ethyl magnesium bromide.

(b) Low and medium molecular weight triacylglycerols

About 40 mg TG dissolved in 0.1 ml hexane was incubated for 70 s at 37°C, under vigorous shaking, with 15 mg pancreatic lipase (previously extracted with diethyl ether) dispersed in 1.0 ml 0.05 M-Tris buffer (pH 8.0), 0.2 ml 0.2% (w/v) sodium cholate and 0.1 ml 22% (w/v) $CaCl_2$. The reaction was stopped by the addition of 1.5 ml ethanol. The pH of the solution was adjusted to 4.0 with 1 M-HCl and the mixture extracted with three 20 ml portions of diethyl ether. The total ether extract was washed with 2 ml portions of water until the washings were neutral. The ether was evaporated at room temperature under $N_2$ and the lipid residue disso. oroform was applied to a thin layer of silica gel G impregnated with 3% (w/w) boric acid and developed in hexane-diethyl ether (1:1, v/v). The 1,2(2,3)-DGs isolated from the plates were extracted with diethyl ether as described for the high mol. wt. fraction. (Section 2.5.1.a.).

## 2.5.2. Preparation of 2-acyl-sn-glycerols

2-MGs were prepared from the TGs of the fractions of high, medium and low mol. wt. by hydrolysis with pancreatic lipase as in Section 2.5.1.b. After extraction, and purification on t.l.c. the 2-MGs were analysed by g.l.c. as their constituent FA methyl esters, as for the 1,2(2,3)-DGs. (Section 2.5.1.a.).

## 2.5.3. Preparation of phosphatidyl phenols

DGs (5-10 mg) dissolved in 1 ml ethanol-free chloroform were added slowly with cooling to a solution containing 1 ml freshly distilled

pyridine, 1 ml chloroform and 0.2 ml phenyldichlorophosphate. After 90 min at room temperature with occasional swirling, 2 ml pyridine and 1 ml water were added dropwise with cooling (in ice). The phosphatidyl phenols were then extracted with 30 ml chloroform, 30 ml methanol, 25 ml water and 0.5 ml triethylamine. After thorough mixing the chloroform layer was recovered and the methanol - water layer re-extracted with 30 ml chloroform. The chloroform extracts were combined and evaporated below 35°C on a rotary evaporator, or under $N_2$. The phosphatidyl phenols were purified by t.l.c. on silica gel G plates developed in chloroform-methanol-14M-ammonia (80:20:2, by vol.). The 1,2 and 2,3-PLs identified by spraying the edges of the plate with a phosphate ester spray (Vaskovsky and Kostetsky, 1968) were recovered by elution from the adsorbent with 15 ml portions of chloroform-methanol-water (60:30:3, by v followed by two 15 ml portions of chloroform-methanol-water (3 :3, by vol.) and the solvent evaporated as before.

2.5.4. Hydrolysis of phosphatidyl phenols by phospholipase A

The triethylammonium salts of the phosphatidyl phenols were dissolved in 3 ml diethyl ether and 0.5 ml 0.5M-Tris buffer at pH 7.5 containing 2 x $10^{-3}$ M $CaCl_2$, and 1 mg snake venom (Ophiophagus hannah) added.

After gently shaking overnight, 10 ml isopropanol was added and most of the solvent removed under vacuum below 35°C, before taking to dryness under $N_2$ with the aid of small volumes of absolute ethamol. The residue, dissolved in 0.5 ml chloroform-methanol (2:1, v/v) and 0.02 ml acetic acid, was applied to a 3% (w/w) boric acid - silica gel G plate which was developed in hexane - diethyl ether (1:1, v/v). The FAs liberated by the phospholipase treatment, identified by spraying

the top half of the plate with 2',7'-dichlorofluorescein, were isolated from the plates and extracted with two 20 ml portions of 10% (v/v) methanol in diethyl ether. The phosphatidyl phenols were recovered from the origin by elution with two 15 ml portions of chloroform - methanol - water (60:30:3, by vol.) and two 15 ml portions of chloroform - methanol - water (30:60:3, by vol.) as before. After evaporation of the solvent and dissolving in chloroform - methanol (2:1, v/v), the phosphatidyl phenols were reapplied to a silica gel G plate. The plate was developed in chloroform - methanol - 14M-ammonia (80:20:2, by vol.) and the phosphatidyl phenols detected by spraying both edges of the plate with the phosphate ester spray. The unchanged phosphatidyl phenol and lysophosphatidyl phenol had $R_f$ values of about 0.5 and 0.25 respectively under these conditions. The 2,3-PLs and the 1-PLs were recovered from the plate as before and analysed, after transesterification, by g.l.c. The FAs isolated after phospholipase A hydrolysis were converted to methyl esters by refluxing with 14% (w/v) boron trifluoride in methanol and analysed by g.l.c. The FA composition obtained was found not to be representative of the FAs at the 2 position as determined by pancreatic lipase hydrolysis, because of the loss of short chain FAs during extraction and during conversion to methyl esters.

Section 2.6. Thermal analysis of milk fat

A differential scanning calorimeter (Perkin - Elmer D.S.C. model 1B) was used to carry out the thermal analyses of the total milk fats and their constituent fractions. Calibration of the D.S.C. for power and temperature readout were carried out according to the method of Norris et al (1973).

2.6.1. Analysis of triacylglycerol samples

About 20 - 40 mg TG dissolved in hexane was transferred to a 0.3 ml reaction vial. The sample was added in 0.1 ml aliquots, and the solvent evaporated under nitrogen after each addition. Traces of solvent still remaining were removed by placing the sample in a vacuum desiccator overnight. Samples were stored until required for thermal analysis at -20°C under nitrogen.

A glass capillary was used to transfer 5 - 8 mg of the melted TG to a preweighed sample pan. The sample pan was then sealed and loaded into the D.S.C.. The sample was cooled to -100°C and after equilibration at this temperature a heating thermogram was recorded up to 50 - 60°C at a rate of 16°C/min.

When samples showed an exothermic transition on the heating thermogram, which could result from a polymorphic transition of the sample from a less to a more stable crystalline form, a tempering procedure was adopted so as to obtain the heating thermogram for the more stable polymorphic form. This tempering procedure consisted of heating the sample from -100°C at 16°C/min to the temperature of the exothermic transition, holding at this temperature for 5 min, followed by shock cooling to -100°C, equilibrating at this temperature for 5 min and then recording the heating thermogram as before.

2.6.2. Analysis of data

The raw data from the D.S.C. was corrected for thermal lag, temperature and power calibration using a special program developed for use on a Hewlett - Packard 9830 calculator fitted with paper tape reader, thermal line printer and graphic plotter (Munro D.S. and Tuttiett P.T., D.R.I., New Zealand), and used to produce a corrected melting curve and its integral.

Section 2.7. Triacylglycerol biosynthesis in freshly secreted milk

2.7.1. Materials

(a) Preparation of milk samples

Milk was obtained from cows or goats as follows:

(1) cows were milked at 6.30 a.m., using an intramuscular injection of oxytocin (20 U.S.P. units) (Butocin; Burns-Biotec, Oakland, U.S.A.) to aid total milk letdown; milked again at 8.00 a.m. with oxytocin injections, and the sample collected at 9.30 a.m. by handmilking or machine milking, again with the use of oxytocin.

(2) goats were handmilked to completion at 7.30 a.m. and 8.30 a.m., then the milk sample was taken from a 9.30 a.m. milking.

The freshly secreted milk was immediately placed on ice for transport to the laboratory.

In most experiments, the milk was centrifuged at 0°C for 10 min at 700 xg. This centrifugation allowed most of the cream to form in a layer on the surface, and the skim milk was readily removed from beneath the layer using a pipette.

(b) Preparation of substrate

Two different substrate preparations were used in the experiments. All substrates were diluted with an appropriate amount of the particular FA before preparation of the substrate solution. The two methods for preparation of the substrate were: (a) the $[^{14}C]$ - labelled FA (5 μmoles) was dissolved in 2.5 ml of ethanol. (b) The $[^{14}C]$ - labelled FA (5 μmoles) was solubilized with warming if necessary in 0.8 ml 0.1M-phosphate buffer (pH 7.4) made slightly alkaline by the addition of 1M-KOH, and then the pH was carefully adjusted to neutrality by the dropwise addition of 0.5 M-HCl. The solution was added to 1 ml of bovine serum albumen (10 μmoles) (defatted by the method of Goodman, 1957) in 0.1 M-phosphate buffer (pH 7.4) with vigorous shaking on a

vortex mixer, and the volume adjusted to 2 ml with the addition of 0.1M-phosphate buffer (pH 7.4).

(c) Cofactors

The cofactors used in the various experiments were ATP (1 µmole/10 ml), CoA (0.3 µmole/10 ml), glycerol (20 µmole/10 ml), glycerol-3-phosphate (10 µmole/10 ml), dithiothreitol (0.5 µmole/10 ml), EDTA (5 µmole/10 ml), NaF (5 µmole/10 ml) and $MgCl_2$ (0.5 µmole/10 ml). They were dissolved in 0.1 M - phosphate buffer (pH 7.4) before addition to the milk.

2.7.2. Methods

(a) Incubation

Between 50 and 100 µl of the substrate solution and 0.1 ml of the appropriate cofactors were added to 10 - 15 ml of skim milk in a 50 ml Erlenmeyer flask. The flasks were incubated with gentle agitation at 37°C in a water bath for periods of up to 120 min. When time-course experiments were conducted, 1.0 ml samples were removed, by pipette, from the flasks at the appropriate times.

(b) Lipid extraction

Each aliquot of the incubation mixture was immediately extracted with chloroform-methanol using a modified Bligh-Dyer procedure. To each 1 ml of milk in a 40 ml glass-stoppered tube was added 3 ml chloroform-methanol (1:2, v/v). After shaking and allowing to stand for 5 min., 1 ml chloroform and 1 ml water were added for each 1 ml of milk. The lower (chloroform) layer was then removed and the sample re-extracted with 1 ml chloroform to each original 1 ml of milk, plus sufficient 6M-HCl to lower the pH to 4. The tube was shaken and the chloroform layer removed.

After evaporation of the chloroform, the sample was dissolved in

chloroform-methanol (2:1, v/v) and aliquots taken for scintillation counting and separation of lipid classes.

(c) Separation of lipid classes

Samples, after the addition of unlabelled FAs and DGs to act as carriers, were dissolved in 0.2 ml chloroform-methanol (2:1 v/v) and spotted on to 20 x 20 cm t.l.c. plates, coated with a 0.5 mm thickness of silica gel G (Merck), along with the appropriate standards. The plates were developed in hexane - diethyl ether - formic acid (70:30:0.5, by vol.) and the lipids detected under u.v. light after spraying with 2',7'-dichlorofluorescein. After identification, the appropriate bands (TGs, FAs and DGs), were scraped from the plate, directly into scintillation vials. Phospholipids were extracted, from the silica gel G, with chloroform - methanol - water (60:30:3, by vol.), before counting a suitable aliquot.

(d) Measurement of radioactivity

Samples were counted in a Packard Tricarb Model 2425 liquid scintillation counter using 10 ml of toluene containing 0.6% PPO (2,5-diphenyloxazole) and 0.02% POPOP (1,4-di-(2-[5-phenyloxazoyl]))-benzene. Counting efficiencies were determined by means of an automatic external standard and checked by the use of [$^{14}$C] hexadecane as an internal standard.

Section 2.8. Positional analysis of radioactive triacylglycerols

The TGs were reacted with pancreatic lipase as in Section 2.5.1.(b). Special care was taken in the extraction of products to minimize losses. Water washings were partitioned against hexane to recover any lipid lost from the ether phase. Aliquots of all products were counted by scintillation counting. Amounts of TGs and MGs present were determined by g.l.c. of the FA methyl esters (Section 2.3.2.1.), after the addition

of methyl heptadecanoate as an internal standard.

The following calculation allowed determination of the proportion of the FA in position 2 of the TGs.

$$\text{Percentage of FA in position 2} = \frac{\frac{\text{Cpm MG}}{\text{moles MG}}}{\frac{\text{Cpm TG}}{\text{moles TG}}} \times \frac{100}{1}$$

Cpm MG = counts per min in MG fraction

Cpm TG = counts per min in TG fraction

moles MG = no. of moles of FA in MG fraction

moles TG = no. of moles of FA in TG fraction

# Chapter 3

# RESULTS

## Section 3.1. Composition of milk samples

In order to ascertain the effect of the intake of elevated levels of 18:2 on the composition and the thermal properties of milk fat, the FA and TG compositions, the positional distribution of FAs in the TGs, the levels of the TG classes of differing levels of unsaturation, and the thermal properties as determined by differential scanning calorimetry were used to compare a milk fat high in 18:2 with a normal milk fat.

Milk fat was collected from the pair of monozygous twin cows on selected days throughout the trial period. After the level of 18:2 in the milk fat of the cow fed the protected supplement of sunflower seed had reached 15 - 17 mole % and had remained at this level for four days (Figure 2a), the milk fats collected from the two cows on the fourth day (day 19) were selected for further comparison of the two milk fats.

### 3.1.1. Fatty acid composition

#### (a) Proportions of fatty acids

Milk fat from the two cows showed similar proportions of FAs at the start of the trial period (Figures 2a and 2b)(Appendix 1). Over the trial period the FA composition of milk fat from the control cow varied within a narrow range (Figures 2a and 2b) (Appendix 1) e.g. 4:0 varied from 10.5 to 12.5%, 16:0 from 20.3 to 23.5% and 18:1 from 17.9 to 22.5%. The proportions of 18:1 in the milk fat from the control cow appeared to decrease over the trial period but other FAs tended to exhibit only day to day variations.

Over the period of supplement feeding the milk fat of the

experimental cow exhibited day to day variations in the levels of most FAs, but the distinguishable trends were the increasing proportions of 18:2, which were accompanied by decreasing proportions of 14:0 and 16:0 and possible slight decreases in the proportions of 4:0, 10:0 and 12:0 (Figures 2a and 2b) (Appendix 1).

Table 5. Fatty acid composition of the milk fat of the control and experimental cows on the last day of the trial period

| | Fatty acid composition (mole %) | |
|---|---|---|
| FA | Milk from Control cow | Milk from Experimental cow |
| 4:0 | 11.9 | 10.4 |
| 6:0 | 5.3 | 5.4 |
| 8:0 | 2.3 | 2.1 |
| 10:0 | 4.1 | 3.2 |
| 10:1 | 0.3 | 0.2 |
| 12:0 | 3.5 | 2.6 |
| 14:0 | 11.9 | 7.7 |
| 14:1 | 1.0 | 0.5 |
| 15:0 | 1.0 | 0.7 |
| 16:0 | 22.1 | 14.1 |
| 16:1 | 2.0 | 1.1 |
| 17:0 | 0.9 | 0.5 |
| 18:0 | 11.9 | 14.1 |
| 18:1 | 18.6 | 20.6 |
| 18:2 | 1.8 | 15.5 |
| 18:3 | 0.8 | 0.7 |
| 20:? | 0.5 | 0.7 |

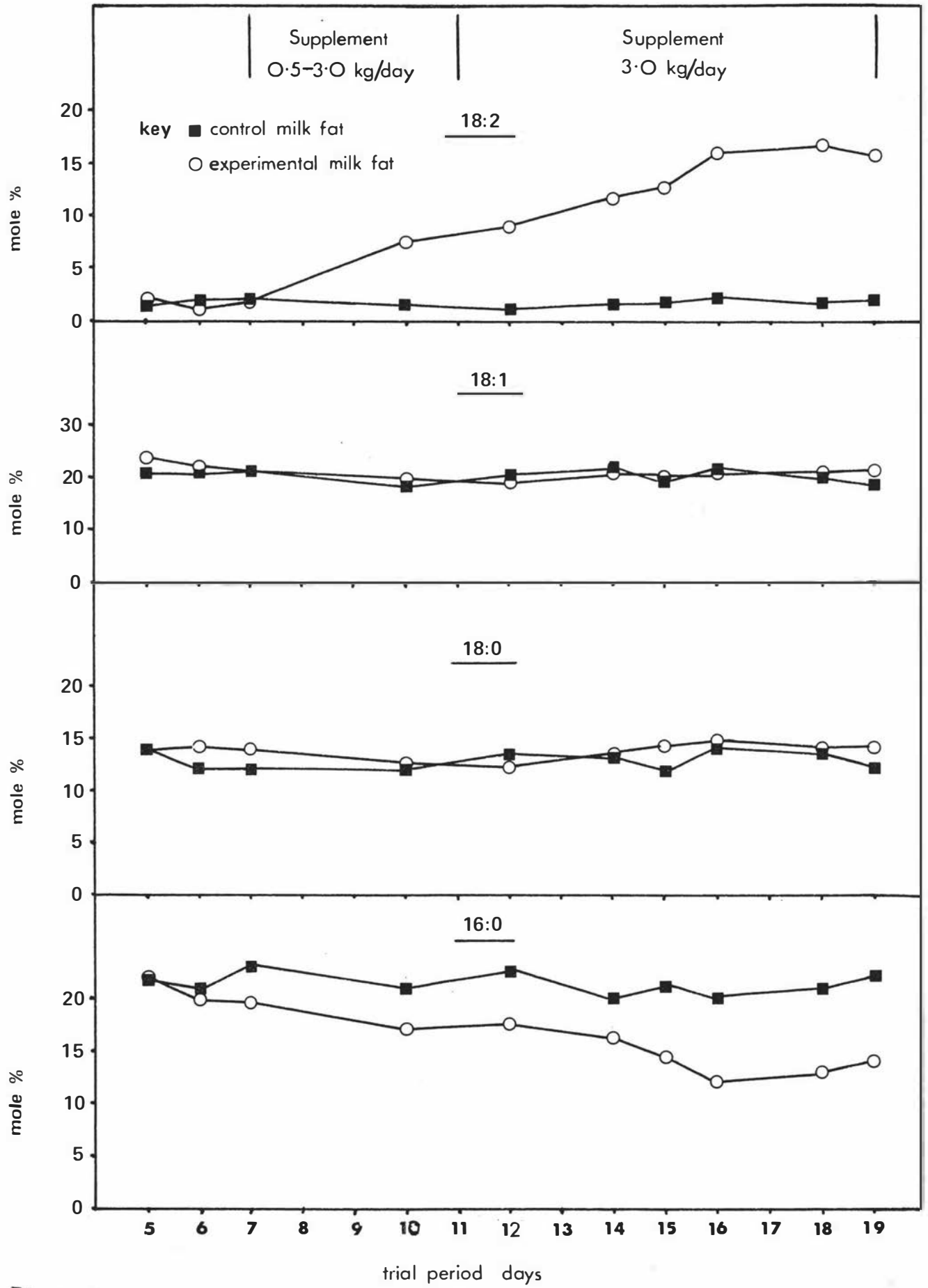



Figure 2a. Proportions of fatty acids in the triacylglycerols of milk fat from the control and experimental cows over the trial period.

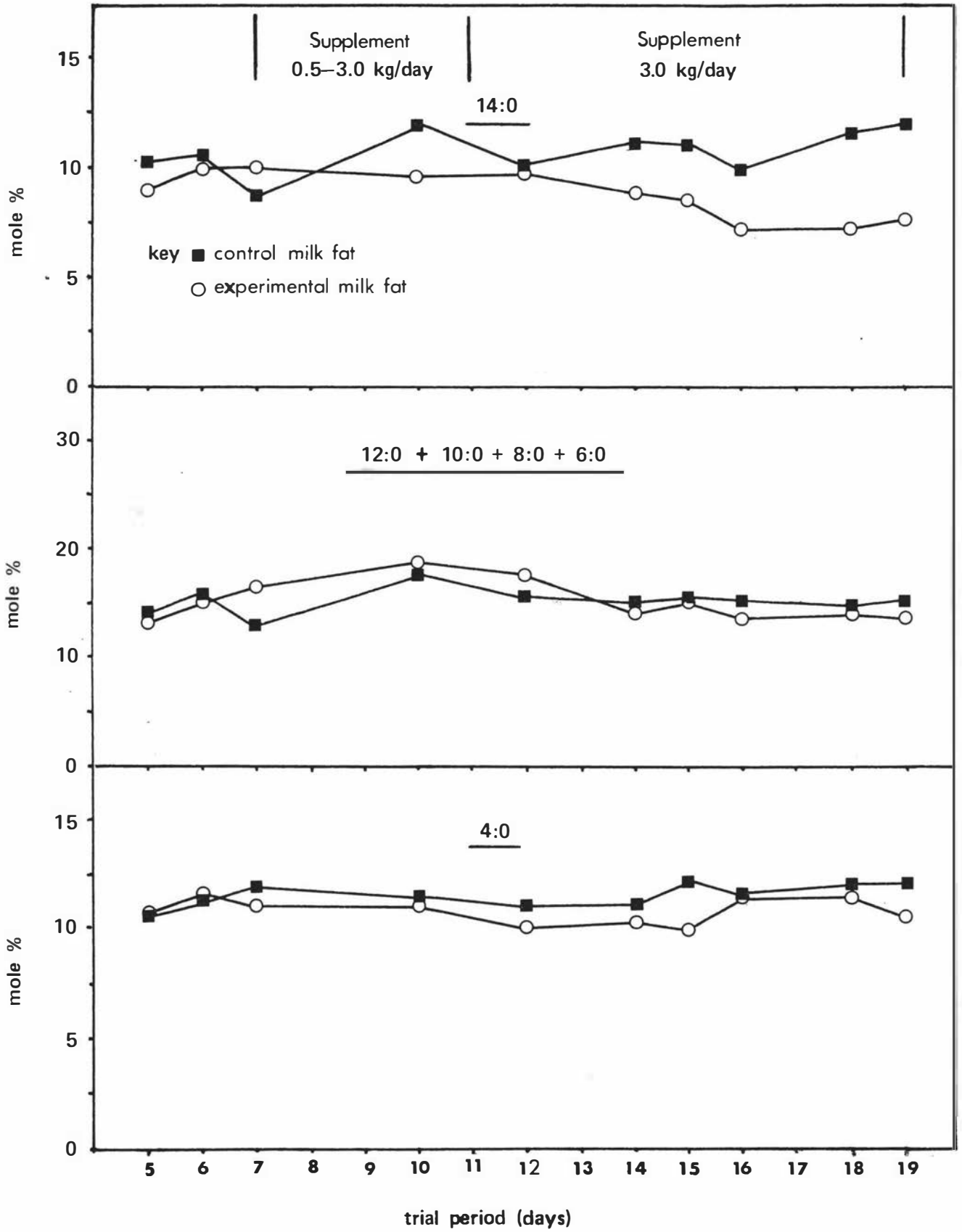



Figure 2b. Proportions of fatty acids in the triacylglycerols of milk fat from the control and experimental cows over the trial period.

Comparison of the proportions of FAs in the milk fat of the control and experimental cows on the last day of the trial (Table 5) (Figures 2a and 2b), revealed that milk fat from the experimental cow contained higher proportions of 18:2 (15.5% compared to 1.8%), 18:0 (14.1% compared to 11.9%) and 18:1 (20.6% compared to 18.6%) than milk fat from the control cow. The milk fat from the experimental cow also contained lower proportions of 14:0 (7.7% compared to 11.9%), 16:0 (14.1% compared to 22.1%) and 4:0 (10.4% compared to 11.9%). Proportions of minor FAs were similar except for noticeably lower levels of 10:0 (3.2% compared to 4.1%) and 12:0 (2.6% compared to 3.5%) in the milk fat of the experimental cow (Figures 3 and 4).

As a consequence of the high level of 18:2 in this milk fat of the experimental cow, the milk fat from the last day of the trial is henceforth referred to as the 18:2-rich milk fat.

(b) Amounts of fatty acids secreted

The average daily yield of FAs over the pre-experimental and the experimental periods (Table 6) was calculated using the respective FA compositions and fat yields. In the pre-experimental period the amounts of the FAs, 4:0 to 16:0, secreted by the two cows were similar, but the control cow produced smaller amounts of 18:0 and 18:1 than the experimental cow. The yields of 4:0 to 16:0 in this period were 297.0 g/day for the control cow and 293.8 g/day for the experimental cow. On the other hand the yield of 18 carbon FAs was 278.4 g/day and 297.5 g/day for the control and the experimental cow respectively. The difference in the overall fat yield (Appendix 2) of the two cows appeared to arise mainly from the higher yield of 18 carbon FAs in the milk of the experimental cow.

Over the last four days of the trial the average yields of individual FAs in the milk of the control cow were very similar to the

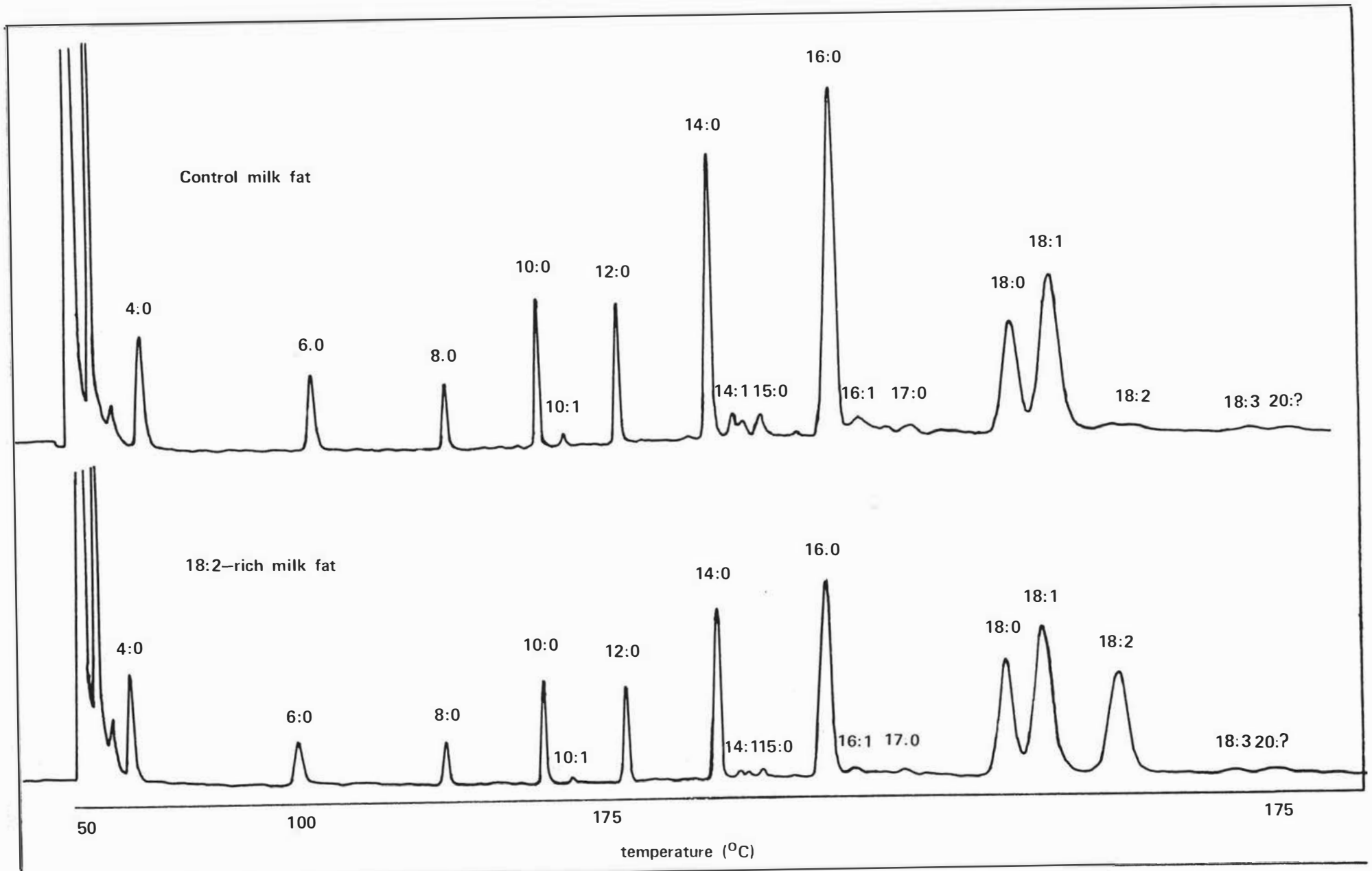



Figure 3. Gas-liquid chromatograms of the fatty acid methyl esters of the control and 18:2-rich milk fats.

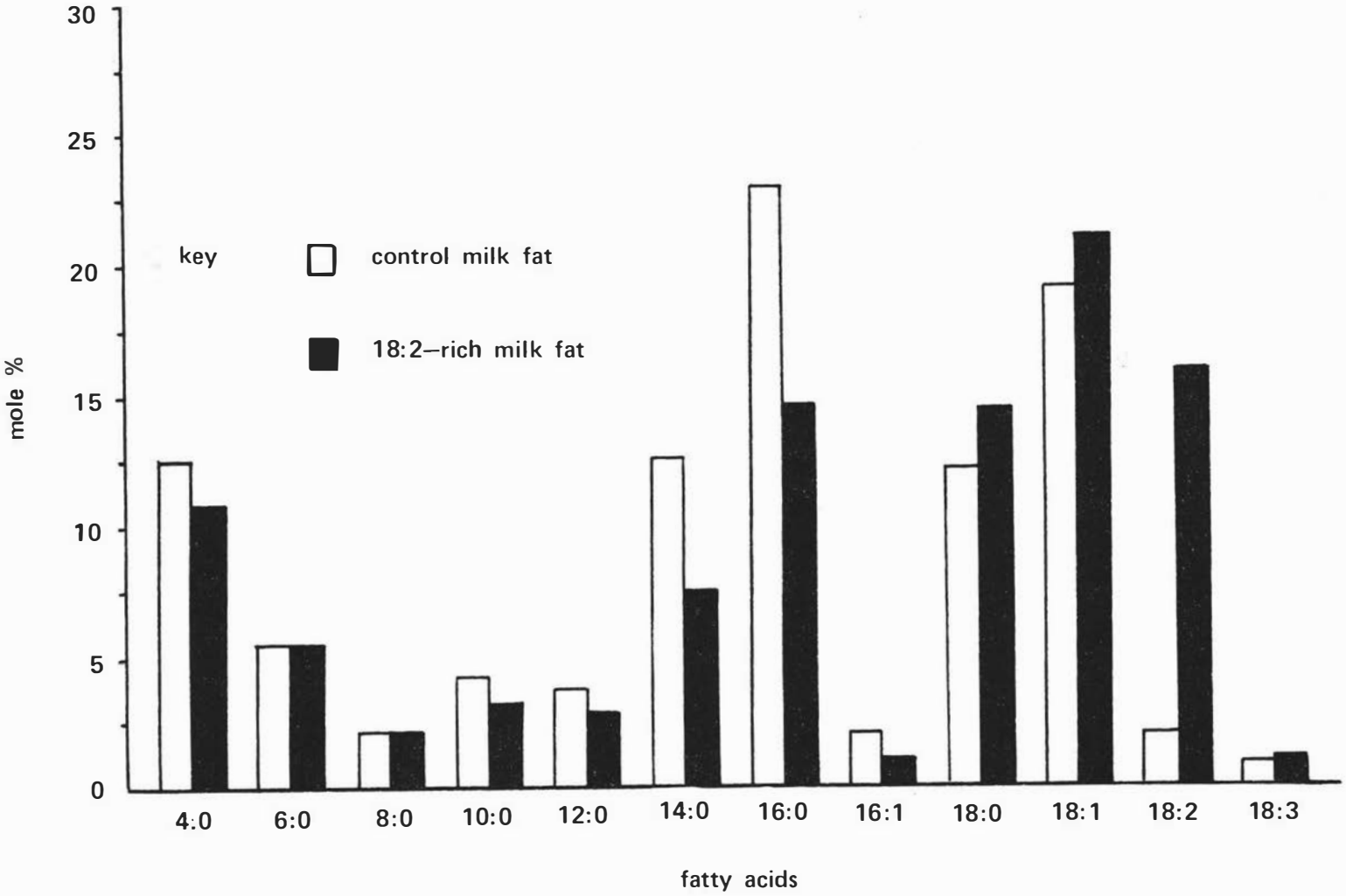



Figure 4. Fatty acid compositions of the control and 18:2-rich milk fats.

Table 6. Yields of major fatty acids in milk of cows fed the control and experimental diets.[a]

| FA | Yield (g) | | | |
|---|---|---|---|---|
| | Pre-experimental period | | Experimental period | |
| | Control | Expt. | Control | Expt. |
| 4:0 | 25.7 | 27.1 | 25.4 | 28.3 |
| 6:0 | 13.4 | 15.7 | 14.9 | 14.2 |
| 8:0 | 6.3 | 8.8 | 8.3 | 9.7 |
| 10:0 | 17.1 | 18.3 | 17.1 | 16.5 |
| 12:0 | 20.2 | 22.6 | 17.1 | 15.9 |
| 14:0 | 59.4 | 57.0 | 60.8 | 48.9 |
| 16:0 | 149.9 | 144.3 | 133.9 | 97.9 |
| 18:0 | 94.8 | 107.1 | 87.4 | 116.5 |
| 18:1 | 159.7 | 173.3 | 139.9 | 173.7 |
| 18:2 | 13.5 | 9.5 | 11.1 | 128.9 |
| 18:3 | 10.4 | 7.6 | 5.5 | 5.5 |
| Others | 36.5 | 38.8 | 31.7 | 33.3 |
| Fat yield[b] | 611.9 | 630.1 | 553.1 | 689.3 |

[a] Composition of polyunsaturated supplement in the experimental diet = 41.4% lipid

| FA | 14:0 | 16:0 | 18:0 | 18:1 | 18:2 |
|---|---|---|---|---|---|
| mole % | 0.1 | 8.1 | 4.9 | 18.8 | 68.1 |

[b] Average yields over the pre-experimental and experimental periods, - remaining data given in Appendix 2.

yields over the pre-experimental period (Table 6).

Comparison of the milk from the control cow and the experimental cow over the last four days of the trial indicated that the amounts produced, of FAs in the range 4:0 to 10:0 were almost unaltered by feeding the polyunsaturated supplement. In contrast there were lower yields of 12:0 (15.9 g compared to 17.1 g), 14:0 (48.9 g compared to 60.8 g) and 16:0 (97.9 g compared to 133.9 g) and greater yields of 18:2 (128.9 g compared to 11.1 g) in the milk fat from the experimental cow (18:2-rich milk fat) than in the milk fat from the control cow. Although the amounts of 18:0 and 18:1 secreted in the milk of the experimental cow were greater than in the milk of the control cow, the yield of these two FAs in the milk of both cows was similar to that in the respective milk samples in the pre-experimental period.

(c) Trans 18:1 content

The control and 18:2-rich milk fats contained different proportions of trans 18:1 as determined by argentation t.l.c. (Section 2.4.3.). In the control milk fat trans 18:1 comprised 22.3% of the total 18:1 compared with trans 18:1 comprising 18.8% of the total 18:1 in the 18:2-rich milk fat. This was equivalent to 4.2% and 3.9% of the FAs of the control and 18:2-rich milk fats respectively. On the other hand, the yield of trans 18:1 in the two milk samples was similar with a yield of about 30 g/day in both milk samples.

### 3.1.2. Triacylglycerol composition

The TG compositions of the two milk fats, as determined by separation of the TGs by g.l.c. on the basis of acyl carbon number, were almost identical in the pre-experimental period (Table 7). At the end of the experimental period the milk fat from the control cow contained similar proportions of all TGs to those in the pre-experimental period,

Table 7. Triacylglycerol composition of milk fats from the pair of monozygous twin cows fed on the control and experimental diets.

| | Triacylglycerol composition (mole %) | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-experimental period | | | | | | | | Experimental period | | | | | | | | | | | | | |
| Day | 5 | | 6 | | 7 | | Average[c] | | 10 | | 12 | | 14 | | 15 | | 16 | | 18 | | 19[d] | |
| Cow | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] |
| TG[e] | | | | | | | | | | | | | | | | | | | | | | |
| 26 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.1 |
| 28 | 0.2 | 0.6 | 0.2 | 0.5 | 0.2 | 0.7 | 0.2 | 0.6 | 0.3 | 1.4 | 0.4 | 0.4 | 0.2 | 0.8 | 0.3 | 0.7 | 1.9 | 0.7 | 0.4 | 1.0 | 0.7 | 0.6 |
| 30 | 1.0 | 1.3 | 0.7 | 1.2 | 0.8 | 1.2 | 0.8 | 1.2 | 0.9 | 1.3 | 1.2 | 1.2 | 1.2 | 1.2 | 0.8 | 1.0 | 1.6 | 1.0 | 1.2 | 1.0 | 1.4 | 1.1 |
| 32 | 2.3 | 2.7 | 1.6 | 2.5 | 2.0 | 2.6 | 2.0 | 2.6 | 2.2 | 2.5 | 2.5 | 2.3 | 3.0 | 2.4 | 2.2 | 2.1 | 2.4 | 1.8 | 2.6 | 1.8 | 3.0 | 2.1 |
| 34 | 4.9 | 5.8 | 4.4 | 5.3 | 4.8 | 5.6 | 4.7 | 5.6 | 5.7 | 4.9 | 5.2 | 4.5 | 6.6 | 4.3 | 5.6 | 4.0 | 4.7 | 3.1 | 6.0 | 3.1 | 6.7 | 3.6 |
| 36 | 10.3 | 11.3 | 10.0 | 10.7 | 10.9 | 11.0 | 10.4 | 11.0 | 11.4 | 9.9 | 11.7 | 9.1 | 12.0 | 8.6 | 11.5 | 8.2 | 10.6 | 6.7 | 12.2 | 6.9 | 12.3 | 7.5 |
| 38 | 15.6 | 14.8 | 14.5 | 14.5 | 15.8 | 14.7 | 15.3 | 14.7 | 14.5 | 14.1 | 14.8 | 13.1 | 15.2 | 12.5 | 15.2 | 12.3 | 14.3 | 11.5 | 15.1 | 11.2 | 14.5 | 11.7 |
| 40 | 14.2 | 13.0 | 12.9 | 12.7 | 14.3 | 13.1 | 13.8 | 12.9 | 13.0 | 14.8 | 12.9 | 14.6 | 12.9 | 14.9 | 13.2 | 15.5 | 12.9 | 17.1 | 12.9 | 17.1 | 12.0 | 16.9 |
| 42 | 7.9 | 7.7 | 7.9 | 7.8 | 8.1 | 8.0 | 8.0 | 7.8 | 8.9 | 8.5 | 8.2 | 9.2 | 8.7 | 8.7 | 8.5 | 8.7 | 7.6 | 8.4 | 8.2 | 8.2 | 7.9 | 9.0 |
| 44 | 5.6 | 6.0 | 6.0 | 6.1 | 5.9 | 6.1 | 5.8 | 6.1 | 7.1 | 6.0 | 6.6 | 6.7 | 7.2 | 6.0 | 6.7 | 5.6 | 5.9 | 4.8 | 6.4 | 4.8 | 6.5 | 5.5 |
| 46 | 5.5 | 5.7 | 5.9 | 5.9 | 5.7 | 5.9 | 5.7 | 5.8 | 6.7 | 5.6 | 6.5 | 6.4 | 6.8 | 6.0 | 6.3 | 5.5 | 5.9 | 4.8 | 5.3 | 4.8 | 6.2 | 5.6 |
| 48 | 6.4 | 6.4 | 6.8 | 6.8 | 6.3 | 6.3 | 6.5 | 6.5 | 6.8 | 5.7 | 7.2 | 6.5 | 7.4 | 6.0 | 7.0 | 5.4 | 6.9 | 4.6 | 7.2 | 4.7 | 6.9 | 5.1 |
| 50 | 9.6 | 9.0 | 10.2 | 9.5 | 9.1 | 8.9 | 9.6 | 9.1 | 8.5 | 8.0 | 9.3 | 8.6 | 8.6 | 8.5 | 8.9 | 8.3 | 9.7 | 8.1 | 9.0 | 8.0 | 8.6 | 7.8 |
| 52 | 10.3 | 9.4 | 11.2 | 10.0 | 9.9 | 9.4 | 10.5 | 9.6 | 8.9 | 8.8 | 8.5 | 9.0 | 7.0 | 9.7 | 8.5 | 10.3 | 9.3 | 11.2 | 8.0 | 10.6 | 7.7 | 9.5 |
| 54 | 6.2 | 6.2 | 7.6 | 6.4 | 6.2 | 6.5 | 6.7 | 6.4 | 5.1 | 8.5 | 5.0 | 8.5 | 3.2 | 10.3 | 5.3 | 12.5 | 6.3 | 16.1 | 5.5 | 16.8 | 5.6 | 13.9 |

a Milk fat from control cow

b Milk fat from experimental cow

c Average TG composition in the pre-experimental period

d Milk fat selected for comparison of the control and experimental milk fats

e Only TGs of even acyl carbon no. considered

except for higher proportions of $C_{34}$ (6.7% compared to 4.7%) and $C_{36}$ (12.3% compared to 10.4%) and lower proportions of $C_{52}$ (7.7% compared to 10.5%) (Table 7). It was evident that throughout the trial period the TG composition of the control milk fat showed only slight variation.

As the proportions of 18:2 in the milk fat of the experimental cow increased as a result of supplement feeding, the major effects on the TG composition were to increase the levels of $C_{54}$ (6.4% to 16.8%) and $C_{40}$ (12.9% to 17.1%) and to decrease the levels of $C_{34}$ (5.6% to 3.1%), $C_{36}$ (11.0% to 6.9%), $C_{38}$ (14.7% to 11.2%) and $C_{50}$ (9.1% to 8.0%). The levels of $C_{44}$, $C_{46}$ and $C_{48}$ also tended to decrease with increasing 18:2 content of the milk fat (Table 7).

Substantial differences were evident between the TG compositions of the milk fats collected from the control and experimental cows on the last day of the trial (day 19). The milk fat from the experimental cow (18:2-rich milk fat) contained higher proportions of $C_{40}$ (16.9% compared to 12.0%), $C_{52}$ (9.5% compared to 7.7%) and $C_{54}$ (13.9% compared to 5.6%), and lower proportions of $C_{34}$ (3.6% compared to 6.7%), $C_{36}$ (7.5% compared to 12.3%) and $C_{38}$ (11.7% compared to 14.5%) than the milk fat from the control cow. The proportions of $C_{50}$ in the two milk fats were similar, comprising 8.6 and 7.8% of the control and 18:2-rich milk fats respectively. Levels of $C_{44}$, $C_{46}$ and $C_{48}$, which were only minor constituents, were slightly lower in the 18:2-rich milk fat than in the control milk fat e.g. $C_{44}$ comprised 6.5% of the control milk fat and 5.5% of the 18:2-rich milk fat (Table 7) (Figures 5 and 6).

## Section 3.2. Fractionation of milk fat samples

### 3.2.1. Proportions of the fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

The proportions of the high, medium and low mol. wt. fractions

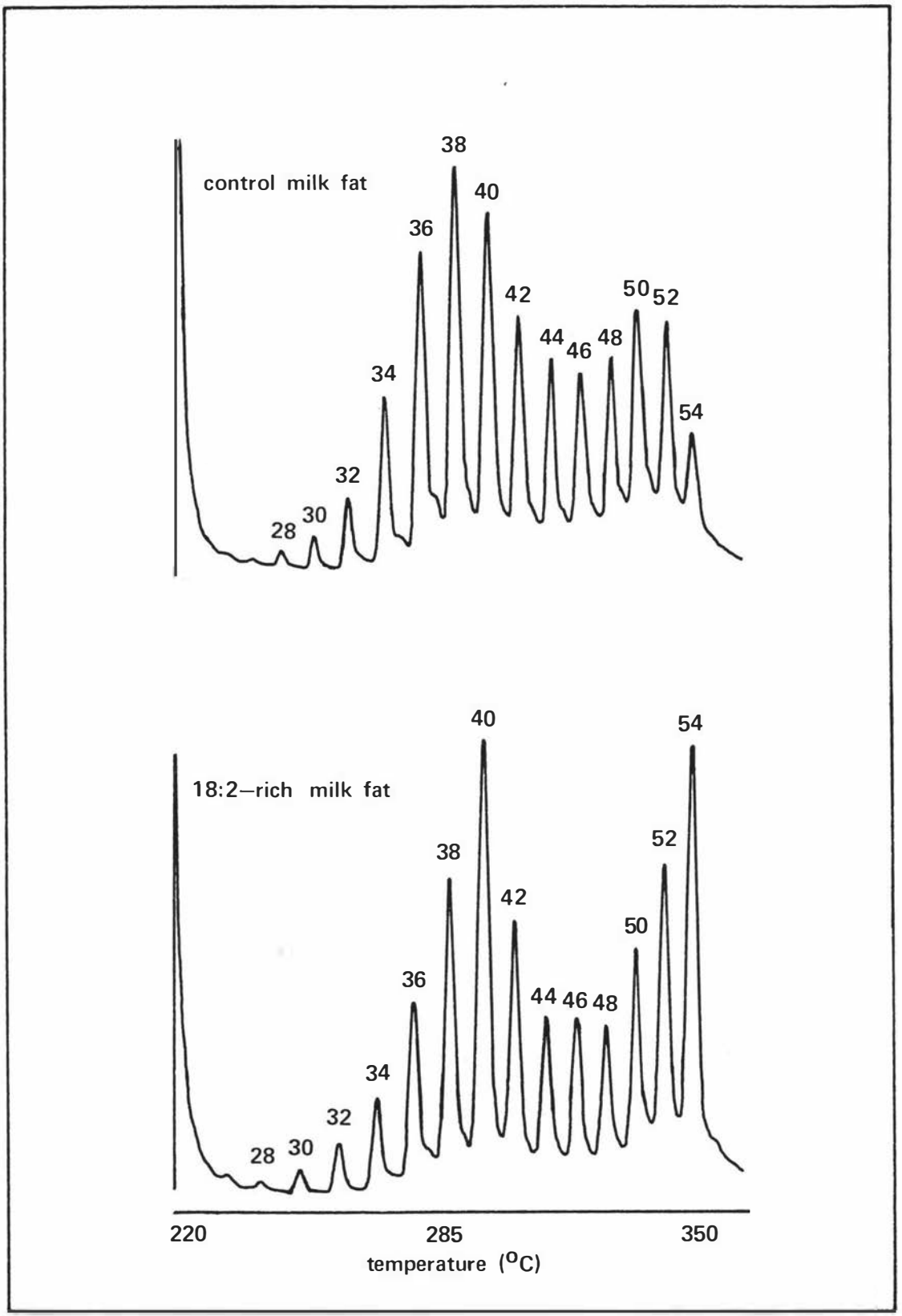



Figure 5. Gas-liquid chromatograms of the triacylglycerols of the control and 18:2-rich milk fats.

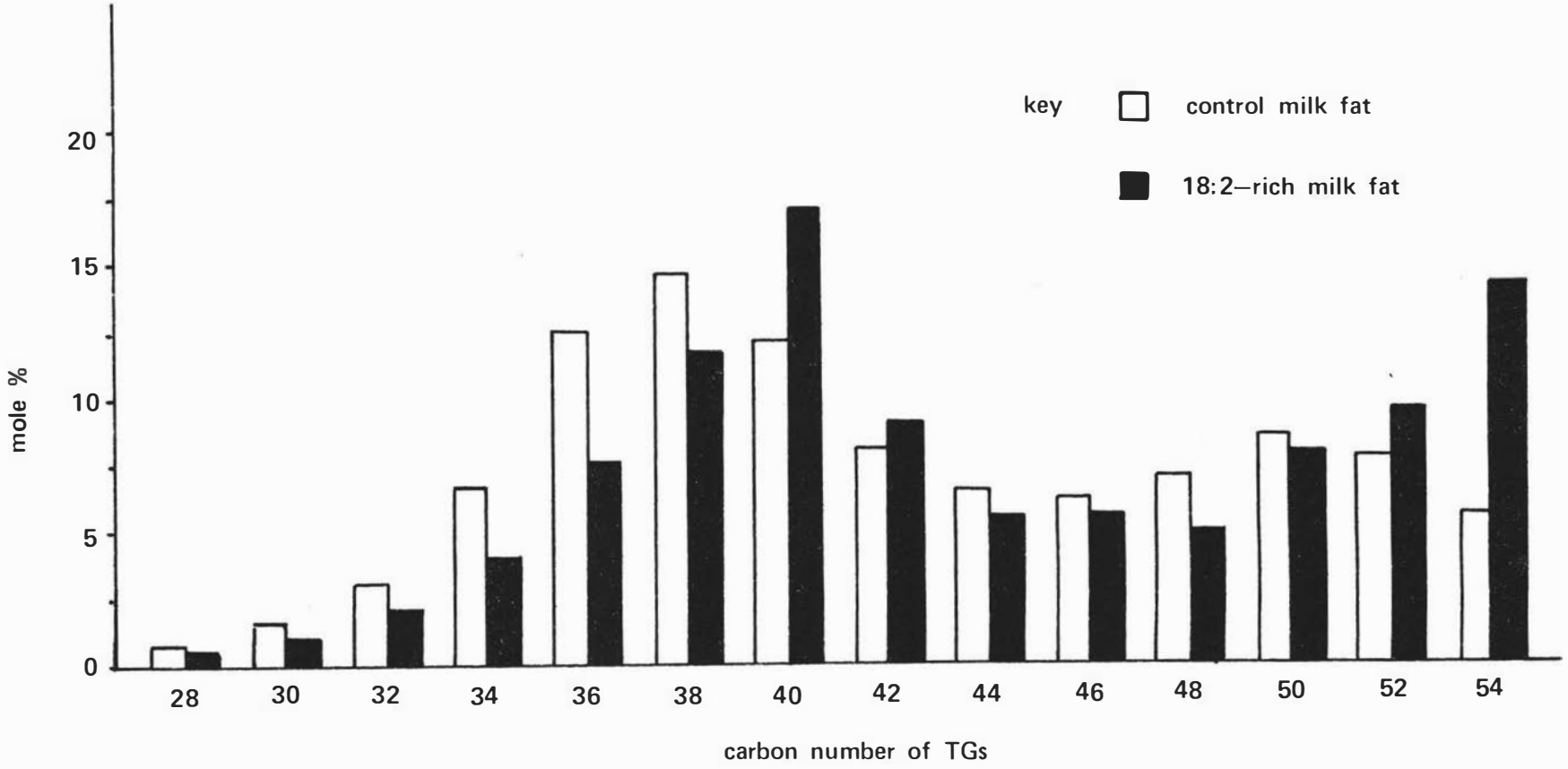



Figure 6. Triacylglycerol compositions of the control and 18:2-rich milk fats.

obtained by silicic acid chromatography were determined by weighing, and the molar proportions calculated using the respective FA compositions of each fraction. The weights, the weight proportions, and the molar proportions obtained are shown in Table 8.

The final data for the 18:2-rich milk fat was compiled from duplicate column runs (Table 8). TG compositions (Table 9) of the TG fractions from the two column runs were almost identical and it was considered valid to bulk the two samples to provide sufficient sample for subsequent experimentation.

As shown in Table 8, the fractions of medium mol. wt. were of comparable size, but the high mol. wt. fraction was of greater proportion in the 18:2-rich milk fat, at the expense of the low mol. wt. fraction.

### 3.2.2. Fatty acid composition of the fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

The FA compositions of the TG fractions of high, medium and low mol. wt. are given in Table 10. The most obvious difference between the two milk fats was the higher levels of 18:2 in each fraction of the 18:2-rich milk fat in comparison to the same fraction of the control milk fat. There were corresponding lower levels of 16:0, and to a lesser extent 14:0, in each fraction of the 18:2-rich milk fat.

In the high mol. wt. fraction, the 18:2-rich milk fat contained higher levels of 18:2 (16.1% compared to 1.7%) and lower levels of 16:0 (16.1% compared to 27.3%) and 14:0 (8.7% compared to 13.3%) than the control milk fat, but the proportions of other FAs were quite similar except for a slight increase in the level of 18:0 (18.5% compared to 16.4%) in the 18:2-rich milk fat. The major FAs of both high mol. wt. fractions were 16:0, 18:0 and 18:1 with the addition of 18:2 in the

Table 8. Proportions of the fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

| | Control milk fat | | | 18:2-rich milk fat | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | weight (mg) | wt. % | mole % | weight (mg) | | | wt. % | | | mole % |
| | | | | (a)[e] | (b)[f] | Total | (a) | (b) | Average | |
| TG fraction of high mol. wt. | 381 | 41.1 | 36.1 | 350 | 280 | 630 | 49.0 | 48.7 | 48.8 | 43.0 |
| TG fraction of medium mol. wt. | 175 | 18.9 | 19.7 | 130 | 105 | 235 | 18.2 | 18.3 | 18.3 | 19.4 |
| TG fraction of low mol. wt. | 370 | 40.0 | 44.2 | 235 | 190 | 425 | 32.7 | 33.0 | 32.9 | 37.6 |
| TGs recovered (mg). | 926 | | | 715 | 575 | 1290 | | | | |
| Milk fat placed on column (mg). | 960 | | | 745 | 600 | 1345 | | | | |
| Percentage recovery from column | 96.5% | | | 96.0% | 95.8% | 95.9% | | | | |

[e] First column run

[f] Second column run

Table 9. Triacylglycerol composition of the fractions of high, medium and low molecular weight of the 18:2-rich milk fat obtained from two silicic acid column runs

| TG[a] (Carbon no.) | Triacylglycerol composition (mole %) | | | | | |
|---|---|---|---|---|---|---|
| | High mol. wt. fraction | | Medium mol. wt. fraction | | Low mol. wt. fraction | |
| | RUN 1 | RUN 2 | RUN 1 | RUN 2 | RUN 1 | RUN 2 |
| 24 | - | - | - | - | 0.2 | - |
| 26 | - | - | - | - | 0.9 | 0.5 |
| 28 | - | - | - | - | 2.8 | 1.9 |
| 30 | - | - | - | - | 4.8 | 3.7 |
| 32 | - | - | 0.5 | - | 7.6 | 6.9 |
| 34 | - | - | 2.8 | 1.2 | 10.8 | 10.5 |
| 36 | - | - | 9.6 | 7.9 | 17.0 | 17.8 |
| 38 | 0.7 | 0.2 | 21.5 | 20.7 | 21.0 | 22.0 |
| 40 | 2.1 | 1.6 | 29.0 | 28.1 | 30.5 | 31.3 |
| 42 | 4.8 | 4.2 | 18.5 | 18.3 | 4.0 | 4.9 |
| 44 | 5.9 | 5.8 | 6.3 | 8.0 | 0.4 | 0.4 |
| 46 | 8.1 | 8.0 | 4.1 | 5.5 | - | - |
| 48 | 9.6 | 9.9 | 1.6 | 2.1 | - | - |
| 50 | 17.2 | 17.3 | 1.8 | 2.3 | - | - |
| 52 | 22.1 | 22.6 | 1.5 | 1.9 | - | - |
| 54 | 29.5 | 30.3 | 2.8 | 4.1 | - | - |

[a] Only TGs of even acyl carbon no. considered

Table 10. Fatty acid composition of the triacylglycerol fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

| FA | Fatty acid composition (mole %) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | High mol. wt. fraction | | Medium mol. wt. fraction | | Low mol. wt. fraction | | Milk fat | | | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | | 18:2-rich | |
| | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 4:0 | - | - | 5.3 | 8.2 | 25.0 | 24.3 | 11.9 | 12.1 | 10.4 | 10.7 |
| 6:0 | 0.4 | 1.1 | 10.4 | 12.3 | 6.2 | 5.9 | 5.3 | 4.9 | 5.4 | 5.1 |
| 8:0 | 1.1 | 1.5 | 4.3 | 3.8 | 2.3 | 2.3 | 2.3 | 2.3 | 2.1 | 2.2 |
| 10:0 | 3.8 | 3.5 | 5.9 | 4.5 | 3.6 | 3.7 | 4.1 | 4.1 | 3.2 | 3.8 |
| 10:1 | - | - | 0.5 | 0.3 | 0.5 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 |
| 12:0 | 4.0 | 3.2 | 4.5 | 2.6 | 3.8 | 3.4 | 3.5 | 4.0 | 2.6 | 3.2 |
| 14:0 | 13.3 | 8.8 | 11.8 | 7.3 | 11.5 | 9.1 | 11.9 | 12.2 | 7.7 | 8.6 |
| 14:1 | 0.7 | 0.6 | 1.4 | 0.4 | 0.9 | 0.6 | 1.0 | 0.9 | 0.5 | 0.6 |
| 15:0 | 1.3 | 0.7 | 1.0 | 0.6 | 0.9 | 0.6 | 1.0 | 1.1 | 0.7 | 0.6 |
| 16:0 | 27.3 | 16.2 | 23.6 | 14.0 | 20.2 | 13.1 | 22.1 | 23.4 | 14.1 | 14.6 |
| 16:1 | 1.9 | 1.4 | 2.1 | 0.9 | 1.4 | 1.0 | 2.0 | 1.7 | 1.1 | 1.2 |
| 17:0 | 0.9 | 0.7 | 0.8 | 0.6 | 0.4 | 0.2 | 0.9 | 0.7 | 0.5 | 0.5 |
| 18:0 | 16.4 | 18.6 | 11.1 | 15.3 | 7.6 | 8.0 | 11.9 | 11.5 | 14.1 | 14.0 |
| 18:1 | 26.5 | 26.5 | 15.3 | 15.9 | 12.6 | 13.2 | 18.6 | 18.2 | 20.6 | 19.4 |
| 18:2 | 1.7 | 16.1 | 1.0 | 11.6 | 1.2 | 13.5 | 1.8 | 1.3 | 15.5 | 14.2 |
| 18:3 | 0.7 | 1.1 | 0.8 | 0.7 | 1.2 | 0.8 | 0.8 | 0.9 | 0.7 | 0.9 |
| 20:? | - | - | 0.2 | 1.0 | 0.8 | - | 0.5 | 0.4 | 0.7 | 0.2 |

18:2-rich milk fat.

The medium mol. wt. fractions of both milk fats contained a similar range of FAs (4:0 to 18:3), but in the 18:2-rich milk fat, the medium mol. wt. fraction contained higher levels of 4:0 (8.2% compared to 5.3%), 18:0 (15.3% compared to 11.1%) and 18:2 (11.6% compared to 1.0%), and lower levels of 12:0 (2.6% compared to 4.5%), 14:0 (7.3% compared to 11.8%) and 16:0 (14.0% compared to 23.6%) than the medium mol. wt. fraction of the control milk fat. The levels of other FAs, including 18:1, were quite similar. In both fractions, FAs in the range 4:0 to 12:0, comprised about 30% of the total FAs.

The low mol. wt. fraction of the 18:2-rich milk fat, contained higher levels of 18:2 (13.5% compared to 1.2%) and lower levels of 16:0 (13.1% compared to 20.2%) than this fraction in the control milk fat. All other FAs were of similar proportions. 4:0 and 6:0 comprised about 30% of the low mol. wt. fractions from both sources.

The highest level of 18:2 in the 18:2-rich milk fat was in the high mol. wt. fraction (16.1%), followed by the low mol. wt. fraction (13.5%).

### 3.2.3. Triacylglycerol composition of the fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

The dominant feature of the high mol. wt. fractions was the greater proportion of TGs containing 54 acyl carbons in the 18:2-rich milk fat compared with the control milk fat (29.4% compared to 10.7%) (Table 11). This was accompanied by lower proportions of $C_{48}$ (9.6% compared to 17.1%) and $C_{50}$ (17.0% compared to 25.9%). In the 18:2-rich milk fat fraction, TGs with 50 - 54 acyl carbons were the only major species, comprising 68.7% of the total TGs, but in the control milk fat fraction $C_{48}$ (17.1%) was also a major TG, with $C_{50}$ - $C_{54}$

comprising only 58.7% of the total (Figure 7).

The TG compositions of the medium mol. wt. fraction of the two milk fats were quite similar, the only noticeable difference being a slightly greater proportion of $C_{40}$ (28.8% compared to 24.8%) in the 18:2-rich milk fat compared to the control milk fat. The proportions of the other major TGs, in the 18:2-rich and control milk fats respectively, were 20.8% and 21.6% $C_{38}$ and 19.7% and 19.5% $C_{42}$.

In the low mol. wt. fraction, the TG composition of the 18:2-rich milk fat was markedly different to that of the control milk fat. The increased availability of 18:2 resulted in higher levels of $C_{40}$ (30.5% compared to 13.3%) and lower proportions of $C_{36}$ (18.3% compared to 28.0%) in the 18:2-rich milk fat. The proportions of $C_{34}$ were also lower in the 18:2-rich milk fat (10.9% compared to 18.8%). The only major TG component to be unaffected by the greater incorporation of 18:2 was $C_{38}$ which had proportions of 22.9% and 24.9% in the 18:2-rich and control milk fat fractions respectively.

## Section 3.3. Proportions of the triacylglycerol classes of differing levels of unsaturation in the high, medium and low molecular weight fractions of the control and 18:2-rich milk fats

The proportions, in the two fats and their respective fractions, of the TG classes prepared by argentation t.l.c. are presented in Table 12.

The high mol. wt. fraction of the 18:2-rich milk fat and the control milk fat contained 20.0% and 29.5% saturated TGs respectively. Similarly, the proportions of *trans* - monoene TGs in these two milk fat fractions were 3.6% and 9.9%, with the proportions of *cis* - monoene TGs being 21.1% and 34.1% respectively. Greater proportions of diene TGs

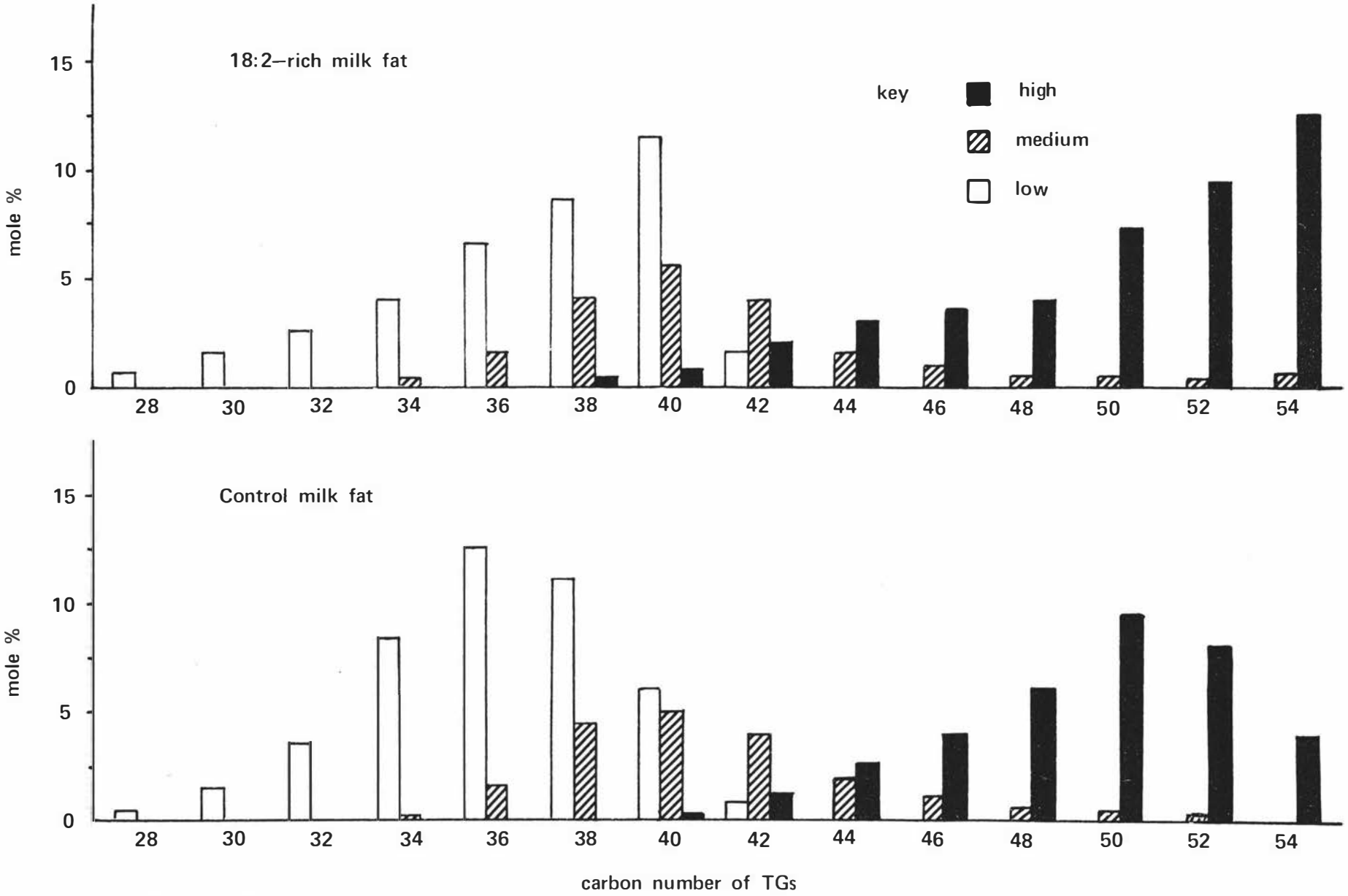



Figure 7. Triacylglycerol compositions of the triacylglycerol fractions of the control and 18:2-rich milk fats.

Table 11. Triacylglycerol composition of the triacylglycerol fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

| TG[a] (Carbon no.) | Triacylglycerol composition (mole %) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | High mol. wt. fraction | | Medium mol. wt. fraction | | Low mol. wt. fraction | | Milk fat | | | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | | 18:2-rich | |
| | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 26 | - | - | - | - | 0.2 | 0.2 | - | 0.1 | 0.1 | 0.1 |
| 28 | - | - | - | - | 1.3 | 1.9 | 0.7 | 0.6 | 0.6 | 0.7 |
| 30 | - | - | - | - | 3.4 | 3.9 | 1.4 | 1.5 | 1.1 | 1.5 |
| 32 | - | - | 0.1 | 0.1 | 8.1 | 7.2 | 3.0 | 3.6 | 2.1 | 2.7 |
| 34 | - | - | 1.1 | 1.6 | 18.8 | 10.9 | 6.7 | 8.5 | 3.6 | 4.4 |
| 36 | - | - | 7.7 | 8.3 | 28.0 | 18.3 | 12.3 | 13.9 | 7.5 | 8.5 |
| 38 | - | 0.9 | 21.6 | 20.8 | 24.9 | 22.9 | 14.5 | 15.3 | 11.7 | 13.0 |
| 40 | 0.8 | 1.9 | 24.8 | 28.8 | 13.3 | 30.5 | 12.0 | 11.1 | 16.9 | 17.9 |
| 42 | 3.8 | 4.5 | 19.5 | 19.7 | 1.8 | 4.1 | 7.9 | 6.0 | 9.0 | 7.3 |
| 44 | 7.8 | 6.2 | 10.4 | 7.4 | 0.2 | 0.1 | 6.5 | 5.0 | 5.5 | 4.1 |
| 46 | 11.8 | 8.3 | 6.1 | 4.9 | - | - | 6.2 | 5.5 | 5.6 | 4.5 |
| 48 | 17.1 | 9.6 | 3.5 | 1.8 | - | - | 6.9 | 6.9 | 5.1 | 4.5 |
| 50 | 25.9 | 17.0 | 2.9 | 1.9 | - | - | 8.6 | 9.9 | 7.8 | 7.7 |
| 52 | 22.1 | 22.3 | 1.8 | 1.6 | - | - | 7.7 | 8.3 | 9.5 | 9.9 |
| 54 | 10.7 | 29.4 | 0.6 | 3.1 | - | - | 5.6 | 4.0 | 13.9 | 13.2 |

[a] Only TGs of even carbon number considered

(27.5% compared to 17.5%) and triene TGs (15.6% compared to 8.9%) occurred in the 18:2-rich milk fat than in the control milk fat. Tetraene TGs were of quantitative importance in the 18:2-rich milk fat contributing 12.1% of the total TGs.

In the medium mol. wt. fraction the proportions of saturated TGs were lower in the 18:2-rich milk fat than in the control milk fat (28.0% compared to 46.9%) with the proportion of diene TGs being higher (22.5% compared to 13.2%) but the proportions of trans - monoene (7.9% compared to 7.8%), cis - monoene (24.1% compared to 23.9%) and triene TGs (9.0% compared to 8.1%) were similar. A tetraene TG class, comprising 8.5% of the medium mol. wt. TGs, was present in the 18:2-rich milk fat.

In the low mol. wt. fraction, the proportions of saturated (31.2% compared to 47.2%), trans - monoene (5.0% compared to 9.3%) and cis - monoene TGs (20.1% compared to 28.0%) were lower in the 18:2-rich milk fat, while proportions of diene TGs were higher (27.7% compared to 9.2%). In the more unsaturated TG classes, the level of triene TGs was only slightly higher (9.1% compared to 6.3%) but a significant tetraene class, comprising 6.9% of the low mol. wt. TGs again became apparent in the 18:2-rich milk fat.

The same trends as observed in the individual fractions were observed in the composition of the total milk fats as calculated from the proportions in the composite fractions. The 18:2-rich milk fat contained smaller proportions of saturated (25.8% compared to 40.7%), trans - monoene (5.0% compared to 9.2%) and cis - monoene TGs (21.3% compared to 29.4%) than in the control milk fat. The proportions of diene (26.6% compared to 13.0%) and triene TGs (11.9% compared to 7.6%) were greater in the 18:2-rich milk fat. This latter milk fat also contained 9.5% tetraene TGs.

Table 12. Proportions of the triacylglycerol classes of differing levels of unsaturation in the high, medium, and low molecular weight fractions of the control and 18:2-rich milk fats[c]

mole %

| Triacylglycerols | Control milk fat %F[a] | Control milk fat %T[b] | 18:2-rich milk fat %F[a] | 18:2-rich milk fat %T[b] |
|---|---|---|---|---|
| TG fraction of high mol. wt. | 100.0 | 36.1 | 100.0 | 43.0 |
| Saturated TGs | 29.5 | 10.6 | 20.0 | 8.6 |
| Trans - monoene TGs | 9.9 | 3.6 | 3.6 | 1.5 |
| Cis - monoene TGs | 34.1 | 12.3 | 21.1 | 9.1 |
| Diene TGs | 17.5 | 6.3 | 27.5 | 11.8 |
| Triene TGs | 8.9 | 3.2 | 15.6 | 6.7 |
| Tetraene TGs | - | - | 12.1 | 5.2 |
| TG fraction of medium mol. wt. | 100.0 | 19.7 | 100.0 | 19.5 |
| Saturated TGs | 46.9 | 9.2 | 28.0 | 5.5 |
| Trans - monoene TGs | 7.8 | 1.5 | 7.9 | 1.5 |
| Cis - monoene TGs | 23.9 | 4.7 | 24.1 | 4.7 |
| Diene TGs | 13.2 | 2.6 | 22.5 | 4.4 |
| Triene TGs | 8.1 | 1.6 | 9.0 | 1.8 |
| Tetraene TGs | - | - | 8.5 | 1.7 |
| TG fraction of low mol. wt. | 100.0 | 44.2 | 100.0 | 37.5 |
| Saturated TGs | 47.2 | 20.9 | 31.2 | 11.7 |
| Trans - monoene TGs | 9.3 | 4.1 | 5.0 | 1.9 |
| Cis - monoene TGs | 28.0 | 12.4 | 20.1 | 7.5 |
| Diene TGs | 9.2 | 4.1 | 27.7 | 10.4 |
| Triene TGs | 6.3 | 2.8 | 9.1 | 3.4 |
| Tetraene TGs | - | - | 6.9 | 2.6 |
| Milk fat | | 100.0 | | 100.0 |
| Saturated TGs | | 40.7 | | 25.8 |
| Trans - monoene TGs | | 9.2 | | 5.0 |
| Cis - monoene TGs | | 29.4 | | 21.3 |
| Diene TGs | | 13.0 | | 26.6 |
| Triene TGs | | 7.6 | | 11.9 |
| Tetraene TGs | | - | | 9.5 |

[a] Percentage of triacylglycerol fraction

[b] Percentage of milk fat

[c] Results means of two determinations

## Section 3.4. Composition of the TG classes of differing levels of unsaturation in the high, medium and low molecular weight fractions of the control and 18:2-rich milk fats

### 3.4.1. Composition of the TG classes of the fraction of high molecular weight in the control and 18:2-rich milk fats

(a) Fatty acid composition

Compared to the control milk fat, the proportions of 16:0 in the saturated TGs of the high mol. wt. fraction of the 18:2-rich milk fat were lower (29.0% compared to 39.1%) but the proportions of 18:0 were higher (30.5% compared to 26.8%) (Table 13). The proportions of the other major FA, i.e. 14:0, were similar in the two milk fats comprising 16.0% and 17.8% of the saturated TGs of the 18:2-rich and control milk fats respectively.

The FA compositions of the trans - monoene and cis - monoene TGs were quite similar in the two milk fats. The trans - monoene TGs of the 18:2-rich milk fat contained greater proportions of 18:0 (25.3% compared to 19.2%) and lower proportions of 14:0 (10.3% compared to 13.2%) and 16:0 (22.5% compared to 26.7%), than in the equivalent fraction of the control milk fat. There were higher proportions of 18:0 (19.8% compared to 16.7%) and lower proportions of 14:0 (9.4% compared to 12.0%) and 16:0 (20.7% compared to 28.4%) in the cis - monoene TGs of the 18:2-rich milk fat as compared to the control milk fat. The other FA of importance in these two TG classes was 18:1, comprising 25.8% and 33.0% of the trans - monoene and cis - monoene TGs respectively, in the high mol. wt. fraction of the 18:2-rich milk fat, and 28.0% and 30.8% of the trans - monoene and cis - monoene TGs respectively in the high mol. wt. fraction of the control milk fat.

The major difference between the diene TGs of the two milk fat fractions was the higher level of 18:2 (18.2% compared to 1.7%) and

Table 13. Fatty acid composition of the triacylglycerol classes of differing levels of unsaturation in the high molecular weight fraction of the control and 18:2-rich milk fats[d]

| FA | Fatty acid composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Fraction[c] | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 4:0 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 6:0 | 0.2 | 4.1 | - | 1.9 | - | 1.9 | - | 0.8 | - | 0.6 | - | - | 0.4 | 0.1 | 1.1 | 1.6 |
| 8:0 | 1.3 | 4.0 | 1.0 | 2.3 | 0.4 | 2.7 | - | 1.7 | - | 0.9 | - | - | 1.1 | 0.6 | 1.5 | 2.1 |
| 10:0 | 6.0 | 7.9 | 2.9 | 3.8 | 2.6 | 5.0 | 2.2 | 3.6 | 0.6 | 2.8 | - | 0.7 | 3.8 | 3.4 | 3.7 | 4.3 |
| 10:1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 12:0 | 6.9 | 7.2 | 3.8 | 3.3 | 3.6 | 4.2 | 2.6 | 3.0 | 1.1 | 2.2 | - | 0.9 | 4.0 | 4.2 | 3.2 | 3.7 |
| 14:0 | 17.8 | 16.0 | 13.2 | 10.3 | 12.0 | 9.4 | 7.9 | 7.0 | 4.5 | 5.1 | - | 3.1 | 13.3 | 12.4 | 8.7 | 8.7 |
| 14:1 | - | - | 1.1 | - | 1.8 | 1.3 | 1.7 | 0.8 | 1.7 | 0.6 | - | 0.5 | 0.7 | 1.2 | 0.6 | 0.6 |
| 15:0 | 1.8 | 1.3 | 1.6 | 1.4 | 1.6 | 0.8 | 0.9 | 0.7 | 0.6 | 0.6 | - | 0.4 | 1.3 | 1.4 | 0.7 | 0.8 |
| 16:0 | 39.1 | 29.0 | 26.7 | 22.5 | 28.4 | 20.7 | 17.9 | 15.8 | 11.4 | 11.0 | - | 7.6 | 27.3 | 28.0 | 16.1 | 18.0 |
| 16:1 | - | - | 2.6 | 3.3 | 2.2 | 1.1 | 3.5 | 1.6 | 2.2 | 1.7 | - | 1.1 | 1.9 | 1.8 | 1.4 | 1.2 |
| 17:0 | - | - | - | - | - | - | - | - | - | - | - | - | 0.9 | - | 0.7 | - |
| 18:0 | 26.8 | 30.5 | 19.2 | 25.3 | 16.7 | 19.8 | 10.5 | 17.3 | 9.1 | 11.3 | - | 7.6 | 16.4 | 18.1 | 18.5 | 18.6 |
| 18:1 | - | - | 28.0 | 25.8 | 30.8 | 33.0 | 51.1 | 29.4 | 46.0 | 32.9 | - | 25.9 | 26.5 | 26.3 | 26.5 | 24.2 |
| 18:2 | - | - | - | - | - | - | 1.7 | 18.2 | 15.9 | 29.2 | - | 48.6 | 1.7 | 1.7 | 16.1 | 15.5 |
| 18:3 | - | - | - | - | - | - | - | - | 6.8 | 1.2 | - | 3.6 | 0.7 | 0.6 | 1.1 | 0.6 |

[a] Trans - monoene TGs
[b] Cis - monoene TGs
[c] High mol. wt. fraction
[d] Results means of two determinations.
[e] Control milk fat
[f] 18:2-rich milk fat

lower level of 18:1 (29.4% compared to 51.1%) in the 18:2-rich milk fat than in the control milk fat. The proportions of 16:0 were lower (15.8% compared to 17.9%) and the proportions of 18:0 were higher (17.3% compared to 10.5%) in the 18:2-rich milk fat. Levels of 14:0 were similar with 7.0% and 7.9% in the 18:2-rich and the control milk fats respectively.

In the triene TGs, there were higher proportions of 18:2 (29.2% compared to 15.9%) and lower proportions of 18:1 (32.9% compared to 46.0%) in the 18:2-rich milk fat than in the control milk fat. The ratio of 18:1 to 18:2 was close to 1:1 in the 18:2-rich milk fat but in the control milk fat this ratio was almost 3:1, signifying a definite change in the character of these TGs.

The major proportion of the FAs in the tetraene TGs of the 18:2-rich milk fat consisted of 18:1 (25.9%) and 18:2 (48.6%).

(b) Triacylglycerol composition

The saturated TGs of the high mol. wt. fraction from the two milk fats were of similar composition, except for a trend towards higher levels of $C_{42}$ (20.1% compared to 14.0%) and lower levels of $C_{46}$ (15.5% compared to 21.0%) and $C_{48}$ (13.5% compared to 19.5%) in the 18:2-rich milk fat (Table 14). The proportions of the other major TGs were 18.7% and 19.8% $C_{44}$ and 13.0% and 14.9% $C_{50}$ in the 18:2-rich and the control milk fats respectively.

The relative differences between the respective *trans* - monoene and *cis* - monoene TGs of the two milk fats were similar in both monoene classes. In the *trans* - monoene TGs, the 18:2-rich milk fat had greater proportions of $C_{54}$ (13.3% compared to 5.3%) and lesser proportions of $C_{50}$ (22.1% compared to 35.2%) and $C_{48}$ (12.1% compared to 21.6%). The other major TG was $C_{52}$, comprising 24.3% and 25.0% of the *trans* - monoene TGs in the 18:2-rich and the control milk fats

Table 14. Triacylglycerol composition of the triacylglycerol classes of differing levels of unsaturation in the high molecular weight fraction of the control and 18:2-rich milk fats

| TG (Carbon No.) | Triacylglycerol composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Fraction[c] | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 36 | - | - | - | 1.3 | - | - | - | - | - | - | - | - | - | - | - | - |
| 38 | - | 1.3 | - | 4.3 | - | - | - | 0.1 | - | - | - | - | - | - | 0.9 | 0.5 |
| 40 | 2.6 | 9.8 | - | 5.7 | - | 0.7 | - | 0.1 | - | - | - | - | 0.8 | 0.8 | 1.9 | 2.4 |
| 42 | 14.0 | 20.1 | 0.1 | 4.5 | 0.3 | 4.3 | 0.2 | 1.6 | 0.1 | - | - | - | 3.8 | 4.3 | 4.5 | 5.5 |
| 44 | 19.8 | 18.7 | 3.6 | 4.8 | 4.3 | 8.3 | 2.3 | 4.9 | 0.2 | 1.0 | - | - | 7.8 | 8.1 | 6.2 | 7.2 |
| 46 | 21.0 | 15.5 | 9.2 | 7.6 | 10.7 | 12.1 | 4.7 | 8.9 | 1.5 | 3.7 | - | 1.0 | 11.8 | 11.7 | 8.3 | 9.1 |
| 48 | 19.5 | 13.5 | 21.6 | 12.1 | 22.3 | 16.4 | 6.9 | 11.4 | 5.2 | 4.9 | - | 2.0 | 17.1 | 17.1 | 9.6 | 10.7 |
| 50 | 14.9 | 13.0 | 35.2 | 22.1 | 33.1 | 24.6 | 22.4 | 20.5 | 16.5 | 15.1 | - | 7.0 | 25.9 | 24.5 | 17.0 | 17.4 |
| 52 | 6.8 | 7.4 | 25.0 | 24.3 | 23.2 | 22.6 | 41.7 | 26.5 | 28.6 | 29.4 | - | 16.9 | 22.1 | 22.2 | 22.3 | 21.2 |
| 54 | 1.4 | 0.6 | 5.3 | 13.3 | 6.0 | 11.0 | 21.8 | 25.9 | 48.0 | 45.9 | - | 73.0 | 10.7 | 11.1 | 29.4 | 26.0 |

[a] Trans - monoene TGs

[b] Cis - monoene TGs

[c] High mol. wt. fraction

[e] Control milk fat

[f] 18:2-rich milk fat

respectively. In the cis - monoene TGs, the major TGs were $C_{48}$ (16.4%), $C_{50}$ (24.6%) and $C_{52}$ (22.6%) in the 18:2-rich milk fat and $C_{48}$ (22.3%), $C_{50}$ (33.1%) and $C_{52}$ (23.2%) in the control milk fat.

The proportions of $C_{50}$ (20.5% compared to 22.4%) and $C_{54}$ (25.9% compared to 21.8%) were reasonably similar in the diene TGs of the 18:2-rich and the control milk fats, but the 18:2-rich milk fat had a much lower proportion of $C_{52}$ (26.5% compared to 41.7%) with correspondingly higher proportions of the $C_{44}$ to $C_{48}$ TGs (25.2% compared to 13.9%).

The TG compositions of the two classes of triene TGs were almost identical with the proportions of $C_{50}$, $C_{52}$ and $C_{54}$ being 15.1%, 29.4% and 45.9% respectively in the 18:2-rich milk fat and 16.5%, 28.6% and 48.0% respectively in the control milk fat. The $C_{52}$ and $C_{54}$ TGs together comprised about 75% of the triene TGs in both milk fats.

The major TGs in the tetraene TGs of the 18:2-rich milk fat were $C_{54}$ (73.0%) with lesser proportions of $C_{52}$ (16.9%).

### 3.4.2. Composition of the TG classes of the fraction of medium molecular weight in the control and 18:2-rich milk fats

#### (a) Fatty acid composition

The saturated, trans - monoene and cis - monoene TGs in the medium mol. wt. fractions of the two milk fats had similar FA compositions although these TGs in the 18:2-rich milk fat tended to have greater proportions of 18:0 and lesser proportions of 14:0 and 16:0 than the control milk fat (Table 15).

In the saturated TGs the proportions of 14:0, 16:0 and 18:0 in the 18:2-rich and control milk fats respectively were 11.9% and 14.3% 14:0, 25.7% and 30.3% 16:0, and, 21.8% and 14.9% 18:0. The FAs in the range 4:0 to 8:0, comprised 28.8% and 26.0% of the 18:2-rich and control milk

Table 15. Fatty acid composition of the triacylglycerol classes of differing levels of unsaturation in the medium molecular weight fraction of the control and 18:2-rich milk fats [d]

| FA | Fatty acid composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Fraction[c] | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 4:0 | 7.5 | 11.2 | 1.6 | 9.7 | 2.3 | 8.0 | 0.2 | 3.0 | - | - | - | - | 5.3 | 4.2 | 8.2 | 6.5 |
| 6:0 | 12.3 | 12.8 | 8.4 | 10.0 | 9.3 | 12.7 | 6.8 | 9.5 | 2.4 | 12.8 | - | 3.0 | 10.4 | 9.7 | 12.3 | 11.0 |
| 8:0 | 6.2 | 4.8 | 4.7 | 4.1 | 5.7 | 4.4 | 4.0 | 4.5 | 2.4 | 7.5 | - | 3.0 | 4.3 | 5.4 | 3.8 | 4.7 |
| 10:0 | 8.2 | 5.7 | 6.3 | 5.0 | 6.7 | 4.8 | 5.5 | 6.5 | 4.9 | 6.6 | - | 5.2 | 5.9 | 7.1 | 4.5 | 5.6 |
| 10:1 | - | - | - | - | 0.2 | 0.4 | 3.3 | 0.7 | 0.9 | 0.5 | - | 1.1 | 0.5 | 0.6 | 0.3 | 0.4 |
| 12:0 | 5.0 | 4.7 | 4.3 | 3.5 | 4.1 | 3.0 | 3.7 | 3.4 | 3.9 | 2.5 | - | 2.8 | 4.5 | 4.5 | 2.6 | 3.5 |
| 14:0 | 14.3 | 11.9 | 10.3 | 6.3 | 9.7 | 6.0 | 7.1 | 5.2 | 8.7 | 3.0 | - | 4.8 | 11.8 | 11.5 | 7.3 | 7.1 |
| 14:1 | - | - | 1.0 | 0.4 | 2.6 | 0.8 | 2.8 | 0.8 | 2.1 | - | - | 1.0 | 1.4 | 1.2 | 0.4 | 0.5 |
| 15:0 | 1.4 | 1.4 | 1.3 | 0.4 | 1.0 | 0.6 | 0.7 | 0.9 | 1.0 | 0.7 | - | 0.6 | 1.0 | 1.2 | 0.6 | 0.9 |
| 16:0 | 30.3 | 25.7 | 21.4 | 16.6 | 16.5 | 13.8 | 14.4 | 10.7 | 18.1 | 3.6 | - | 7.5 | 23.6 | 23.2 | 14.0 | 15.2 |
| 16:1 | - | - | 3.1 | 1.5 | 2.6 | 1.6 | 3.7 | 0.6 | 3.0 | 1.3 | - | 0.8 | 2.1 | 1.6 | 0.9 | 0.8 |
| 17:0 | - | - | - | - | - | - | - | - | - | - | - | - | 0.8 | - | 0.6 | - |
| 18:0 | 14.9 | 21.8 | 11.1 | 14.5 | 8.5 | 11.4 | 5.4 | 12.1 | 7.9 | 1.9 | - | 4.6 | 11.1 | 11.2 | 15.3 | 13.3 |
| 18:1 | - | - | 26.5 | 28.0 | 30.7 | 32.4 | 35.1 | 14.3 | 21.1 | 27.3 | - | 11.2 | 15.3 | 15.7 | 15.9 | 16.6 |
| 18:2 | - | - | - | - | - | - | 6.2 | 27.9 | 8.9 | 31.0 | - | 46.8 | 1.0 | 1.5 | 11.6 | 13.1 |
| 18:3 | - | - | - | - | - | - | 1.0 | - | 14.7 | 1.2 | - | 7.5 | 0.8 | 1.3 | 0.7 | 0.8 |
| 20:? | - | - | - | - | - | - | - | - | - | - | - | - | 0.2 | - | 1.0 | - |

[a] Trans - monoene TGs
[b] Cis - monoene TGs
[c] Medium mol. wt. fraction
[d] Results means of two determinations
[e] Control milk fat
[f] 18:2-rich milk fat

fats respectively.

The trans - monoene TGs of the 18:2-rich milk fat contained higher proportions of 18:0 (14.5% compared to 11.1%), and lower proportions of 14:0 (6.3% compared to 10.3%) and 16:0 (16.6% compared to 21.4%). The FA composition of the cis - monoene TGs was similar to that of the trans - monoene TGs, with higher proportions of 18:0 (11.4% compared to 8.5%) and lower proportions of 14:0 (6.0% compared to 9.7%) and 16:0 (13.8% compared to 16.5%) in the 18:2-rich milk fat compared to the control milk fat. 4:0 to 8:0 together comprised 23.8% of the 18:2-rich milk fat and 14.7% of the control milk fat in the trans - monoene TGs and 25.1% of the 18:2-rich milk fat and 17.3% of the control milk fat in the cis - monoene TGs.

The FA compositions of the diene TGs of medium mol. wt. showed considerable differences with the 18:2-rich milk fat having higher proportions of 18:2 (27.9% compared to 6.2%) and correspondingly lower proportions of 18:1 (14.3% compared to 35.1%) than the control milk fat. The other major FA was 16:0 comprising 10.7% and 14.4% of the 18:2-rich and control milk fats respectively.

The triene TGs were also of very different FA compositions with the 18:2-rich milk fat being comprised mostly of 18:1 (27.3%) and 18:2 (31.0%), while the control milk fat was comprised of 16:0 (18.1%), 18:1 (21.1%) and 18:3 (14.7%). 6:0 was present, comprising 12.8% of the 18:2-rich milk fat and only 2.4% of the control milk fat.

In the tetraene TGs, of the 18:2-rich milk fat, the predominant FA was 18:2 (46.8%), along with a smaller amount of 18:1 (11.2%).

(b) Triacylglycerol composition

The TG compositions of the saturated TGs of medium mol. wt. from the two milk fats were quite similar except for slightly higher proportions in the 18:2-rich milk fat of $C_{38}$ (36.7% compared to 33.5%)

and $C_{36}$ (26.1% compared to 17.0%), and lower proportions of $C_{40}$ (20.9% compared to 24.8%) than in the control milk fat (Table 16). These three TG species i.e. $C_{36}$, $C_{38}$ and $C_{40}$ were the only major TGs in the saturated TGs of the medium mol. wt. fractions.

Both the trans and cis - monoene TGs of the 18:2-rich milk fat had higher proportions of $C_{38}$ and $C_{40}$ TGs than the corresponding TG classes of the control milk fat. The respective proportions of the major TGs in the trans - monoene TGs of the 18:2-rich and control milk fats were 23.0% and 19.3% $C_{38}$, 41.8% and 31.7% $C_{40}$ and 20.1% and 20.3% $C_{42}$. In the cis - monoene TGs, the major TGs were $C_{38}$ (23.6%), $C_{40}$ (44.7%) and $C_{42}$ (20.2%) in the 18:2-rich milk fat, and $C_{38}$ (15.0%), $C_{40}$ (32.1%), $C_{42}$ (23.4%) and $C_{44}$ (13.5%) in the control milk fat.

The diene TGs of the medium mol. wt. fraction of the 18:2-rich milk fat had a narrow range of TG species, with $C_{40}$ (34.5%) and $C_{42}$ (36.4%) comprising 70.9% of the total, whereas in the medium mol. wt. fraction of the control milk fat $C_{40}$ (11.5%) and $C_{42}$ (21.1%) made up only 32.6% of the total. The other major diene TGs in the control milk fat fraction were $C_{44}$ (18.2%) and $C_{46}$ (16.7%).

The same trend existed in the triene TGs. In the 18:2-rich milk fat $C_{42}$ (40.2%), $C_{44}$ (19.4%) and $C_{46}$ (17.5%) together comprised 77.1% of the total, but in the control milk fat $C_{42}$ (6.5%), $C_{44}$ (7.8%) and $C_{46}$ (12.7%) made up only 27.0% of the total. The major TGs in the control milk fat comprised $C_{46}$ (12.7%), $C_{48}$ (12.9%), $C_{50}$ (18.3%), $C_{52}$ (16.7%) and $C_{54}$ (12.1%).

The tetraene TGs of the 18:2-rich milk fat were comprised of a wider range of TG species than the corresponding triene TGs. The major TGs were $C_{46}$ (12.9%), $C_{50}$ (11.1%), $C_{52}$ (13.3%) and $C_{54}$ (39.0%).

Table 16. Triacylglycerol composition of the triacylglycerol classes of differing levels of unsaturation in the medium molecular weight fraction of the control and 18:2-rich milk fats

| TG (Carbon No.) | Triacylglycerol composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Fraction[c] | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 30 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 32 | 0.1 | 2.0 | - | - | - | - | - | - | - | - | - | - | 0.1 | - | 0.1 | 0.6 |
| 34 | 3.1 | 8.1 | 0.3 | 0.9 | - | 0.2 | 0.2 | - | 0.8 | - | - | - | 1.1 | 1.6 | 1.6 | 2.4 |
| 36 | 17.0 | 26.1 | 8.3 | 5.0 | 1.2 | 3.6 | 0.8 | 0.2 | 2.3 | - | - | - | 7.7 | 9.2 | 8.3 | 8.6 |
| 38 | 33.5 | 36.7 | 19.3 | 23.0 | 15.0 | 23.6 | 3.4 | 5.1 | 3.5 | - | - | - | 21.6 | 21.5 | 20.8 | 18.9 |
| 40 | 24.8 | 20.9 | 31.7 | 41.8 | 32.1 | 44.7 | 11.5 | 34.5 | 4.3 | 2.9 | - | 1.2 | 24.8 | 23.6 | 28.8 | 28.0 |
| 42 | 12.3 | 5.0 | 20.3 | 20.1 | 23.4 | 20.2 | 21.1 | 36.4 | 6.5 | 40.2 | - | 5.8 | 19.5 | 16.3 | 19.7 | 20.2 |
| 44 | 5.4 | 0.9 | 9.4 | 6.1 | 13.5 | 4.6 | 18.2 | 12.0 | 7.8 | 19.4 | - | 7.6 | 10.4 | 9.5 | 7.4 | 6.9 |
| 46 | 2.3 | 0.3 | 5.1 | 2.9 | 7.4 | 1.9 | 16.7 | 6.3 | 12.7 | 17.5 | - | 12.9 | 6.1 | 6.5 | 4.9 | 4.9 |
| 48 | 1.1 | - | 3.4 | 0.2 | 4.7 | 0.6 | 11.1 | 2.5 | 12.9 | 5.5 | - | 6.8 | 3.5 | 4.4 | 1.8 | 1.8 |
| 50 | 0.4 | - | 2.0 | 0.1 | 2.5 | 0.5 | 10.1 | 1.9 | 18.3 | 6.0 | - | 11.1 | 2.9 | 3.8 | 1.9 | 2.1 |
| 52 | - | - | 0.3 | - | 0.3 | 0.1 | 5.5 | 0.9 | 16.7 | 4.3 | - | 13.3 | 1.8 | 2.2 | 1.6 | 1.8 |
| 54 | - | - | - | - | - | - | 1.5 | 0.3 | 12.1 | 4.2 | - | 39.0 | 0.6 | 1.2 | 3.1 | 3.8 |
| 56 | - | - | - | - | - | - | - | - | 2.0 | - | - | 2.2 | - | - | - | 0.2 |

a Trans - monoene TGs

b Cis - monoene TGs

c Medium mol. wt. fraction

e Control milk fat

f 18:2-rich milk fat

### 3.4.3. Composition of the TG classes of the fraction of low molecular weight in the control and 18:2-rich milk fats

(a) Fatty acid composition

The FA composition of the saturated, trans - monoene and cis - monoene TGs in the two milk fats were similar, with a tendency for the TGs of the 18:2-rich milk fat to have higher levels of 18:0 and lower levels of 16:0 (Table 17).

The respective proportions of 16:0 in the saturated TGs of the 18:2-rich and control milk fats were 23.5% and 28.2% and those of 18:0 were 14.0% and 10.3%, while the other major FAs present were 4:0 (21.6% of the 18:2-rich milk fat and 23.8% of the control milk fat) and 14:0 (18.3% of the 18:2-rich milk fat and 17.4% of the control milk fat).

The respective proportions of the major FAs of the trans - monoene TGs in the 18:2-rich and control milk fats, were 21.9% and 22.4% 4:0, 15.0% and 16.5% 16:0, and, 25.3% and 27.1% 18:1. In the cis - monoene TGs the major FAs in the 18:2-rich milk fat were 4:0 (26.7%), 16:0 (12.4%) and 18:1 (30.1%) and the major FAs in the control milk fat were 4:0 (23.7%), 16:0 (15.6%) and 18:1 (29.4%).

The diene TGs of the low mol. wt. fraction of the 18:2-rich milk fat contained 27.9% 18:2 and 9.3% 18:1, in contrast to the low mol. wt. fraction of the control milk fat where the diene TGs contained 33.7% 18:1 and 7.3% 18:2. There were similar proportions of 4:0 in the diene TGs of the 18:2-rich and control milk fats i.e. 22.7% and 22.4% respectively.

The FA composition of the triene TGs was also different, with the 18:2-rich milk fat containing 24.1% 4:0, 22.7% 18:1 and 32.4% 18:2 compared to 16.0% 4:0, 17.9% 18:1 and 12.7% 18:3 in the control milk fat.

The FAs of the tetraene TGs of the low mol. wt. fraction of the 18:2-rich milk fat consisted mainly of 4:0 (19.2%) and 18:2 (41.5%) i.e.

Table 17. Fatty acid composition of the triacylglycerol classes of differing levels of unsaturation in the low molecular weight fraction of the control and 18:2-rich milk fats [d]

| FA | Fatty acid composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Fraction[c] | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 4:0 | 23.8 | 21.6 | 22.4 | 21.9 | 23.7 | 26.7 | 22.4 | 22.7 | 16.0 | 24.1 | - | 19.2 | 25.0 | 23.0 | 24.3 | 23.0 |
| 6:0 | 6.1 | 6.2 | 5.2 | 5.0 | 5.4 | 4.8 | 5.2 | 5.5 | 8.2 | 4.8 | - | 6.8 | 6.2 | 5.9 | 5.9 | 5.6 |
| 8:0 | 2.4 | 3.2 | 1.5 | 2.5 | 1.5 | 1.7 | 2.1 | 1.5 | 2.3 | 1.3 | - | 1.5 | 2.3 | 2.0 | 2.3 | 2.1 |
| 10:0 | 4.2 | 5.8 | 2.3 | 3.6 | 2.2 | 2.8 | 2.2 | 1.8 | 2.4 | 1.3 | - | 1.4 | 3.6 | 3.2 | 3.7 | 3.3 |
| 10:1 | - | - | - | - | 0.4 | 0.2 | 2.3 | 0.2 | 0.9 | 0.3 | - | 0.5 | 0.5 | 0.4 | 0.3 | 0.2 |
| 12:0 | 6.0 | 6.4 | 3.1 | 3.7 | 2.8 | 3.6 | 2.3 | 2.2 | 2.8 | 1.3 | - | 1.1 | 3.8 | 4.3 | 3.4 | 3.7 |
| 14:0 | 17.4 | 18.3 | 11.4 | 9.9 | 8.8 | 8.1 | 4.5 | 5.6 | 5.4 | 2.4 | - | 2.6 | 11.5 | 12.3 | 9.1 | 9.8 |
| 14:1 | - | - | - | 0.1 | 1.6 | 0.7 | 1.4 | 0.4 | 1.3 | 0.3 | - | 0.3 | 0.9 | 0.7 | 0.6 | 0.3 |
| 15:0 | 1.5 | 1.1 | 0.7 | 0.7 | 0.8 | 0.7 | 0.7 | 0.6 | 0.8 | 0.3 | - | 0.4 | 0.9 | 1.1 | 0.6 | 0.7 |
| 16:0 | 28.2 | 23.5 | 16.5 | 15.0 | 15.6 | 12.4 | 8.4 | 11.6 | 11.4 | 4.1 | - | 5.5 | 20.2 | 20.7 | 13.1 | 14.5 |
| 16:1 | - | - | 3.7 | 2.6 | 3.0 | 2.0 | 4.1 | 1.0 | 3.3 | 1.5 | - | 1.3 | 1.4 | 1.8 | 1.0 | 1.0 |
| 17:0 | - | - | - | - | - | - | - | - | - | - | - | - | 0.4 | - | 0.2 | - |
| 18:0 | 10.3 | 14.0 | 6.2 | 9.7 | 4.7 | 6.2 | 3.3 | 9.7 | 5.4 | 2.2 | - | 3.0 | 7.6 | 7.4 | 8.0 | 9.2 |
| 18:1 | - | - | 27.1 | 25.3 | 29.4 | 30.1 | 33.7 | 9.3 | 17.9 | 22.7 | - | 7.1 | 12.6 | 15.0 | 13.2 | 12.4 |
| 18:2 | - | - | - | - | - | - | 7.3 | 27.9 | 8.5 | 32.4 | - | 41.5 | 1.2 | 1.2 | 13.5 | 13.5 |
| 18:3 | - | - | - | - | - | - | - | - | 12.7 | 0.9 | - | 7.7 | 1.2 | 0.8 | 0.8 | 0.5 |
| 20:? | - | - | - | - | - | - | - | - | 0.8 | - | - | - | 0.8 | 0.1 | - | - |

a Trans - monoene TGs

b Cis - monoene TGs

c Low mol. wt. fraction

d Results means of two determinations

e Control milk fat

f 18:2-rich milk fat

60.7% of the total. Low levels of 18:1 and 18:3 were also present.

(b) Triacylglycerol composition

The TG compositions of the saturated, trans - monoene and cis - monoene TGs of the two low mol. wt. fractions, like the FA compositions, were similar except for slightly lower levels of $C_{36}$ in the saturated TGs of the 18:2-rich milk fat, and lower levels of $C_{38}$ and higher levels of $C_{40}$ in the trans - monoene and cis - monoene TGs of the 18:2-rich milk fat (Table 18).

The proportions of the major TG species in the saturated TGs were 16.7% and 13.9% $C_{32}$, 27.4% and 30.2% $C_{34}$, and 28.8% and 35.2% $C_{36}$, in the 18:2-rich and control milk fats respectively.

In the trans - monoene TGs, the 18:2-rich milk fat contained 28.8% $C_{36}$, 40.0% $C_{38}$ and 19.8% $C_{40}$, and the control milk fat, 27.5% $C_{36}$, 47.0% $C_{38}$ and 11.1% $C_{40}$. The proportions of TGs in the cis - monoene TGs of the 18:2-rich and the control milk fats respectively were 27.1% and 29.4% $C_{36}$, 37.7% and 45.6% $C_{38}$ and 18.0% and 12.0% $C_{40}$.

The diene TGs in the low mol. wt. fraction of the 18:2-rich milk fat consisted mostly of $C_{38}$ (35.2%) and $C_{40}$ (45.5%), while the diene TGs of the low mol. wt. fraction of the control milk fat contained $C_{38}$ (15.4%) and $C_{40}$ (50.1%).

In the triene TGs of the 18:2-rich milk fat, 76.2% of the TGs contained 40 acyl carbons and together with $C_{42}$ (15.4%) comprised 91.6% of the total. In the control milk fat, the same TGs comprised only 54.0% of the total (47.2% $C_{40}$ and 6.8% $C_{42}$), with $C_{38}$ (25.5%) being the other major TG present.

The most abundant TG species in the tetraene TGs of the 18:2-rich milk fat were $C_{40}$ (64.9%) along with $C_{42}$ (20.8%).

Table 18. Triacylglycerol composition of the triacylglycerol classes of differing levels of unsaturation in the low molecular weight fraction of the control and 18:2-rich milk fats

| TG (Carbon No.) | Triacylglycerol composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Fraction[c] | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 26 | - | 0.5 | - | - | - | - | - | - | - | - | - | - | 0.2 | - | 0.2 | 0.2 |
| 28 | 1.8 | 4.3 | - | - | - | - | - | - | - | - | - | - | 1.3 | 0.9 | 1.9 | 1.3 |
| 30 | 5.7 | 9.7 | - | - | 0.4 | 0.7 | - | - | - | - | - | - | 3.4 | 2.9 | 3.9 | 3.2 |
| 32 | 13.9 | 16.7 | 0.4 | 2.2 | 3.2 | 5.4 | 8.2 | 0.8 | 3.3 | - | - | 0.8 | 8.1 | 8.7 | 7.2 | 6.7 |
| 34 | 30.2 | 27.4 | 14.0 | 9.2 | 9.2 | 11.2 | 12.0 | 3.2 | 3.4 | 1.2 | - | 0.8 | 18.8 | 19.8 | 10.9 | 12.3 |
| 36 | 35.2 | 28.8 | 27.5 | 28.8 | 29.4 | 27.1 | 10.5 | 11.6 | 12.5 | 2.1 | - | 2.4 | 28.0 | 29.2 | 18.3 | 19.4 |
| 38 | 12.4 | 11.8 | 47.0 | 40.0 | 45.6 | 37.7 | 15.4 | 35.2 | 25.5 | 4.1 | - | 5.7 | 24.9 | 25.2 | 22.9 | 23.8 |
| 40 | 0.7 | 0.8 | 11.1 | 19.8 | 12.0 | 18.0 | 50.1 | 45.5 | 47.2 | 76.2 | - | 64.9 | 13.3 | 12.3 | 30.5 | 28.9 |
| 42 | - | - | - | - | 0.2 | - | 3.7 | 3.7 | 6.8 | 15.4 | - | 20.8 | 1.8 | 0.9 | 4.1 | 3.9 |
| 44 | - | - | - | - | - | - | - | 0.1 | 1.4 | 1.0 | - | 3.1 | 0.2 | 0.1 | 0.1 | 0.3 |
| 46 | - | - | - | - | - | - | - | - | - | - | - | 1.5 | - | - | - | 0.1 |
| 48 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 50 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 52 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 54 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

a Trans - monoene TGs

b Cis - monoene TGs

c Low mol. wt. fraction

e Control milk fat

f 18:2-rich milk fat

### 3.4.4. Composition of the TG classes of the total milk fat

The total composition of each class of TG was reconstructed from the composition of each class in the fractions of high, medium and low mol. wt., to provide additional information about the composition of the different TG classes and additional evidence for the validity of separating two milk fats of such widely differing composition.

(a) Fatty acid composition

(1) Control milk fat

The major FAs in the saturated TGs of the control milk fat were 4:0 (13.9%), 14:0 (16.8%), 16:0 (31.5%) and 18:0 (15.7%)(Table 19). In the trans - monoene and cis - monoene TGs, the respective proportions of the major FAs were 10.2% and 10.4% 4:0, 11.9% and 10.3% 14:0, 21.3% and 21.1% 16:0, 12.1% and 10.3% 18:0 and, 27.4% and 30.2% 18:1. The most abundant FAs in the diene TGs were 16:0 (14.2%) and 18:1 (42.3%), and in the triene TGs, 16:0 (12.8%), 18:1 (30.4%), 18:2 (11.7%) and 18:3 (10.6%).

(2) 18:2-rich milk fat

The proportions of the most abundant FAs in the saturated TGs of the total 18:2-rich milk fat were 12.2% 4:0, 16.2% 14:0, 25.8% 16:0, and 21.2% 18:0 (Table 19). Respective proportions of the major FAs in the trans - monoene and cis - monoene TGs were: 11.2% and 11.2% 4:0, 17.9% and 16.3% 16:0, 16.1% and 13.2% 18:0 and, 26.3% and 31.9% 18:1. Major FAs in the diene TGs were 16:0 (13.3%), 18:0 (13.5%), 18:1 (19.0%) and 18:2 (23.6%), in the triene TGs, 18:1 (29.1%) and 18:2 (30.4%) and in the tetraene TGs, 18:1 (18.1%) and 18:2 (46.3%). 4:0 to 8:0 together comprised 15.6, 12.5 and 8.6% of the diene, triene and tetraene TGs respectively.

Table 19. Fatty acid composition of the triacylglycerol classes of differing levels of unsaturation in the control and 18:2-rich milk fats [c]

| FA | Fatty acid composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Milk fat | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 4:0 | 13.9 | 12.2 | 10.2 | 11.2 | 10.4 | 11.2 | 7.2 | 9.4 | 5.9 | 6.9 | - | 5.3 | 11.9 | 11.0 | 10.4 | 9.9 |
| 6:0 | 6.0 | 6.9 | 3.7 | 5.5 | 3.8 | 5.3 | 3.0 | 4.1 | 3.5 | 3.6 | - | 2.4 | 5.3 | 4.5 | 5.4 | 4.9 |
| 8:0 | 3.0 | 3.8 | 1.8 | 2.9 | 1.7 | 2.7 | 1.5 | 2.1 | 1.3 | 2.0 | - | 0.9 | 2.3 | 2.2 | 2.1 | 2.6 |
| 10:0 | 5.6 | 6.5 | 3.2 | 4.1 | 3.1 | 4.2 | 2.9 | 3.4 | 2.2 | 3.0 | - | 1.7 | 4.1 | 4.0 | 3.2 | 4.2 |
| 10:1 | - | - | - | - | 0.2 | 0.2 | 1.4 | 0.2 | 0.5 | 0.2 | - | 0.3 | 0.3 | 0.3 | 0.2 | 0.1 |
| 12:0 | 6.0 | 6.3 | 3.6 | 3.5 | 3.3 | 3.7 | 2.7 | 2.8 | 2.3 | 2.0 | - | 1.3 | 3.5 | 4.3 | 2.6 | 3.7 |
| 14:0 | 16.8 | 16.2 | 11.9 | 9.0 | 10.3 | 8.2 | 6.7 | 6.2 | 5.7 | 4.0 | - | 3.3 | 11.9 | 12.3 | 7.7 | 8.8 |
| 14:1 | - | - | 0.6 | 0.2 | 1.8 | 1.0 | 1.8 | 0.6 | 1.6 | 0.4 | - | 0.5 | 1.0 | 0.9 | 0.5 | 0.5 |
| 15:0 | 1.6 | 1.2 | 1.2 | 0.8 | 1.2 | 0.7 | 0.8 | 0.7 | 0.8 | 0.5 | - | 0.4 | 1.0 | 1.3 | 0.7 | 0.8 |
| 16:0 | 31.5 | 25.8 | 21.3 | 17.9 | 21.1 | 16.3 | 14.2 | 13.3 | 12.8 | 7.9 | - | 7.0 | 22.1 | 23.8 | 14.1 | 16.2 |
| 16:1 | - | - | 3.2 | 2.5 | 2.6 | 1.5 | 3.7 | 1.2 | 2.8 | 1.6 | - | 1.1 | 2.0 | 1.8 | 1.1 | 1.1 |
| 17:0 | - | - | - | - | - | - | - | - | - | - | - | - | 0.9 | - | 0.5 | - |
| 18:0 | 15.7 | 21.2 | 12.1 | 16.1 | 10.3 | 13.2 | 7.2 | 13.5 | 7.5 | 7.3 | - | 5.8 | 11.9 | 12.0 | 14.1 | 14.1 |
| 18:1 | - | - | 27.4 | 26.3 | 30.2 | 31.9 | 42.3 | 19.0 | 30.4 | 29.1 | - | 18.1 | 18.6 | 19.3 | 20.6 | 18.3 |
| 18:2 | - | - | - | - | - | - | 4.4 | 23.6 | 11.7 | 30.4 | - | 46.3 | 1.8 | 1.5 | 15.5 | 14.3 |
| 18:3 | - | - | - | - | - | - | 0.2 | - | 10.6 | 1.1 | - | 5.4 | 0.8 | 0.8 | 0.7 | 0.6 |
| 20:? | - | - | - | - | - | - | - | - | 0.2 | - | - | - | 0.5 | - | 0.7 | - |

[a] Trans - monoene TGs

[b] Cis - monoene TGs

[c] Calculated from the FA Compositions of the TG classes of the respective TG fractions

[e] Control milk fat

[f] 18:2-rich milk fat

(3) Comparison of the fatty acid compositions of the triacylglycerol classes of differing levels of unsaturation in the control and 18:2-rich milk fats

In the saturated, and monoene TGs, the FA compositions of the two milk fats were quite similar, except for the tendency of the 18:2-rich milk fat to have slightly higher levels of 18:0 and lower levels of 16:0.

In the two diene TG classes, the proportions of FAs from 4:0 to 16:0 were almost identical, the difference between the two milk fats being in the higher proportions of 18:0 (13.5% compared to 7.2%) and 18:2 (23.6% compared to 4.4%) and the lower proportions of 18:1 (19.0% compared to 42.3%) in the 18:2-rich milk fat (Table 19).

The major differences in the triene TGs were: the lower proportions of 18:3 (1.1% compared to 10.6%) and 16:0 (7.9% compared to 12.8%) and higher proportions of 18:2 (30.4% compared to 11.7%) in the 18:2-rich milk fat than in the control milk fat.

Proceeding from the saturated to the triene or tetraene TGs, in both milk fats, the TGs tended to have lower levels of the shorter chain FAs (such as 4:0), with increasing levels of the 18 carbon unsaturated FAs. In other words, with increasing unsaturation, the average mol. wt. of the FAs in each TG class increased. As shown in Table 19 the proportions of most FAs especially the saturated FAs, were similar in the equivalent TG classes of the two milk fats, but the diene and triene TGs of the 18:2-rich milk fat contained considerably higher proportions of 18:2, which was compensated by a decrease in the proportion of 18:1 in the diene TGs and by a decrease in the proportion of 18:3 in the triene TGs.

In summary, the relative differences between the FA compositions of the respective TG classes of the two total milk fats were similar to

the relative differences between the FA compositions of the respective TG classes from the respective TG fractions of high, medium and low mol. wt..

(b) Triacylglycerol composition

(1) Control milk fat

The saturated TGs of the control milk fat contained 16.1% $C_{34}$, 21.8% $C_{36}$ and 13.9% $C_{38}$ (Table 20). The proportions of the major TGs in the trans - monoene TGs were 13.6% $C_{36}$, 24.1% $C_{38}$, 10.1% $C_{40}$ and 14.1% $C_{50}$; and the cis - monoene TGs contained 12.6% $C_{36}$, 21.6% $C_{38}$, 10.2% $C_{40}$, 10.1% $C_{48}$ and 14.2% $C_{50}$. The diene TGs contained 18.3% $C_{40}$ 12.8% $C_{50}$, 21.1% $C_{52}$ and 10.8% $C_{54}$, and the triene TGs, 10.1% $C_{38}$, 18.3% $C_{40}$, 10.8% $C_{50}$, 15.6% $C_{52}$ and 22.8% $C_{54}$.

(2) 18:2-rich milk fat

In the 18:2-rich milk fat, the most abundant saturated TGs were $C_{34}$ (14.2%), $C_{36}$ (18.6%) and $C_{38}$ (13.6%) (Table 20). The respective proportions of the major TGs in the trans - monoene and cis - monoene TG classes were 12.9% and 10.3% $C_{36}$, 23.5% and 18.5% $C_{38}$, and 21.8% and 16.5% $C_{40}$. The major diene TGs were $C_{38}$ (14.7%), $C_{40}$ (23.5%), $C_{52}$ (11.9%) and $C_{54}$ (11.5%), and the major triene TGs were $C_{40}$ (22.2%), $C_{42}$ (10.5%), $C_{52}$ (17.2%) and $C_{54}$ (26.5%). In the tetraene TGs, the only major TG species present, were $C_{40}$ (18.0%), $C_{52}$ (11.6%) and $C_{54}$ (46.9%), together comprising 76.5% of the total tetraene TGs.

(3) Comparison of the triacylglycerol composition of the triacylglycerol classes of differing levels of unsaturation in the control and 18:2-rich milk fats

The TG compositions of the saturated TGs of the two milk fats were similar, except for slightly lower proportions of $C_{34}$ and $C_{36}$ in the 18:2-rich milk fat (Table 20). In the trans and cis - monoene TGs, the 18:2-rich milk fat tended to have higher proportions of the low mol.

Table 20. Triacylglycerol composition of the triacylglycerol classes of differing levels of unsaturation in the control and 18:2-rich milk fats [c]

| TG (Carbon no.) | Triacylglycerol composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Saturated TGs | | Monoene TGs[a] | | Monoene TGs[b] | | Diene TGs | | Triene TGs | | Tetraene TGs | | Milk fat | | | |
| | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Cont.[e] | 18:2[f] | Control | | 18:2-rich | |
| | | | | | | | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 26 | - | 0.2 | - | - | - | - | - | - | - | - | - | - | - | - | 0.1 | 0.1 |
| 28 | 0.9 | 2.0 | - | - | - | - | - | - | - | - | - | - | 0.7 | 0.4 | 0.6 | 0.5 |
| 30 | 2.9 | 4.4 | - | - | 0.2 | 0.2 | - | - | - | - | - | - | 1.4 | 1.2 | 1.1 | 1.2 |
| 32 | 7.1 | 8.0 | 0.2 | 0.8 | 1.4 | 1.9 | 2.6 | 0.3 | 1.2 | - | - | 0.2 | 3.0 | 3.8 | 2.1 | 2.6 |
| 34 | 16.1 | 14.2 | 6.3 | 3.7 | 3.9 | 4.0 | 3.9 | 1.3 | 1.4 | 0.3 | - | 0.2 | 6.7 | 8.9 | 3.6 | 5.1 |
| 36 | 21.8 | 18.6 | 13.6 | 12.9 | 12.6 | 10.3 | 3.5 | 4.6 | 5.1 | 0.6 | - | 0.7 | 12.3 | 14.7 | 7.5 | 9.0 |
| 38 | 13.9 | 13.6 | 24.1 | 23.5 | 21.6 | 18.5 | 5.6 | 14.7 | 10.1 | 1.2 | - | 1.6 | 14.5 | 15.7 | 11.7 | 12.8 |
| 40 | 6.6 | 8.1 | 10.1 | 21.8 | 10.2 | 16.5 | 18.3 | 23.5 | 18.3 | 22.2 | - | 18.0 | 12.0 | 10.4 | 16.9 | 17.3 |
| 42 | 6.5 | 7.8 | 3.4 | 7.5 | 4.0 | 6.3 | 5.5 | 8.2 | 3.9 | 10.5 | - | 6.7 | 7.9 | 5.2 | 9.0 | 7.8 |
| 44 | 6.4 | 6.4 | 2.9 | 3.4 | 4.0 | 4.6 | 4.7 | 4.2 | 2.2 | 3.8 | - | 2.2 | 6.5 | 4.8 | 5.5 | 4.6 |
| 46 | 6.0 | 5.2 | 4.4 | 3.3 | 5.7 | 5.6 | 5.6 | 5.0 | 3.3 | 4.7 | - | 3.3 | 6.2 | 5.5 | 5.6 | 4.9 |
| 48 | 5.4 | 4.5 | 9.0 | 3.9 | 10.1 | 7.1 | 5.5 | 5.5 | 4.9 | 3.6 | - | 2.3 | 6.9 | 7.1 | 5.1 | 5.0 |
| 50 | 4.0 | 4.3 | 14.1 | 7.1 | 14.2 | 10.6 | 12.8 | 9.4 | 10.8 | 9.4 | - | 5.8 | 8.6 | 9.6 | 7.8 | 7.9 |
| 52 | 1.8 | 2.5 | 9.8 | 7.8 | 9.8 | 9.7 | 21.1 | 11.9 | 15.6 | 17.2 | - | 11.6 | 7.7 | 8.5 | 9.5 | 9.4 |
| 54 | 0.4 | 0.2 | 2.0 | 4.3 | 2.5 | 4.7 | 10.8 | 11.5 | 22.8 | 26.5 | - | 46.9 | 5.6 | 4.2 | 13.9 | 11.9 |
| 56 | - | - | - | - | - | - | - | - | 0.4 | - | - | 0.4 | - | - | - | - |

[a] Trans - monoene TGs

[b] Cis - monoene TGs

[c] Calculated from the TG compositions of the TG classes of the respective TG fractions

[e] Control milk fat

[f] 18:2-rich milk fat

wt. TG, $C_{40}$, and lower proportions of the high mol. wt. TG, $C_{50}$, than the corresponding TG classes in the control milk fat. There were appreciable differences between the TG compositions of the diene TGs of the control and 18:2-rich milk fats, with the 18:2-rich milk containing higher proportions of $C_{38}$ (14.7% compared to 5.6%) and $C_{40}$ (23.5% compared to 18.3%) and lower proportions of $C_{50}$ (9.4% compared to 12.8%) and $C_{52}$ (11.9% compared to 21.1%).

The triene TGs of the 18:2-rich milk fat contained lower proportions of $C_{38}$ (1.2% compared to 10.1%) and higher proportions of $C_{40}$ (22.2% compared to 18.3%) and $C_{42}$ (10.5% compared to 3.9%) than in the triene TGs of the control milk fat. The proportions of $C_{50}$, $C_{52}$ and $C_{54}$ were similar.

The monoene, diene and triene TG classes of the 18:2-rich milk fat generally contained higher proportions of the TGs of lower mol. wt. ($C_{40}$ and $C_{42}$), and, in the case of the monoene and diene TG classes, lower proportions of the TGs of higher mol. wt. ($C_{50}$ and $C_{52}$), suggesting an increase in low mol. wt. TGs. However when the relative contribution of these TG classes to the total milk fats was taken into account, it became evident that because of the increase in size of the diene and triene TG classes and the emergence of a tetraene TG class the level of $C_{50}$ and $C_{52}$ TGs in the total milk fats was the same, and the level of $C_{54}$ TGs in the 18:2-rich milk fat was actually higher than the control milk fat. It would appear that the contribution of the $C_{50}$ and $C_{52}$ TGs of the monoene and diene TGs to the total milk fat was relatively unaltered by feeding high levels of 18:2, but the contribution of $C_{40}$ TGs in the monoene, diene and triene TGs to the total milk fat was much increased. The increased proportions of $C_{54}$ in the 18:2-rich milk fat mainly arose from the presence of the tetraene TGs which were comprised of 47% $C_{54}$ and contributed almost 40%

of the $C_{54}$ in the 18:2-rich milk fat.

In conclusion, the separation of two milk fats of differing composition using the methods described, allowed a valid comparison of the levels and compositions of the TG classes of differing levels of unsaturation. Evidence for this comes from the observation that the same trends that appeared in the respective TG fractions were also evident in the total milk fats. The range of TG species was similar in the equivalent TG classes from the two milk fats, and also, except for the presence of very low mol. wt. TGs in the saturated TGs of both milk fats, the range of TGs ($C_{34}$ to $C_{54}$) was similar in all TG classes.

### 3.4.5. Purity of the triacylglycerols of differing levels of unsaturation in the control and 18:2-rich milk fats

The average number of double bonds per TG molecule in the TG classes of differing levels of unsaturation in the control and 18:2-rich milk fats and their respective fractions is shown in Table 21. In the control and 18:2-rich milk fats the saturated and *cis* - monoene TGs are, within experimental error, pure TG classes. In contrast the *trans* - monoene TGs of all TG fractions tended to have a value slightly lower than 1.00 indicating possible contamination by saturated TGs. The diene and triene TGs of control milk fat fractions had, in most instances, values slightly less than 2.00 and 3.00 respectively indicating some contamination of the diene TGs by *cis* - monoene TGs and some contamination of the triene TGs by diene TGs. On the other hand, the diene TGs of the 18:2-rich milk fat fractions had values close to or above 2.00 indicating some contamination by triene TGs. The triene TGs of 18:2-rich milk fat fractions had values slightly lower than expected. These values experienced for the diene and triene TGs of the 18:2-rich milk fat fractions would perhaps indicate

Table 21. Degree of purity of the triacylglycerols of differing levels of unsaturation in the control and 18:2-rich milk fats

| | Average number of double bonds per TG molecule | |
|---|---|---|
| | Control[a] | 18:2-rich[a] |
| High mol. wt. fraction | | |
| Saturated TGs | 0.00 | 0.00 |
| Trans - monoene TGs | 0.95 | 0.87 |
| Cis - monoene TGs | 1.05 | 1.06 |
| Diene TGs | 1.79 | 2.05 |
| Triene TGs | 3.07 | 2.92 |
| Tetraene TGs | - | 4.07 |
| Medium mol. wt. fraction | | |
| Saturated TGs | 0.00 | 0.00 |
| Trans - monoene TGs | 0.92 | 0.90 |
| Cis - monoene TGs | 1.08 | 1.06 |
| Diene TGs | 1.81 | 2.17 |
| Triene TGs | 2.67 | 2.84 |
| Tetraene TGs | - | 3.91 |
| Low mol. wt. fraction | | |
| Saturated TGs | 0.00 | 0.00 |
| Trans - monoene TGs | 0.92 | 0.84 |
| Cis - monoene TGs | 1.03 | 0.99 |
| Diene TGs | 1.68 | 2.02 |
| Triene TGs | 2.41 | 2.78 |
| Tetraene TGs | - | 3.47 |
| Total milk fat | | |
| Saturated TGs | 0.00 | 0.00 |
| Trans - monoene TGs | 0.94 | 0.87 |
| Cis - monoene TGs | 1.05 | 1.04 |
| Diene TGs | 1.76 | 2.05 |
| Triene TGs | 2.71 | 2.86 |
| Tetraene TGs | - | 3.87 |

[a] Calculated from the respective FA compositions

the lack of a distinct separation of diene and triene TGs. The tetraene TGs of high and medium mol. wt. of the 18:2-rich milk fat had close to four double bonds per TG molecule, but those of low mol. wt. were slightly below this figure indicating in this case some contamination by triene TGs.

## Section 3.5. Stereospecific analysis of triacylglycerols

### 3.5.1. Stereospecific analysis of synthetic triacylglycerols

Racemic and stereospecific synthetic TGs (supplied by R. Norris, D.R.I. (N.Z.) ) were subjected to hydrolysis by pancreatic lipase to check the specificity of this enzyme. The results agreed closely with that expected for the composition of position 2 of these TGs (Table 22).

Table 22. Positional analysis of synthetic triacylglycerols

| | FA Composition (mole %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | TG Composition | | | | 2-MG Composition[c] | | | |
| FA | 4:0 | 16:0 | 18:0 | 18:1 | 4:0 | 16:0 | 18:0 | 18:1 |
| TG | | | | | | | | |
| rac - StPP[ab] | - | 67.1 | 32.9 | - | - | 96.8 | 2.1 | 1.1 |
| rac - POP | - | 66.7 | tr. | 33.3 | - | 2.8 | 0.8 | 96.4 |
| rac - PPO | - | 66.4 | - | 33.6 | - | 96.6 | 1.1 | 2.3 |
| rac - OOSt | - | 33.4 | - | 66.6 | - | 2.5 | 1.2 | 96.3 |
| rac - OStP | - | 34.0 | 33.0 | 33.0 | - | 1.0 | 99.0 | - |
| sn - StStB | 33.7 | - | 66.3 | tr. | - | 0.6 | 99.4 | tr. |
| sn - StStO | - | 0.5 | 66.3 | 33.2 | - | 2.2 | 97.8 | tr. |

a St = stearate

b TGs abbreviated as from Litchfield (1972)

c position 2

The 1,2(2,3)-DGs produced from the hydrolysis of sn - StStB by pancreatic lipase and from the hydrolysis of sn - StStO by a Grignard reagent, were subjected to stereospecific analysis as a check on the method being employed. The FA composition of the 1,2(2,3)-DGs had a FA composition (Table 23) very similar to that calculated from the equation

$$1,2(2,3)\text{-DGs} = \frac{3 \times (\text{TGs}) + (2\text{-MGs})}{4}$$

suggesting that representative DGs were being produced.

Table 23. Composition of 1,2(2,3)-diacylglycerols produced by the hydrolysis of synthetic triacylglycerols

| | | Fatty acid composition (mole %) | | | |
|---|---|---|---|---|---|
| FA | | 4:0 | 16:0 | 18:0 | 18:1 |
| sn - StStO | | | | | |
| 1,2(2,3)-DGs | observed | - | 0.5 | 73.6 | 25.9 |
| | calculated | - | 0.9 | 74.2 | 24.9 |
| sn - StStB | | | | | |
| 1,2(2,3)-DGs | observed | 27.0 | 0.8 | 72.1 | tr. |
| | calculated | 25.3 | 0.2 | 74.6 | tr. |

The FA composition of positions 1, 2 and 3 of sn - StStO and sn - StStB (Table 24) was close to that expected, and provided confirmation of the specificity of the method.

Table 24. Stereospecific analysis of sn - StStO and sn - StStB

| | Fatty acid composition (mole %) | | | |
|---|---|---|---|---|
| | 4:0 | 16:0 | 18:0 | 18:1 |
| sn - StStO | | | | |
| TG | - | 0.5 | 66.3 | 33.2 |
| TG (Recalc)[g] | - | 2.3 | 66.9 | 30.8 |
| position 1[a] | - | 2.0 | 96.6 | 1.4 |
| 2[b] | - | 2.2 | 97.8 | tr. |
| 2[c] | - | 1.9 | 98.1 | tr. |
| 3[d] | - | -2.7 | 4.5 | 98.2 |
| 3[e] | - | 2.6 | 6.4 | 91.0 |
| 3[f] | - | 0.0(±2.7)[h] | 5.5(±1.0)[h] | 94.6(±3.6)[h] |
| sn - StStB | | | | |
| TG | 33.7 | - | 66.3 | tr. |
| TG (Recalc)[g] | 31.0 | 1.6 | 66.4 | 0.9 |
| position 1[a] | 5.9 | 1.5 | 92.5 | tr. |
| 2[b] | - | 0.6 | 99.4 | tr. |
| 3[d] | 95.2 | -2.1 | 7.0 | tr. |
| 3[e] | 87.2 | 2.8 | 7.4 | 2.6 |
| 3[f] | 91.2(±4.0)[h] | 0.4(±2.5)[h] | 7.2(±0.2)[h] | 1.3(±1.3)[h] |

a 1-PLs

b 2-MGs

c FA released by phospholipase A

d 3 x (TGs) - (1-PLs) - (2-MGs)

e 2 x (2,3-PLs) - (2-MGs)

f Average of two calculations for position 3.

g 2 x (2,3-PLs) + (1-PLs) /3

h Difference between position 3 by the two methods of calculation.

3.5.2. Stereospecific analysis of the triacylglycerol fractions of high, medium and low molecular weight of the control and 18:2-rich milk fats

The results of the stereospecific analyses of the milk fat fractions of the two milk fats are given in Tables 26-31 and Figures 8-9.

For the stereospecific analysis of TGs to be quantitative, the FAs of the 1,2(2,3)-DGs formed as intermediates must be representative of the FAs in the original TG. The FA composition of the 1,2(2,3)-DGs prepared from the fractions of high mol. wt. by chemical hydrolysis with a Grignard reagent gave results consistent with those determined for the 1,2(2,3)-DGs by calculation from the TG and 2-MG compositions (Table 25). The FA compositions of the 1,2(2,3)-DGs obtained from the TGs of the low and medium mol. wt. fractions by hydrolysis with pancreatic lipase were not as close to the calculated compositions but were still acceptable. Deviations of up to 2% were present between the proportions of major components (>10% of the total) with the exception of 4:0 in the low mol. wt. fractions and 6:0 in the medium mol. wt. fractions. Values for 4:0 obtained by experiment were 20 - 21% higher in the fractions of low mol. wt. and values for 6:0 were 17 - 24% higher in the fractions of medium mol. wt., than those determined by calculation.

Only those 1,2(2,3)-DGs that were within the limits set out above for the various TG fractions were used in the subsequent steps of a stereospecific analysis.

When duplicate analyses were carried out the FA composition of 2-MGs, 1-PLs and 2,3-PLs agreed within 3% (absolute) for the major FAs (>10% of total).

The accuracy of a stereospecific analysis can be determined from the comparison of the FA composition of position 3 obtained by two

Table 25. Fatty acid composition of the diacylglycerol intermediates in the stereospecific analysis of the triacylglycerol fractions of the control and 18:2-rich milk fats

| FA | Fatty acid composition (mole %) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | High mol. wt. fraction | | | | | | | | Medium mol. wt. fraction | | | | Low mol. wt. fraction | | | |
| | 1,2(2,3)-DGs | | | | 1,3-DGs | | | | 1,2(2,3)-DGs | | | | 1,2(2,3)-DGs | | | |
| | Control | | 18:2-rich | | Control | | 18:2-rich | | Control | | 18:2-rich | | Control | | 18:2-rich | |
| | Exp.[a] | Calc.[b] | Exp.[a] | Calc.[b] | Exp.[a] | Calc.[c] | Exp.[a] | Calc.[c] | Exp.[a] | Calc.[b] | Exp.[a] | Calc.[b] | Exp.[a] | Calc.[b] | Exp.[a] | Calc.[b] |
| 4:0 | - | - | - | - | - | - | 0.1 | - | 4.6 | 4.0 | 7.0 | 6.1 | 22.5 | 18.8 | 22.0 | 18.2 |
| 6:0 | 0.2 | 0.3 | 0.9 | 0.8 | 0.3 | 0.6 | 0.4 | 1.7 | 10.0 | 8.3 | 11.9 | 9.6 | 5.9 | 5.5 | 4.6 | 5.8 |
| 8:0 | 1.4 | 1.0 | 1.8 | 1.5 | 0.8 | 1.3 | 1.1 | 1.5 | 3.2 | 4.0 | 3.3 | 4.0 | 2.4 | 3.1 | 2.3 | 3.3 |
| 10:0 | 4.3 | 3.6 | 3.8 | 3.8 | 3.3 | 4.2 | 2.8 | 2.9 | 5.1 | 6.0 | 4.7 | 5.0 | 4.5 | 4.9 | 3.9 | 5.1 |
| 10:1 | - | - | 0.1 | - | 0.6 | - | 0.1 | - | 0.4 | 0.4 | 0.2 | 0.3 | 0.7 | 0.6 | 0.2 | 0.4 |
| 12:0 | 4.3 | 4.4 | 3.6 | 4.0 | 3.0 | 3.0 | 2.6 | 1.7 | 4.9 | 5.2 | 3.6 | 3.3 | 5.2 | 5.1 | 4.2 | 4.4 |
| 14:0 | 16.1 | 16.1 | 10.4 | 10.7 | 9.2 | 7.8 | 6.1 | 5.0 | 13.5 | 14.5 | 8.8 | 9.0 | 16.1 | 14.2 | 9.5 | 10.5 |
| 14:1 | 1.3 | 1.0 | 1.1 | 0.5 | 2.3 | 0.6 | 0.3 | 0.5 | 0.8 | 1.3 | 0.8 | 0.5 | 1.0 | 1.0 | 0.4 | 0.7 |
| 15:0 | 1.6 | 1.3 | 1.0 | 0.7 | 1.1 | 1.0 | 0.8 | 0.7 | 1.1 | 1.1 | 0.7 | 0.7 | 1.0 | 1.2 | 0.7 | 0.7 |
| 16:0 | 28.0 | 29.7 | 17.7 | 18.0 | 22.0 | 22.5 | 14.6 | 12.7 | 26.2 | 25.7 | 15.3 | 15.6 | 21.0 | 21.5 | 13.6 | 13.3 |
| 16:1 | 2.0 | 2.0 | 1.7 | 1.5 | 1.6 | 1.7 | 1.6 | 1.3 | 2.7 | 2.0 | 1.5 | 1.0 | 1.3 | 1.5 | 1.5 | 1.1 |
| 17:0 | 1.3 | 0.8 | 0.8 | 0.6 | 2.7 | 1.1 | 2.5 | 0.9 | 0.8 | 0.7 | 0.5 | 0.6 | 0.1 | 0.4 | 0.5 | 0.2 |
| 18:0 | 14.2 | 14.1 | 16.0 | 16.4 | 19.8 | 21.0 | 22.1 | 23.0 | 10.6 | 10.1 | 13.8 | 14.4 | 5.9 | 6.9 | 8.3 | 7.5 |
| 18:1 | 23.3 | 23.5 | 23.8 | 24.5 | 31.2 | 32.5 | 29.0 | 30.6 | 14.3 | 14.7 | 16.5 | 16.3 | 10.3 | 12.4 | 13.4 | 13.3 |
| 18:2 | 1.3 | 1.6 | 16.0 | 16.1 | 1.6 | 2.0 | 15.7 | 16.2 | 1.0 | 1.1 | 10.5 | 12.0 | 1.0 | 1.2 | 14.4 | 14.8 |
| 18:3 | 0.7 | 0.7 | 1.4 | 1.0 | 0.6 | 0.8 | 0.2 | 1.3 | 0.7 | 0.8 | 0.5 | 0.7 | 0.7 | 1.1 | 0.6 | 0.9 |
| 20:? | - | - | - | - | - | - | - | - | 0.2 | 0.2 | 0.5 | 0.8 | 0.3 | 0.7 | - | - |

[a] obtained experimentally [b] 3 x (TGs) + (2-MGs) /4 [c] 3 x (TGs) - (2-MGs) /2

methods of calculation (Section 2.5) (Tables 26 to 31; Appendices 3 to 8). Differences between the two results were greatest in the low and medium mol. wt. fractions where errors of up to 6% (absolute) were evident for major components (>10% of total), and lowest in the high mol. wt. fractions where the maximum difference between the percentages for any major FA was 4.6%. The average difference between the percentages of major FAs (>10% of total) was 2.5 - 2.6%. Negative values (up to 2% absolute) were obtained in position 3 of the low mol. wt. fractions of the two milk fats where 4:0 and 6:0 provided over 80% of the FAs present.

A further check on the accuracy of results is possible by recalculating the FA composition of the TGs using the calculation

$$TGs = [2 \times (2,3\text{-PLs}) + (1\text{-PLs})]/3$$

The results for all analyses agreed within 2% (absolute) (Appendices 3 to 8).

3.5.2.1. Stereospecific analysis of the triacylglycerols in the fraction of high molecular weight of the control and 18:2-rich milk fats

In the high mol. wt. fraction of the control milk fat, position 1 contained, as the predominant fatty acids, 29.1% 16:0, 18.9% 18:0 and 27.2% 18:1, whereas position 1 of the 18:2-rich milk fat contained 21.8% 16:0, 22.0% 18:0, 25.7% 18:1 and 11.7% 18:2 (Table 26). Thus the major differences between the two milk fats in position 1, were the lower proportion of 16:0 and the higher proportion of 18:2 in position 1 of the 18:2-rich milk fat, than in position 1 of the control milk fat. Levels of 18:0 were slightly higher but the other FAs were virtually the same (Figures 8 and 9).

Position 2 of the high mol. wt. fraction of the control milk fat

Table 26. Stereospecific analysis of the triacylglycerols in the fraction of high molecular weight of the control and 18:2-rich milk fats [b]

| FA | Fatty acid composition (mole %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[ac] | | Fraction[d] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | - | - | - ( - ) | - ( - ) | - | - |
| 6:0 | - | - | - | - | 1.7 (± 0.5)[e] | 3.0 (± 0.3) | 0.4 | 1.1 |
| 8:0 | 1.7 | 0.3 | 0.6 | 1.6 | 1.8 (± 0.8) | 2.1 (± 0.5) | 1.1 | 1.5 |
| 10:0 | 3.6 | 1.3 | 3.0 | 4.7 | 6.2 (± 1.4) | 3.1 (± 1.4) | 3.8 | 3.5 |
| 10:1 | - | 0.1 | - | - | - ( - ) | 0.0 (± 0.2) | - | - |
| 12:0 | 4.0 | 2.5 | 5.9 | 6.2 | 3.4 (± 1.3) | 1.3 (± 0.4) | 4.0 | 3.2 |
| 14:0 | 8.2 | 8.0 | 24.4 | 16.4 | 6.8 (± 0.6) | 2.4 (± 0.4) | 13.3 | 8.0 |
| 14:1 | 0.4 | 0.4 | 1.0 | 0.2 | 1.8 (± 1.2) | 0.8 (± 0.4) | 0.7 | 0.6 |
| 15:0 | 1.9 | 1.3 | 1.9 | 0.6 | 0.9 (± 0.8) | 1.1 (± 0.9) | 1.3 | 0.7 |
| 16:0 | 29.1 | 21.8 | 36.9 | 23.3 | 15.7 (± 0.2) | 5.3 (± 1.8) | 27.3 | 16.2 |
| 16:1 | 1.7 | 2.4 | 2.3 | 1.7 | 1.3 (± 0.4) | 0.8 (± 0.7) | 1.9 | 1.4 |
| 17:0 | 0.6 | 1.5 | 0.6 | 0.3 | 1.0 (± 0.5) | 0.8 (± 0.5) | 0.9 | 0.7 |
| 18:0 | 18.9 | 22.0 | 7.2 | 9.8 | 20.9 (± 2.3) | 22.5 (± 1.5) | 16.4 | 18.6 |
| 18:1 | 27.2 | 25.7 | 14.4 | 18.4 | 36.1 (± 1.9) | 34.5 (± 0.9) | 26.5 | 26.5 |
| 18:2 | 1.5 | 11.7 | 1.2 | 16.0 | 1.6 (± 0.8) | 19.6 (± 1.0) | 1.7 | 16.1 |
| 18:3 | 1.1 | 1.1 | 0.6 | 0.8 | 0.9 (± 0.5) | 2.6 (± 1.2) | 0.7 | 1.1 |
| 20:? | - | - | - | - | - ( - ) | - ( - ) | - | - |

[a] Positions relative to sn - glycerol-3-phosphate

[b] Complete results given in Appendices 3 and 4

[c] Average for FA composition of position 3 from two methods of calculation

[d] High mol. wt. fraction

( )[e] Difference between position 3 by the two methods of calculation

was occupied by 24.4% 14:0, 36.9% 16:0, 14.4% 18:1 compared to 16.4% 14:0, 23.3% 16:0, 18.4% 18:1 and 16.0% 18:2 in the high mol. wt. fraction of the 18:2-rich milk fat. Thus the proportions of 14:0 and 16:0 were lower in position 2 of the 18:2-rich milk fat than in position 2 of the control milk fat, and the proportions of 18:0, 18:1 and 18:2 were higher.

The major FAs in position 3 of the control milk fat were 16:0 (15.7%), 18:0 (20.9%) and 18:1 (36.1%), whereas in the 18:2-rich milk fat, the most abundant FAs were 18:0 (22.5%), 18:1 (34.5%) and 18:2 (19.6%). The proportions of 16:0, in position 3, were somewhat lower in the 18:2-rich milk fat (5.3% compared to 15.7%) with correspondingly higher proportions of 18:2 (19.6% compared to 1.6%).

When the proportional distribution of the FAs was calculated (Table 27), 62% and 61% of 14:0 was found to be in position 2 of the high mol. wt. fraction of the control and 18:2-rich milk fats respectively. 16:0 was concentrated in position 2 (45% of the 16:0) more than in position 1 (36% of the 16:0) of the control milk fat, but in the 18:2-rich milk fat, 16:0 was evenly distributed over positions 1 and 2 with 90% of the total 16:0 being in these two positions. 18:0 was preferentially esterified in position 1 (40 - 41% of the 18:0) and position 3 (41 - 45% of the 18:0) in both milk fats. The distribution of 18:1 was also very similar in the two milk fats with 35% and 33% of the 18:1 in position 1, 19% and 23% of the 18:1 in position 2 and 47% and 44% of the 18:1 in position 3 of the control and 18:2-rich milk fats respectively. 18:2 was concentrated at positions 1 and 3 of the control milk fat, and at positions 2 and 3 of the 18:2-rich milk fat with the distribution being quite similar to that of 18:1. In the 18:2-rich milk fat, 25% of the 18:2 was in position 1, 34% in position 2 and 42% in position 3.

Table 27. Proportional distribution of fatty acids in the triacylglycerols of the fraction of high molecular weight of the control and 18:2-rich milk fats

| FA | Fatty acid distribution (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[a] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | - | - | - | - |
| 6:0 | - | - | - | - | 100.0 | 100.0 |
| 8:0 | 41.5 | 7.5 | 14.6 | 40.0 | 43.9 | 52.5 |
| 10:0 | 28.1 | 14.3 | 23.4 | 51.6 | 48.4 | 34.1 |
| 10:1 | - | 100.0 | - | - | - | - |
| 12:0 | 30.1 | 25.0 | 44.4 | 62.0 | 25.5 | 13.0 |
| 14:0 | 20.8 | 29.9 | 61.9 | 61.2 | 17.3 | 8.9 |
| 14:1 | 12.5 | 28.6 | 31.3 | 14.3 | 56.2 | 57.1 |
| 15:0 | 40.4 | 43.3 | 40.4 | 20.0 | 19.1 | 36.7 |
| 16:0 | 35.6 | 43.3 | 45.2 | 46.2 | 19.2 | 10.5 |
| 16:1 | 32.1 | 49.0 | 43.4 | 34.7 | 24.5 | 16.3 |
| 17:0 | 27.3 | 57.7 | 27.3 | 11.5 | 45.4 | 30.8 |
| 18:0 | 40.2 | 40.5 | 15.3 | 18.1 | 44.5 | 41.4 |
| 18:1 | 35.0 | 32.7 | 18.5 | 23.4 | 46.5 | 43.9 |
| 18:2 | 34.9 | 24.7 | 27.9 | 33.8 | 37.2 | 41.5 |
| 18:3 | 42.3 | 24.4 | 23.1 | 17.8 | 34.6 | 57.8 |
| 20:? | - | - | - | - | - | - |

[a] Positions relative to sn - glycerol-3-phosphate

3.5.2.2. Stereospecific analysis of the triacylglycerols in the fraction of medium molecular weight of the control and 18:2-rich milk fats

The major fatty acids in position 1 of the medium mol. wt. fraction of the control milk fat were 16:0 (32.3%), 18:0 (19.8%) and 18:1 (25.7%), whereas 16:0 (19.5%), 18:0 (28.5%), 18:1 (21.4%) and 18:2 (13.4%) were in position 1 of the 18:2-rich milk fat (Table 28). There were much lower proportions of 16:0 in position 1 of the 18:2-rich milk fat than in the control milk fat (19.5% compared to 32.3%), and proportions of 18:2 were higher (13.4% compared to 2.1%). Introduction of 18:2 into position 1 was also accompanied by increased proportions of 18:0 and decreased proportions of 18:1 when compared to the control milk fat (Table 28) (Figures 8 and 9).

In position 2 of the control and 18:2-rich milk fats respectively, the proportions of the major FAs were: 22.6% and 14.3% 14:0, 32.1% and 20.4% 16:0, 12.7% and 17.5% 18:1, with 13.1% 18:2 in the 18:2-rich milk fat. There were lower proportions of 14:0 and 16:0, and higher proportions of 18:0, 18:1 and 18:2 in position 2 of the medium mol. wt. fraction of the 18:2-rich milk fat compared to the medium mol. wt. fraction of the control milk fat.

The major FAs in position 3 of both milk fats were 4:0 and 6:0, with 17.5% and 24.4% 4:0 in the control and 18:2-rich milk fats respectively and 29.8% and 35.8% 6:0 in the two milk fat fractions respectively.

All of the 4:0 and almost all of the 6:0 were confined to position 3 of the medium mol. wt. fraction of both milk fats (Table 29). 8:0 and 10:0 were preferentially esterified in both positions 2 and 3 of the two milk fats. The distribution of 14:0 was almost identical in the two milk fat fractions, with a similar preference for position

Table 28. Stereospecific analysis of the triacylglycerols in the fraction of medium molecular weight of the control and 18:2-rich milk fats [b]

| FA | Fatty acid composition (mole %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[ac] | | Fraction[d] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | - | - | 17.5 (± 1.5)[e] | 24.4 (± 0.2) | 5.3 | 8.2 |
| 6:0 | - | - | 1.8 | 1.4 | 29.8 (± 0.4) | 35.8 (± 0.3) | 10.4 | 12.3 |
| 8:0 | 0.7 | 0.4 | 3.0 | 4.8 | 8.1 (± 1.1) | 4.9 (± 1.3) | 4.3 | 3.8 |
| 10:0 | 0.9 | 1.9 | 6.1 | 6.7 | 8.0 (± 2.7) | 3.9 (± 1.0) | 5.9 | 4.5 |
| 10:1 | - | - | 0.3 | 0.3 | 0.7 (± 0.5) | 0.3 (± 0.3) | 0.5 | 0.3 |
| 12:0 | 2.9 | 2.8 | 7.3 | 5.3 | 2.8 (± 0.5) | 0.6 (± 0.9) | 4.5 | 2.6 |
| 14:0 | 9.8 | 7.6 | 22.6 | 14.3 | 2.4 (± 0.6) | 0.8 (± 0.8) | 11.8 | 7.3 |
| 14:1 | - | - | 1.2 | 0.7 | 1.9 (± 1.1) | 0.3 (± 0.2) | 1.4 | 0.4 |
| 15:0 | 0.4 | 1.0 | 1.6 | 0.9 | 0.7 (± 0.3) | 0.2 (± 0.3) | 1.0 | 0.6 |
| 16:0 | 32.3 | 19.5 | 32.1 | 20.4 | 8.3 (± 1.9) | 5.4 (± 3.3) | 23.6 | 14.0 |
| 16:1 | 1.5 | 1.6 | 1.6 | 1.3 | 2.8 (± 0.4) | 0.2 (± 0.4) | 2.1 | 0.9 |
| 17:0 | 0.3 | 0.9 | 0.5 | 0.5 | 1.0 (± 0.6) | 0.3 (± 0.1) | 0.8 | 0.6 |
| 18:0 | 19.8 | 28.5 | 7.2 | 11.6 | 8.0 (± 1.7) | 7.0 (± 1.2) | 11.1 | 15.3 |
| 18:1 | 25.7 | 21.4 | 12.7 | 17.5 | 7.9 (± 0.4) | 8.5 (± 0.4) | 15.3 | 15.9 |
| 18:2 | 2.1 | 13.4 | 1.3 | 13.1 | 0.3 (± 0.8) | 6.1 (± 2.2) | 1.0 | 11.6 |
| 18:3 | 1.9 | 0.8 | 0.7 | 0.9 | 0.2 (± 0.5) | 0.2 (± 0.2) | 0.8 | 0.7 |
| 20:? | 1.6 | 0.2 | - | 0.3 | -0.5 (± 0.5) | 1.2 (± 1.3) | 0.2 | 1.0 |

[a] Positions relative to sn - glycerol-3-phosphate

[b] Complete data given in Appendices 5 and 6

[c] Average for FA composition of position 3 from two methods of calculation

[d] Medium mol. wt. fraction

( )[e] Difference between position 3 by the two methods of calculation

Table 29. Proportional distribution of fatty acids in the triacylglycerols of the fraction of medium molecular weight of the control and 18:2-rich milk fats

| FA | Fatty acid distribution (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[a] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | - | - | 100.0 | 100.0 |
| 6:0 | - | - | 5.7 | 3.8 | 94.3 | 96.2 |
| 8:0 | 5.9 | 4.0 | 25.4 | 47.5 | 68.7 | 48.5 |
| 10:0 | 6.0 | 15.2 | 40.7 | 53.6 | 53.3 | 31.2 |
| 10:1 | - | - | 30.0 | 50.0 | 70.0 | 50.0 |
| 12:0 | 22.3 | 32.2 | 56.2 | 60.9 | 21.5 | 6.9 |
| 14:0 | 28.2 | 33.5 | 64.9 | 63.0 | 6.9 | 3.5 |
| 14:1 | - | - | 38.7 | 70.0 | 61.3 | 30.0 |
| 15:0 | 14.8 | 47.6 | 59.3 | 42.9 | 25.9 | 9.5 |
| 16:0 | 44.4 | 43.1 | 44.2 | 45.0 | 11.4 | 11.9 |
| 16:1 | 25.4 | 51.6 | 27.1 | 41.9 | 47.5 | 6.5 |
| 17:0 | 16.7 | 52.9 | 27.8 | 29.4 | 55.5 | 17.7 |
| 18:0 | 56.6 | 60.5 | 20.6 | 24.6 | 22.8 | 14.9 |
| 18:1 | 55.5 | 45.2 | 27.4 | 36.9 | 17.1 | 17.9 |
| 18:2 | 56.8 | 41.1 | 35.1 | 40.2 | 8.1 | 18.7 |
| 18:3 | 67.9 | 42.1 | 25.0 | 47.4 | 7.1 | 10.5 |
| 20:? | 100.0 | 11.8 | - | 17.6 | - | 70.6 |

[a] Positions relative to sn - glycerol-3-phosphate

2 where 63 - 65% of the 14:0 was positioned in both milk fats. 16:0 was identically distributed in the two milk fats with 43 - 44% in position 1, 44 - 45% in position 2 and only a small amount in position 3. The distribution of 18:0 in the two milk fats was similar, with 57 - 61% of the 18:0 in position 1 and the remainder spread over positions 2 and 3. On the other hand, 18:1 was differently distributed in the two milk fats with 56% and 27% in positions 1 and 2 respectively in the control milk fat and 45% and 37% in positions 1 and 2 respectively in the 18:2-rich milk fat. 18:2, in the 18:2-rich milk fat, was preferentially esterified in position 1 (41% of the 18:2) and position 2 (40% of the 18:2), with a very similar distribution to that of 18:1 in this milk fat fraction.

3.5.2.3. Stereospecific analysis of the triacylglycerols in the fraction of low molecular weight of the control and 18:2-rich milk fats

The major FAs in position 1 of the low mol. wt. fraction, were: in the control milk fat 16:0 (34.4%), 18:0 (19.8%) and 18:1 (23.5%), and in the 18:2-rich milk fat, 16:0 (23.3%), 18:0 (18.9%), 18:1 (19.6%) and 18:2 (15.5%) (Table 30). The proportions of 16:0 were much lower (23.3% compared to 34.4%) in position 1 of the 18:2-rich milk fat than in the control milk fat, and the proportions of 18:2 were higher (15.5% compared to 2.1%). A small decrease was present in the proportions of 18:1 in position 1 of the low mol. wt. fraction of the 18:2-rich milk fat relative to the proportions in position 1 of the low mol. wt. fraction of the control milk fat (Figures 8 and 9).

In position 2, the major FAs in the low mol. wt. fraction of the control milk fat were 14:0 (22.3%), 16:0 (25.5%) and 18:1 (11.8%). In the 18:2-rich milk fat, the major FAs in position 2 were 14:0 (14.5%),

Table 30. Stereospecific analysis of the triacylglycerols in the fraction of low molecular weight of the control and 18:2-rich milk fats [b]

| FA | Fatty acid composition (mole %) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[ac] | | Fraction[d] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | 0.1 | - | 74.4 (± 0.7)[e] | 72.0 (± 1.0)[e] | 25.0 | 24.3 |
| 6:0 | - | - | 3.2 | 5.6 | 15.2 (± 0.2) | 10.6 (± 1.6) | 6.2 | 5.9 |
| 8:0 | 0.4 | 1.5 | 5.6 | 6.3 | 0.0 (± 0.9) | -1.5 (± 0.6) | 2.3 | 2.3 |
| 10:0 | 1.2 | 2.6 | 8.9 | 9.2 | 0.0 (± 0.7) | -1.1 (± 0.5) | 3.6 | 3.7 |
| 10:1 | 0.1 | 0.1 | 0.8 | 0.6 | 0.3 (± 0.3) | 0.0 (± 0.2) | 0.5 | 0.3 |
| 12:0 | 2.9 | 3.4 | 9.0 | 7.5 | 0.1 (± 0.7) | 0.1 (± 0.8) | 3.8 | 3.4 |
| 14:0 | 10.3 | 8.7 | 22.3 | 14.5 | 2.3 (± 0.4) | 3.7 (± 0.4) | 11.5 | 9.1 |
| 14:1 | 0.2 | 0.3 | 1.4 | 0.8 | 0.6 (± 0.6) | 0.3 (± 0.4) | 0.9 | 0.6 |
| 15:0 | 1.7 | 2.1 | 2.2 | 1.0 | -0.8 (± 0.4) | -0.4 (± 1.0) | 0.9 | 0.6 |
| 16:0 | 34.4 | 23.3 | 25.5 | 13.7 | 3.6 (± 2.9) | 4.9 (± 2.6) | 20.2 | 13.1 |
| 16:1 | 1.6 | 2.1 | 1.6 | 1.5 | 1.2 (± 0.2) | 0.3 (± 1.0) | 1.4 | 1.0 |
| 17:0 | 0.7 | 0.6 | 0.3 | 0.3 | 0.2 (± 0.1) | 0.1 (± 0.4) | 0.4 | 0.2 |
| 18:0 | 19.8 | 18.9 | 4.8 | 5.8 | 0.1 (± 1.9) | 1.4 (± 2.2) | 7.6 | 8.0 |
| 18:1 | 23.5 | 19.6 | 11.8 | 13.7 | 0.8 (± 1.8) | 5.1 (± 1.2) | 12.6 | 13.2 |
| 18:2 | 2.1 | 15.5 | 1.1 | 18.5 | 0.6 (± 0.2) | 4.5 (± 2.0) | 1.2 | 13.5 |
| 18:3 | 0.8 | 1.3 | 0.9 | 1.0 | 0.9 (± 0.6) | 0.0 (± 0.2) | 1.2 | 0.8 |
| 20:? | 0.4 | - | 0.5 | - | 0.5 (± 1.0) | - ( - ) | 0.8 | - |

[a] Positions relative to sn - glycerol-3-phosphate

[b] For complete data see Appendices 7 and 8

[c] Results are the average of FA composition for position 3 from two methods of calculation

[d] Low mol. wt. fraction

( )[e] Difference between position 3 by the two methods of calculation

16:0 (13.7%), 18:1 (13.7%) and 18:2 (18.5%). There were lower proportions of 14:0 (14.5% compared to 22.3%) and 16:0 (13.7% compared to 25.5%) in position 2 of the low mol. wt. fraction of the 18:2-rich milk fat, with higher proportions of 18:2 (18.5% compared to 1.1%) than in position 2 of the corresponding fraction in the control milk fat.

The proportions of 4:0 in position 3 of the control and 18:2-rich milk fats respectively were 74.4% and 72.0%, along with 15.2% 6:0 in the control milk fat and 10.6% 6:0 in the 18:2-rich milk fat. With 4:0 and 6:0 together comprising 89.6% and 82.6% of the FAs in position 3 of the low mol. wt. fraction of the control and 18:2-rich milk fats respectively, only minor amounts of other FAs appeared in position 3.

4:0 was confined almost exclusively to position 3 in the low mol. wt. fractions of the two milk fats, except for 0.1% 4:0 in position 2 of the control milk fat (Table 31). The majority of 6:0 also was esterified at position 3, with 83% and 65% of the 6:0 being esterified in that position in the control and 18:2-rich milk fats respectively. Fatty acids, in the range 8:0 to 14:0, were preferentially esterified at position 2 e.g. 64% and 54% of 14:0 was esterified in position 2 of the control and 18:2-rich milk fats respectively. 16:0 was concentrated in position 1 (54% of the 16:0) and position 2(40% of the 16:0) of the control milk fat and similarly in 18:2-rich milk fat with 56% of the 16:0 in position 1, and 33% of the 16:0 in position 2. The distribution of 18:0 was similar in the two milk fats, with about 75% of the 18:0 in position 1 and about 20% in position 2. 18:1 was esterified preferentially in position 1, with 65% and 51% of the 18:1 in this position in the control and 18:2-rich milk fats respectively. The lower level of 18:1 in position 1 of the 18:2-rich milk fat was compensated by the 13% of the 18:1 in position 3. While a small amount of 18:2 was esterified in position 3, of the low mol. wt.

Table 31. Proportional distribution of fatty acids in the triacylglycerols of the fraction of low molecular weight of the control and 18:2-rich milk fats

| FA | Fatty acid distribution (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[a] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | 0.1 | - | 99.9 | 100.0 |
| 6:0 | - | - | 17.4 | 34.6 | 82.6 | 65.4 |
| 8:0 | 6.7 | 19.2 | 93.3 | 80.8 | - | - |
| 10:0 | 11.9 | 22.0 | 88.1 | 78.0 | - | - |
| 10:1 | 8.3 | 14.3 | 66.7 | 85.7 | 25.0 | - |
| 12:0 | 24.2 | 30.9 | 75.0 | 68.2 | 0.8 | 0.9 |
| 14:0 | 29.5 | 32.3 | 63.9 | 53.9 | 6.6 | 13.8 |
| 14:1 | 9.1 | 21.4 | 63.6 | 57.1 | 27.3 | 21.4 |
| 15:0 | 43.6 | 67.7 | 56.4 | 32.3 | - | - |
| 16:0 | 54.2 | 55.6 | 40.2 | 32.7 | 5.7 | 11.7 |
| 16:1 | 36.3 | 53.8 | 36.3 | 38.5 | 27.4 | 7.7 |
| 17:0 | 58.3 | 60.0 | 25.0 | 30.0 | 16.7 | 10.0 |
| 18:0 | 80.2 | 72.4 | 19.4 | 22.2 | 0.4 | 5.4 |
| 18:1 | 65.1 | 51.0 | 32.7 | 35.7 | 2.2 | 13.3 |
| 18:2 | 55.3 | 40.3 | 28.9 | 48.1 | 15.8 | 11.7 |
| 18:3 | 30.8 | 56.5 | 34.6 | 43.5 | 34.6 | - |
| 20:? | 28.6 | - | 35.7 | - | 35.7 | - |

[a] Positions relative to sn - glycerol-3-phosphate

fraction of the 18:2-rich milk fat, the major proportion was esterified in position 1 (40% of the 18:2) and position 2 (48% of the 18:2) with a slight preference for position 2.

### 3.5.2.4. The arrangement of fatty acids in positions 1, 2 and 3 of the total control and 18:2-rich milk fats

The stereospecific distribution of fatty acids in the three fractions of each milk fat was used to calculate the stereospecific distribution in the total milk fats (Table 32) (Figures 8, 9, and 10).

The salient features of the effect of the increased availability of the 18:2 to the mammary gland for incorporation into milk fat TGs, were the increase in the proportions of 18:2 in each of the three positions of the TGs, the decrease in the proportions of 16:0, from 32.1 to 21.9% in position 1, from 30.9 to 19.1% in position 2 and from 8.9 to 5.2% in position 3, and also the decrease in the proportions of 14:0 in position 2, from 23.1 to 15.3%. Otherwise similar proportions of the major FAs appeared in each position of the control and 18:2-rich milk fats. In position 1 of the control milk fat, the proportions of the most abundant FAs were 32.1% 16:0, 19.5% 18:0 and 25.3% 18:1; and in the 18:2-rich milk fat, 21.9% 16:0, 22.1% 18:0, 22.6% 18:1 and 13.5% 18:2 were the proportions of the most abundant FAs. The predominant FAs in position 2 of the control milk fat were 14:0 (23.1%), 16:0 (30.9%) and 18:1 (12.9%), along with significant proportions of 6:0 to 12:0 comprising a further 19.2% of the total FAs in position 2. In the 18:2-rich milk fat, the proportions of the most abundant FAs in position 2 were 15.3% 14:0, 19.1% 16:0, 16.5% 18:1 and 16.4% 18:2, with FAs from 6:0 to 12:0 again comprising about 20% of the FAs present. In position 3 the major FAs in the control milk fat were 36.3% 4:0, 13.2% 6:0 and 14.9% 18:1, and the major FAs in position 3 of the 18:2-

Figure 8. Stereospecific distribution of the more abundant fatty acids in the triacylglycerols of the control and 18:2-rich milk fats and their respective triacylglycerol fractions

| Fatty acid composition (mole %) | |
|---|---|
| Control | 18:2-rich |
| **High mol. wt.** | |
| 16:0 (29.1%) 18:1 (27.2%) 18:0 (18.9%) | 18:1 (25.7%) 18:0 (22.0%) 16:0 (21.8%) 18:2 (11.7%) |
| 16:0 (36.9%) 14:0 (24.4%) 18:1 (14.4%) | 16:0 (23.3%) 18:1 (18.4%) 14:0 (16.4%) 18:2 (16.0%) |
| 18:1 (36.1%) 18:0 (20.9%) 16:0 (15.7%) | 18:1 (34.5%) 18:0 (22.5%) 18:2 (19.6%) |
| **Medium mol. wt.** | |
| 16:0 (32.3%) 18:1 (25.7%) 18:0 (19.8%) | 18:0 (28.5%) 18:1 (21.4%) 16:0 (19.5%) 18:2 (13.4%) |
| 16:0 (32.1%) 14:0 (22.6%) 18:1 (12.7%) | 16:0 (20.4%) 18:1 (17.5%) 14:0 (14.3%) 18:2 (13.1%) 18:0 (11.6%) |
| 6:0 (29.8%) 4:0 (17.5%) | 6:0 (35.8%) 4:0 (24.4%) |
| **Low mol. wt.** | |
| 16:0 (34.4%) 18:1 (23.5%) 18:0 (19.8%) | 16:0 (23.3%) 18:1 (19.6%) 18:0 (18.9%) 18:2 (15.5%) |
| 16:0 (25.5%) 14:0 (22.3%) 18:1 (11.8%) | 18:2 (18.5%) 14:0 (14.5%) 16:0 (13.7%) 18:1 (13.7%) |
| 4:0 (74.4%) 6:0 (15.2%) | 4:0 (72.0%) 6:0 (10.6%) |
| **Milk fat** | |
| 16:0 (32.1%) 18:1 (25.3%) 18:0 (19.5%) | 18:1 (22.6%) 18:0 (22.1%) 16:0 (21.9%) 18:2 (13.5%) |
| 16:0 (30.9%) 14:0 (23.1%) 18:1 (12.9%) | 16:0 (19.1%) 18:1 (16.5%) 18:2 (16.4%) 14:0 (15.3%) |
| 4:0 (36.3%) 18:1 (14.9%) 6:0 (13.2%) | 4:0 (31.8%) 18:1 (18.4%) 6:0 (12.2%) 18:0 (11.6%) 18:2 (11.3%) |

Table 32. Stereospecific analysis of the triacylglycerols of the control and 18:2-rich milk fats[b]

| FA | Fatty acid composition (mole %) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[a] | | Milk fat | | | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | | 18:2-rich | |
| | | | | | | | Orig. | Recalc. | Orig. | Recalc. |
| 4:0 | - | - | - | - | 36.3 | 31.8 | 11.9 | 12.1 | 10.4 | 10.6 |
| 6:0 | - | - | 1.8 | 2.4 | 13.2 | 12.2 | 5.3 | 5.0 | 5.4 | 4.8 |
| 8:0 | 0.9 | 0.8 | 3.3 | 4.0 | 2.2 | 1.3 | 2.3 | 2.1 | 2.1 | 2.0 |
| 10:0 | 2.0 | 1.9 | 6.2 | 6.8 | 3.8 | 1.7 | 4.1 | 4.0 | 3.2 | 3.5 |
| 10:1 | - | 0.1 | 0.4 | 0.3 | 0.3 | 0.1 | 0.3 | 0.2 | 0.2 | 0.2 |
| 12:0 | 3.3 | 2.9 | 7.5 | 6.5 | 1.8 | 0.7 | 3.5 | 4.2 | 2.6 | 3.4 |
| 14:0 | 9.4 | 8.2 | 23.1 | 15.3 | 3.9 | 2.6 | 11.9 | 12.1 | 7.7 | 8.7 |
| 14:1 | 0.2 | 0.3 | 1.2 | 0.5 | 1.3 | 0.5 | 1.0 | 0.9 | 0.5 | 0.4 |
| 15:0 | 1.5 | 1.5 | 2.0 | 0.8 | 0.1 | 0.4 | 1.0 | 1.2 | 0.7 | 0.9 |
| 16:0 | 32.1 | 21.9 | 30.9 | 19.1 | 8.9 | 5.2 | 22.1 | 24.0 | 14.1 | 15.4 |
| 16:1 | 1.6 | 2.1 | 1.9 | 1.5 | 1.6 | 0.5 | 2.0 | 1.7 | 1.1 | 1.4 |
| 17:0 | 0.6 | 1.0 | 0.4 | 0.3 | 0.6 | 0.4 | 0.9 | 0.5 | 0.5 | 0.6 |
| 18:0 | 19.5 | 22.1 | 6.1 | 8.7 | 9.2 | 11.6 | 11.9 | 11.6 | 14.1 | 14.1 |
| 18:1 | 25.3 | 22.6 | 12.9 | 16.5 | 14.9 | 18.4 | 18.6 | 17.7 | 20.6 | 19.2 |
| 18:2 | 1.9 | 13.5 | 1.2 | 16.4 | 0.9 | 11.3 | 1.8 | 1.3 | 15.5 | 13.7 |
| 18:3 | 1.1 | 1.1 | 0.9 | 0.9 | 0.8 | 1.2 | 0.8 | 0.9 | 0.7 | 1.1 |
| 20:? | 0.5 | - | 0.2 | 0.1 | 0.1 | 0.2 | 0.5 | 0.3 | 0.7 | 0.1 |

[a] Positions relative to sn - glycerol-3-phosphate

[b] Stereospecific distribution of FAs in the TGs calculated from the distribution in the respective TG fractions

Figure 9. Stereospecific distribution of the more abundant fatty acids in the triacylglycerols of the control and 18:2-rich milk fats and their respective triacylglycerol fractions

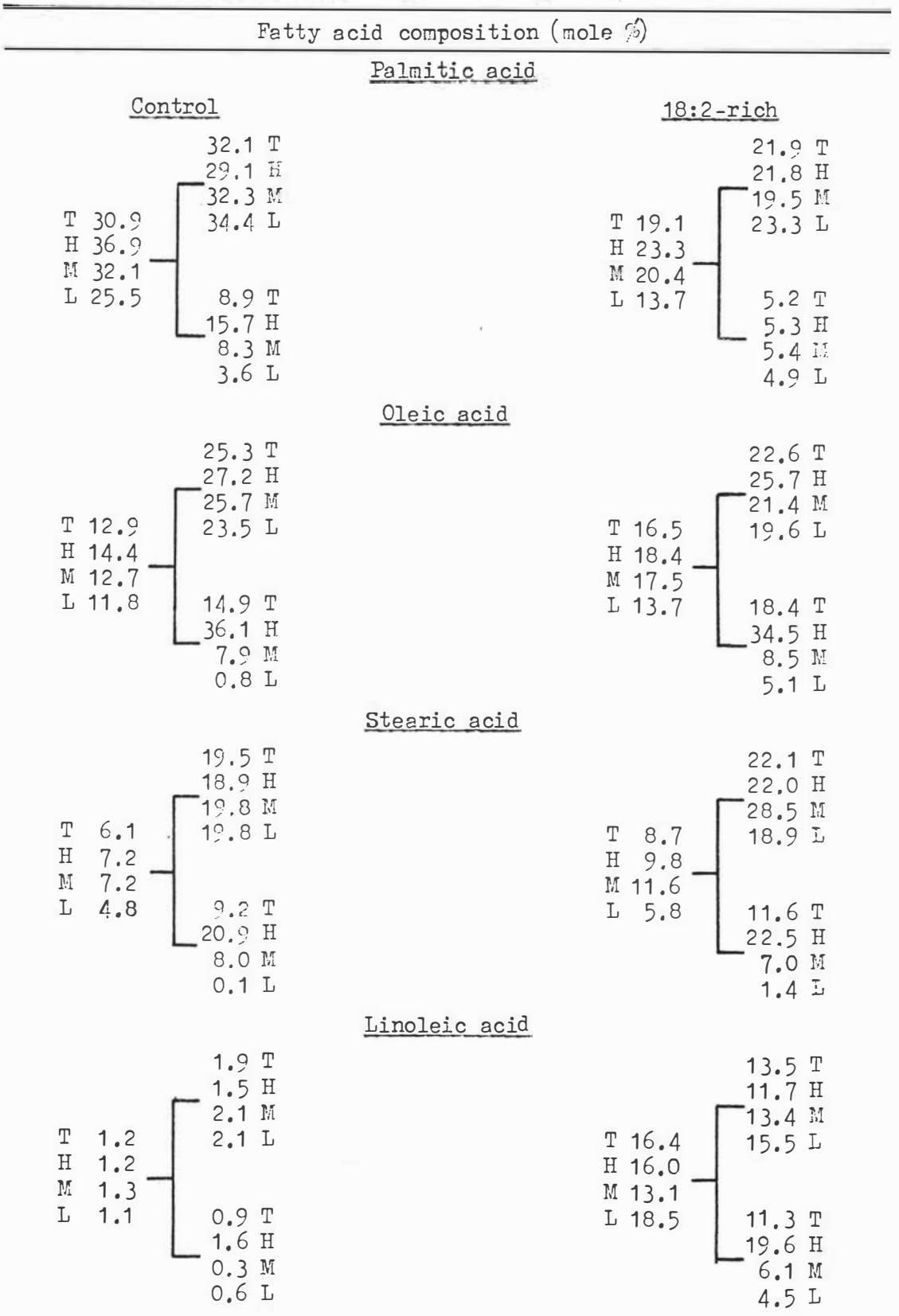
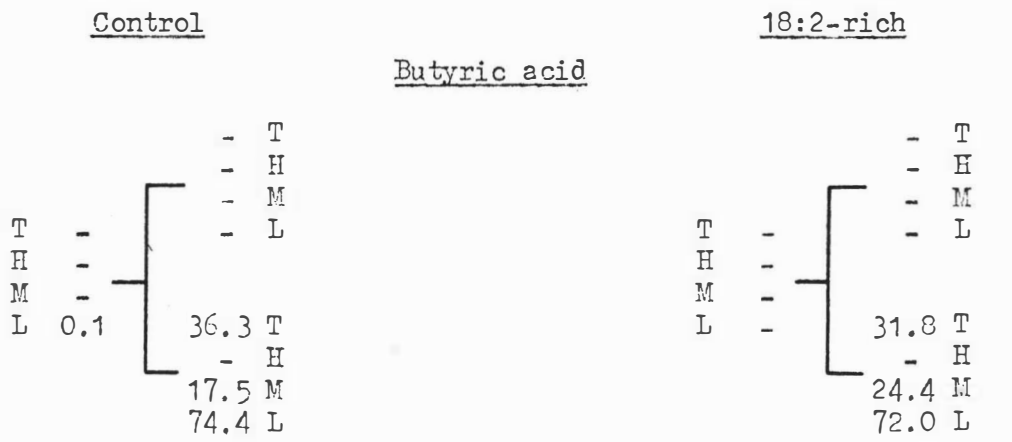


Figure 9.(cont.)




T = Total milk fat.

H = High mol. wt. fraction

M = Medium mol. wt. fraction

L = Low mol. wt. fraction

rich milk fat were 31.8% 4:0, 12.2% 6:0, 11.6% 18:0, 18.4% 18:1 and 11.3% 18:2.

The distribution of FAs in the 18:2-rich milk fat was little changed to that in the control milk fat (Table 33). All of the 4:0 was confined to position 3 of the two milk fats, together with over 80% of the 6:0. The other short and medium chain FAs (8:0 to 14:0) tended to be preferentially esterified in position 2 with lesser amounts in position 1 and position 3. 16:0 was evenly distributed between positions 1 and 2 of the control and 18:2-rich milk fats, with 45 - 47% of the 16:0 in position 1 and 41 - 43% of the 16:0 in position 2 of the two milk fats. Distribution of 18:0 was similar in both milk fats with about 54% of the 18:0 in position 1, and the remainder distributed between positions 2 and 3. The distribution of 18:1 between the 3 positions of the glycerol moiety was slightly altered, with a lower percentage of the 18:1 in position 1 (39% compared to 48%) and correspondingly higher percentages in positions 2 and 3. As in all the fractions of the control and 18:2-rich milk fats, the distribution of FAs contributing only minor amounts to the total composition was difficult to determine with any accuracy but in the control milk fat, 16:1 and 18:3 appeared to be evenly distributed, and 18:2, although present in each position, preferred position 1. On the other hand, in the 18:2-rich milk fat, 16:1 showed a preference for position 1, while 18:3 was again evenly distributed. 18:2, a major component of the 18:2-rich milk fat showed the order of preference 2>1>3, with 40%, 33%, and 27% of the total 18:2 being esterified in each position respectively.

Table 33. Proportional distribution of fatty acids in the triacylglycerols of the control and 18:2-rich milk fats

| FA | Fatty acid distribution (%) | | | | | |
|---|---|---|---|---|---|---|
| | 1[a] | | 2[a] | | 3[a] | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| 4:0 | - | - | - | - | 100.0 | 100.0 |
| 6:0 | - | - | 12.0 | 16.4 | 88.0 | 83.6 |
| 8:0 | 14.0 | 13.1 | 51.6 | 65.6 | 34.4 | 21.3 |
| 10:0 | 16.7 | 18.3 | 56.7 | 65.4 | 31.6 | 16.3 |
| 10:1 | - | 20.0 | 57.1 | 60.0 | 42.9 | 20.0 |
| 12:0 | 26.2 | 28.7 | 59.5 | 64.4 | 14.3 | 6.9 |
| 14:0 | 25.8 | 31.4 | 63.5 | 58.6 | 10.7 | 10.0 |
| 14:1 | 7.4 | 23.1 | 44.4 | 38.5 | 48.2 | 38.5 |
| 15:0 | 41.7 | 55.5 | 55.6 | 29.6 | 2.7 | 14.9 |
| 16:0 | 44.6 | 47.4 | 43.0 | 41.3 | 12.4 | 11.3 |
| 16:1 | 31.4 | 51.2 | 37.3 | 36.6 | 31.3 | 12.2 |
| 17:0 | 37.5 | 58.8 | 25.0 | 17.6 | 37.5 | 23.6 |
| 18:0 | 56.0 | 52.1 | 17.5 | 20.5 | 26.4 | 27.4 |
| 18:1 | 47.6 | 39.3 | 24.3 | 28.7 | 28.1 | 32.0 |
| 18:2 | 47.5 | 32.8 | 30.0 | 39.8 | 22.5 | 27.4 |
| 18:3 | 39.3 | 34.4 | 32.1 | 28.1 | 28.6 | 37.5 |
| 20:? | 62.5 | - | 25.0 | 33.3 | 12.5 | 66.6 |

[a] Positions relative to sn - glycerol-3-phosphate

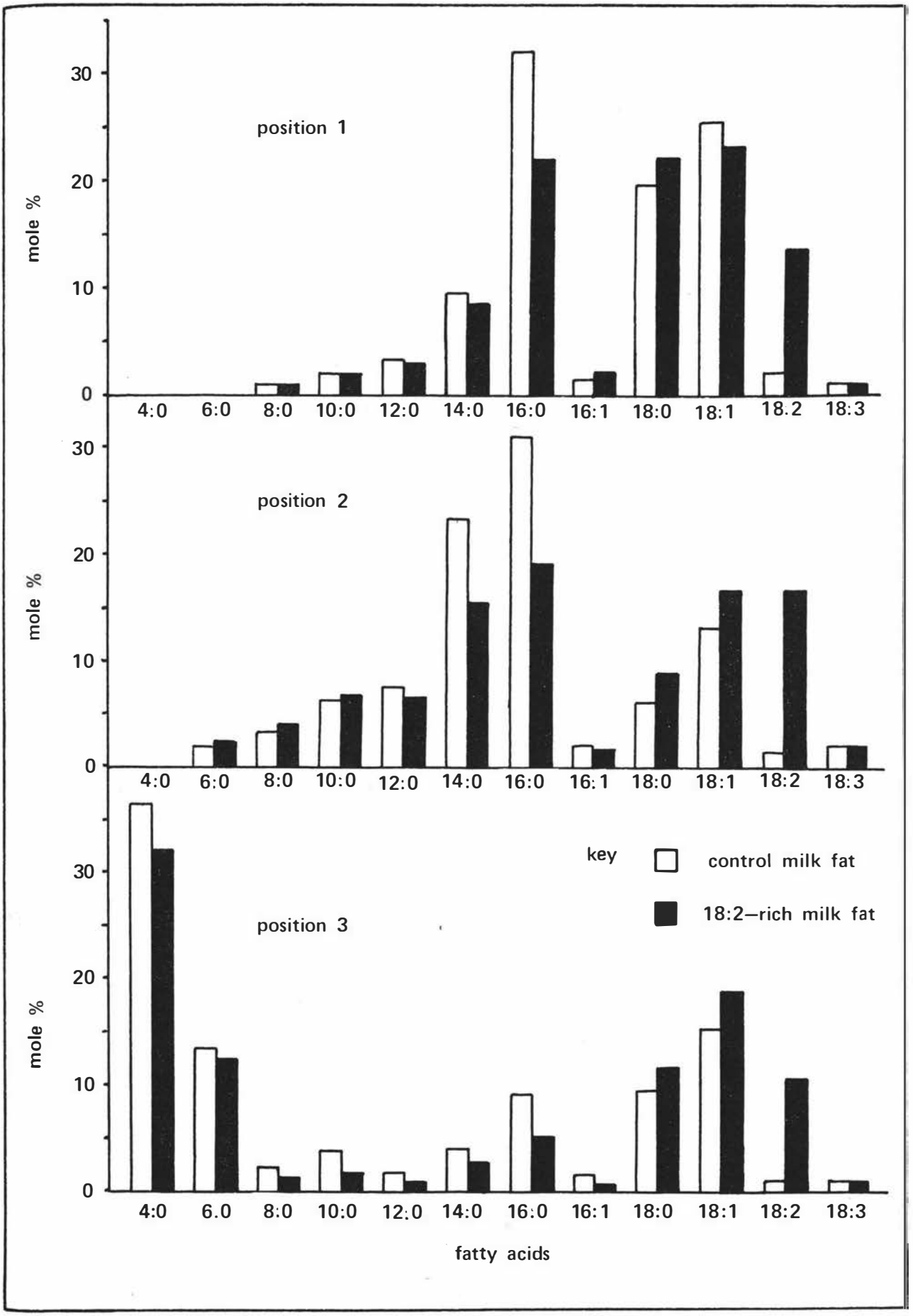



Figure 10. Stereospecific distribution of fatty acids in the triacylglycerols of the control and 18:2-rich milk fats.

## Section 3.6. Comparison of the molecular species of triacylglycerol in the control and 18:2-rich milk fats

The most abundant molecular species of TG in each fraction of the two milk fats were assessed from the proportions and composition of the TG classes of differing levels of unsaturation and from the positional distribution of FAs in the TGs of the fractions of high, medium and low mol. wt. of the control and 18:2-rich milk fats (Figures 11 to 14: Appendices 9 to 11).

### 3.6.1. Molecular species of triacylglycerol in the triacylglycerol fractions of high molecular weight

The saturated TGs of high mol. wt. from both milk fats consisted primarily of 14:0. 16:0 and 18:0 arranged in certain preferred combinations to give major TG species ranging from $C_{42}$ to $C_{52}$ (Figure 11). The relative abundance of TGs as separated by acyl carbon number, and the stereospecific distribution of FAs in the high mol. wt. fractions, indicated that the preferred combinations are likely to be 16:0 - 16:0 - 18:0, 16:0 - 14:0 - 18:0 and 18:0 - 14:0 - 18:0. Very little 18:0 - 18:0 - 18:0 is likely to be present as indicated by the very low proportion of $C_{54}$ TGs in saturated TGs of the high mol. wt. fractions. From the consideration of the similarities, in the stereospecific distribution of FAs in the TGs of the two high mol. wt. fractions and in the FA and TG compositions of the saturated TGs of high mol. wt. it would appear that similar relative proportions of the different molecular species of TG are present in these two TG classes, although the overall amount of saturated TGs was much lower in the 18:2-rich milk fat.

The major *cis* - monoene TGs consisted of 18:1 mainly in either position 1 or position 3 in combinations with 14:0, 16:0 or 18:0

comprising $C_{48}$, $C_{50}$ and $C_{52}$ as the major TGs. The proportion of $C_{48}$ was different in the two milk fats, comprising 7.7 and 3.5% of the high mol. wt. fraction of the control and 18:2-rich milk fats respectively and is most likely to consist of 16:0 - 14:0 - 18:1, whereas the proportion of $C_{50}$, comprising 11.3% and 5.2% of the respective fractions was reduced in the 18:2-rich milk fat and is likely to consist of mainly 16:0 - 16:0 - 18:1, 18:0 - 14:0 - 18:1 and 18:1 - 14:0 - 18:0. In addition the proportions of $C_{52}$ TGs in the 18:2-rich milk fat were reduced (4.8% compared to 7.9% of the fraction) and are likely to contain predominantly 18:1 - 16:0 - 18:0 and 18:0 - 16:0 - 18:1.

The trans - monoene TGs made a significant contribution to the high mol. wt. TGs of the control milk fat with major TG species being $C_{48}$ (2.1%), $C_{50}$ (3.5%) and $C_{52}$ (2.5%). The individual species of TG are likely to be the same as for the cis - monoene TGs.

The stereospecific distribution of the FAs indicated that the principal species of diene TG in the high mol. wt. fraction of the control milk fat are likely to be 16:0 - 18:1 - 18:1 or 18:1 - 16:0 - 18:1, comprising up to 7% of the high mol.wt. fraction, along with 18:1 - 14:0 - 18:1 and 18:1 - 18:0 - 18:1 each comprising up to 4% of the high mol. wt. fraction. The proportion of the TGs in the high mol. wt. fraction of the 18:2-rich milk fat containing a saturated FA and two 18:1s was estimated to be lower (12% compared to 16%), but the proportion of TGs containing two saturated FAs and 18:2 was greatly increased (15% compared to 1%). The 18:2-rich milk fat in addition to the TG species present in the control milk fat is likely to contain predominantly the following TGs: 16:0 - 14:0 - 18:2, 18:0 - 14:0 - 18:2, 16:0 - 16:0 - 18:2, 18:0 - 16:0 - 18:2, 16:0 - 18:2 - 18:0 and 18:0 - 18:2 - 18:0, along with smaller amounts of 18:2 - 16:0 - 18:0 and

Figure 11. Major molecular species of triacylglycerol in the high molecular weight fraction of the control and 18:2-rich milk fats

| Likely major molecular species of triacylglycerol | Carb. no.[a] | Unsatn.[d] | Cont.[b] | 18:2[c] |
|---|---|---|---|---|
| | | | (Approx. %) | |
| 16:0,14:0 (12:0)[e] — 16:0,18:0 / 18:0 | 42-52 | 0 | 28 | 18 |
| 16:0,14:0 — 18:1(trans) / 18:0 ; 16:0,14:0 — 16:0,18:0 / 18:1(trans) | 48-52 | 1 | 8 | 3 |
| 16:0,14:0 — 18:1(cis) / 18:0 ; 16:0,14:0 — 16:0,18:0 / 18:1(cis) | 46-54 | 1 | 32 | 18 |
| 16:0,14:0 (18:0) — 18:1 / 18:1 | 50-54 | 2 | 15 | 12 |
| 16:0,14:0 (12:0) — 18:2 / 18:0 ; 18:2 — 16:0,18:0 / 18:0 ; 16:0,14:0 (12:0) — 16:0,18:0 / 18:2 | 46-54 | 2 | 1 | 14 |
| 18:2 — 16:0,18:0 / 18:1 ; 18:2 — 18:1 / 18:0 ; 16:0,14:0 — 18:1 / 18:2 | 50-54 | 3 | 4 | 13 |
| 18:1 — 18:1 / 18:1 | 54 | 3 | 3 | 1 |
| 18:2 — 16:0,18:0 / 18:2 ; 18:2 — 18:1 / 18:1,18:2 | 52-54 | 4-5 | - | 11 |

[a] Carbon number of TGs
[b] Control milk fat
[c] 18:2-rich milk fat
[d] Number of double bonds per TG molecule
[e] ( ) Minor constituents

18:2 - 14:0 - 18:0.

The most abundant triene TG contained 54 acyl carbons comprising 4.3 and 7.2% of the high mol. wt. fractions of the control and 18:2-rich milk fats respectively and is likely to consist of equal amounts of 18:1 - 18:1 - 18:1 and 18:0 - 18:2 - 18:1 or 18:1 - 18:2 - 18:0 in the control milk fat and 18:0 - 18:2 - 18:1 and 18:1 - 18:2 - 18:0 in the 18:2-rich milk fat. $C_{52}$ TGs were also present in significant amounts and comprised 2.6% of the control and 4.6% of the 18:2-rich high mol. wt. fractions. The predominant species of $C_{52}$ TG is likely to be 16:0 - 18:2 - 18:1.

The "tetraene" TGs of the high mol. wt. fraction of the 18:2-rich milk fat contained predominantly $C_{54}$ TGs which comprised 8.8% of the high mol. wt. fraction, and with reference to the FA composition, the likely TG species are 18:0 - 18:2 - 18:2, 18:1 - 18:2 - 18:1 and 18:1 - 18:2 - 18:2. $C_{52}$ TGs, mainly 16:0 - 18:2 - 18:2 comprised 2.0% of the high mol. wt. fraction.

In summary, on the basis of the composition of the TG classes and the stereospecific distribution of the FAs, the most abundant molecular species of TG contributing to the high mol. wt. fraction of the control and 18:2-rich milk fats are given in Figure 11.

### 3.6.2. Molecular species of triacylglycerol in the triacylglycerol fractions of medium molecular weight

In the saturated TGs of medium mol. wt. of both milk fats, the most abundant TG species was $C_{36}$ comprising 8.0 and 7.3% of the medium mol. wt. fractions of the control and 18:2-rich milk fats respectively. Stereospecific analysis of these TG fractions, and the composition of the TG classes indicated that $C_{36}$ is likely to consist of mainly 16:0 - 16:0 - 4:0 and 16:0 - 14:0 - 6:0. $C_{38}$ was present in lower proportions

in the 18:2-rich milk fat than in the control milk fat (10.3% compared to 15.8% of the fraction) and the main molecular species are likely to be 16:0 - 16:0 - 6:0, 18:0 - 14:0 - 6:0 and 18:0 - 16:0 - 4:0. $C_{40}$, which was also present in lower proportions in the 18:2-rich milk fat (5.9% compared to 11.7% of the medium mol. wt. fraction), is likely to consist of mainly 18:0 - 16:0 - 6:0 and 18:0 - 18:0 - 4:0 (Figure 12).

The major cis - monoene TGs contained 38, 40, 42 and 44 acyl carbons. $C_{38}$ TGs comprised 3.6 and 5.7% of the medium mol. wt. fraction of the control and 18:2-rich milk fats respectively and the most likely individual species with this carbon number are 18:1 - 16:0 - 4:0. 16:0 - 18:1 - 4:0 and 18:1 - 14:0 - 6:0. 18:1 - 16:0 - 6:0, 16:0 - 18:1 - 6:0, 18:0 - 18:1 - 4:0 and 18:1 - 18:0 - 4:0 are the likely most abundant TGs containing 40 acyl carbons which contributed 7.6 and 10.8% of the respective fractions. $C_{42}$ (5.6 and 4.9% of the respective fractions) is likely to consist of mainly 18:1 - 18:0 - 6:0 and 18:0 - 18:1 - 6:0; and $C_{44}$ (3.2 and 1.1% of the respective fractions): 18:1 - 18:0 - 8:0 and 18:0 - 18:1 - 8:0.

The trans - monoene TGs had similar FA and TG compositions to the corresponding cis - monoene TGs, with the major TG species contributing to the medium mol. wt. fraction of the control milk fat being $C_{38}$ (1.5%), $C_{40}$ (2.5%) and $C_{42}$ (1.6%) and to the 18:2-rich milk fat fraction, $C_{38}$ (1.8%), $C_{40}$ (3.3%) and $C_{42}$ (1.6%).

In the control milk fat the most abundant species of diene TG in the medium mol. wt. fraction were $C_{40}$ (1.5%), $C_{42}$ (2.8%), $C_{44}$ (2.4%) and $C_{46}$ (2.2%), consisting of mainly 18:1 - 16:1 - 6:0, 18:1 - 18:1 - 6:0, 18:1 - 18:1 - 8:0 and 18:1 - 18:1 - 10:0 respectively. Similarly in the 18:2-rich milk fat the proportions of these molecular species of TG together comprised about 4% of the medium mol. wt. fraction. However the proportion of the diene TGs containing 18:2 was much higher than in the

Figure 12. Major molecular species of triacylglycerol in the medium molecular weight fraction of the control and 18:2-rich milk fats

| Likely major molecular species of triacylglycerol | Carb. no.[a] | Unsatn.[d] | Cont.[b] (Approx. %) | 18:2[c] (Approx. %) |
|---|---|---|---|---|
| 16:0,14:0 (18:0)[e] — 16:0,18:0 / 4:0,6:0 | 34-42 | 0 | 43 | 27 |
| 16:0,14:0 (18:0) — 18:1(trans) / 4:0,6:0; 18:1 (trans) — 16:0,18:0 / 4:0,6:0 | 38-42 | 1 | 6 | 7 |
| 16:0,14:0 (18:0) — 18:1(cis) / 4:0,6:0 (8:0); 18:1 (cis) — 16:0,18:0 / 4:0,6:0 (8:0) | 38-44 | 1 | 20 | 23 |
| 18:1 — 18:1 / (4:0),6:0,(8:0),(10:0) | 40-46 | 2 | 7 | 4 |
| 16:0,14:0 — 18:2 / 6:0,8:0, (10:0); 18:2 — 16:0,18:0 / 6:0,8:0 (10:0) | 40-46 | 2 | 2 | 16 |
| 18:1 — 18:2 / 6:0,8:0,10:0; 18:2 — 18:1 / 6:0,8:0,10:0 | 42-46 | 3 | 2 | 7 |
| 18:1 — 18:1 / 18:1; 18:2 — 18:1 / 18:0; 16:0,14:0 — 16:0,18:0 / 18:3 | 48-54 | 3 | 5 | 2 |

(to be cont.)

Figure 12 (cont.)

| Likely major molecular species of triacylglycerol | Carb. no.[a] | Unsatn.[d] | Cont.[b] | 18:2[c] (Approx. %) |
|---|---|---|---|---|
| 18:2, 18:2, 6:0,8:0,10:0 | 42-46 | 4 | - | 2 |
| 18:2, 18:2, 18:0 | 50-54 | 4-5 | - | 5 |
| 18:1, 18:1, 18:2 | | | | |
| 18:2, 18:0, 18:2 | | | | |
| 16:0, 18:2, 18:2 | | | | |
| 18:2, 16:0, 18:2 | | | | |

a Carbon number of TGs
b Control milk fat
c 18:2-rich milk fat
d Number of double bonds per TG molecule
( )[e] Minor constituents

control milk fat (19% compared to 2%) with the predominant species likely to be 18:0 - 18:2 - 4:0, 16:0 - 18:2 - 6:0, 18:2 - 16:0 - 4:0, 18:2 - 16:0 - 6:0 and 18:2 - 18:0 - 4:0.

The triene TGs comprised only 8.1% of the medium mol. wt. fraction of the control milk fat and these TGs mostly contained between 46 and 54 acyl carbons. The most likely important TG species are 18:1 - 18:1 - 18:1, 16:0 - 16:0 - 18:3, 16:0 - 18:2 - 18:1 and 18:0 - 18:2 - 18:1. On the other hand, the triene TGs of the medium mol. wt. fraction of the 18:2-rich milk fat consisted primarily of $C_{42}$ (3.6% of the fraction) which is likely to be 18:1 - 18:2 - 6:0 and 18:2 - 18:1 - 6:0, and smaller amounts of $C_{44}$ (1.7% of the fraction) likely to consist of mainly 18:1 - 18:2 - 8:0 and 18:2 - 18:1 - 8:0 and $C_{46}$ (1.6% of the fraction) likely to consist of mainly 18:1 - 18:2 - 10:0 and 18:2 - 18:1 - 10:0.

The class of TG in the medium mol. wt. fraction of the 18:2-rich milk fat designated the "tetraene" TGs contained mainly $C_{54}$ TGs (3.3% of the fraction) and possible major species are 18:0 - 18:2 - 18:2, 18:1 - 18:1 - 18:2, 18:1 - 18:2 - 18:1, 18:1 - 18:2 - 18:2, 18:2 - 18:2 - 18:1 and 18:2 - 18:2 - 18:0. Also present were $C_{52}$ TGs (1.1% of the fraction) and likely to consist of mainly 18:2 - 16:0 - 18:2 and 16:0 - 18:2 - 18:2, $C_{46}$ (1.1%) likely to consist of mainly 18:2 - 10:0 - 18:2 and $C_{50}$ (0.9%), 18:2 - 14:0 - 18:2.

In summary, on the basis of the composition of the TG classes and the stereospecific distribution of FAs, the quantitatively important TG species in the medium mol. wt. fractions are shown in Figure 12.

### 3.6.3. Molecular species of triacylglycerol in the triacylglycerol fractions of low molecular weight

The highly specific placement of 4:0 and other short chain FAs in position 3 of the low mol. wt. TGs assists in assigning the likely

contribution of individual molecular species of TG to the low mol. wt. fraction.

The predominant species of saturated TG are likely to be 16:0 - 16:0 - 4:0, 16:0 - 14:0 - 4:0 and 18:0 - 14:0 - 4:0 together comprising up to 30% of the control fraction and only 18% of the 18:2-rich fraction. Also likely to be present in quantitative amounts are 16:0 - 12:0 - 4:0, 14:0 - 14:0 - 4:0 and 18:0 - 16:0 - 4:0 together comprising about 12 and 9% of the low mol. wt. fractions of the control and 18:2-rich milk fats respectively (Figure 13) (Appendix 11).

Cis - monoene TGs with 38 acyl carbons comprised 12.8 and 7.6% of the low mol. wt. fractions of the control and 18:2-rich milk fats respectively, and are likely to consist of mainly 18:1 - 16:0 - 4:0. Also present were $C_{36}$ (8.2% of control and 5.5% of 18:2-rich fraction) consisting of mainly 18:1 - 14:0 - 4:0 and $C_{40}$ (3.4% and 3.6% of the respective fractions) likely to consist of primarily 18:1 - 18:0 - 4:0.

The trans - monoene TGs contained very similar relative proportions of the major TG species to the cis - monoene TGs, with $C_{36}$ (2.6% of control and 1.4% of 18:2-rich fraction) and $C_{38}$ (4.4% of control and 2.0% of 18:2-rich fraction) making important contributions to the low mol. wt. fractions. The same individual species of TG are likely to be present.

The most abundant TG species in the diene TGs of low mol. wt. in the control milk fat is likely to be 18:1 - 18:1 - 4:0 comprising up to 5% of the low mol. wt. fraction, while in the diene TGs of the 18:2-rich milk fat slightly lower proportions of 18:1 - 18:1 - 4:0 were present (4% compared to 5%) and the TG species 18:2 - 14:0 - 4:0, 16:0 - 18:2 - 4:0, 18:2 - 16:0 - 4:0 and 18:0 - 18:2 - 4:0, because of the increase in the size of the diene TG class, became the most abundant species contributing up to 22% of the low mol. wt. fraction of the 18:2-rich

Figure 13. Major molecular species of triacylglycerol in the low molecular weight fraction of the control and 18:2-rich milk fats

| Likely major molecular species of triacylglycerol | Carb. no.[a] | Unsatn.[d] | Cont.[b] (Approx. %) | 18:2[c] (Approx. %) |
|---|---|---|---|---|
| 16:0,14:0 (18:0)[e] — 16:0,18:0 / 4:0,(6:0) | 30-38 | 0 | 47 | 28 |
| 16:0,14:0 — 18:1(trans) — 4:0,(6:0); 18:1 (trans) — 16:0,18:0 / 4:0,(6:0) | 34-40 | 1 | 9 | 5 |
| 16:0,14:0 (18:0) — 18:1(cis) — 4:0,(6:0); 18:1 (cis) — 16:0,18:0 / 4:0,(6:0) | 34-40 | 1 | 27 | 19 |
| 18:1 — 18:1 / 4:0 | 40 | 2 | 5 | 4 |
| 16:0,14:0 — 18:2 — 4:0; 18:2 — 16:0,18:0 / 4:0 | 36-40 | 2 | 3 | 22 |
| 18:2 — 18:1 — 4:0,(6:0); 18:1 — 18:2 / 4:0,(6:0) | 40-42 | 3 | 2 | 8 |
| 18:2 — 18:2 / 4:0,(6:0) | 40-42 | 4 | - | 6 |

[a] Carbon number of TGs
[b] Control milk fat
[c] 18:2-rich milk fat
[d] Number of double bonds per TG molecule
[e] ( ) Minor constituents

milk fat.

In the control milk fat, 18:1 - 18:2 - 4:0 and 18:3 - 16:0 - 4:0 are likely to be two of the predominant triene TGs comprising about 4.6% of the low mol. wt. fraction, whereas in the 18:2-rich milk fat 18:1 - 18:2 - 4:0 is likely to be the predominant species of triene TG and along with the TG species 18:2 - 18:1 - 4:0 is likely to comprise about 7% of the low mol. wt. fraction of the 18:2-rich milk fat.

In the tetraene TGs of the 18:2-rich milk fat the most abundant TG species contained 40 acyl carbons, comprising 4.5% of the low mol. wt. fraction, and is likely to consist of mainly 18:2 - 18:2 - 4:0.

In summary, the most important TG species of low mol. wt. in the control and 18:2-rich milk fats are shown in Figure 13.

### 3.6.4. Molecular species of triacylglycerol in milk fat

The contributions of individual molecular species of TG to the total control and 18:2-rich milk fats were assessed from the levels of the major TG species present in the respective TG fractions of high, medium and low mol. wt (Figure 14).

Saturated TGs containing 4:0 or 6:0 in position 3 and having between 32 and 42 acyl carbons provided a major proportion of the TGs of both milk fats, comprising about 30% of the TGs in the control and about 17% of the TGs in the 18:2-rich milk fat. In addition the quantitatively important saturated TGs of high mol. wt. ($C_{44}$ - $C_{50}$) comprised a further 8 and 5% of the control and 18:2-rich milk fats respectively. The individual species of saturated TG most likely to contribute substantially to the control and 18:2-rich milk fats, in order of importance, are 16:0 - 14:0 - 4:0, 16:0 - 16:0 - 4:0, 18:0 - 14:0 - 4:0, 16:0 - 16:0 - 6:0 and 18:0 - 14:0 - 6:0. In the high mol. wt. TGs, because of the wider distribution of FAs (Figure 12) no

Figure 14. Major molecular species of triacylglycerol in the control and 18:2-rich milk fats[e]

| Likely major molecular species of triacylglycerol | Carb. no.[a] | Unsatn.[d] | Cont.[b] 18:2[c] (Approx. %) | |
|---|---|---|---|---|
| 16:0,14:0 (18:0)[f] — 16:0,18:0 / 4:0,6:0 | 32-42 | 0 | 30 | 17 |
| 16:0,14:0 (12:0) — 16:0,18:0,(14:0) / 18:0,(16:0) | 44-50 | 0 | 8 | 5 |
| 16:0,14:0 (12:0) — 18:1,(cis) / 4:0,6:0; 18:1 (cis) — 16:0,18:0 / 4:0,6:0 | 34-42 | 1 | 15 | 12 |
| 16:0,14:0 (12:0) — 18:1,(cis) / 18:0; 16:0,14:0 — 16:0,18:0 / 18:1,(cis) | 46-52 | 1 | 11 | 7 |
| 18:1 — 18:1 / 4:0,6:0; 18:2 — 16:0,18:0 / 4:0,6:0; 16:0,14:0 (12:0) — 18:2 / 4:0,6:0 | 36-42 | 2 | 4 | 14 |
| 16:0,14:0 (18:0) — 18:1 / 18:1; 18:2 — 16:0,18:0 / 18:0; 16:0,14:0 — 16:0,18:0 / 18:2 | 48-54 | 2 | 6 | 10 |
| 18:2 — 18:1 / 4:0,6:0 | 40-42 | 3 | 2 | 4 |
| 18:1 — 18:1 / 18:1; 18:2 — 16:0,18:0 / 18:1; 18:2 — 18:1 / 18:0; 16:0,14:0 — 18:1 / 18:2 | 50-54 | 3 | 4 | 7 |

(to be cont.)

Figure 14 (cont.)

| Likely major molecular species of triacylglycerol | Carb. no.[a] | Unsatn.[d] | Cont.[b] | 18:2[c] (Approx. %) |
|---|---|---|---|---|
| 18:2 < 18:2 / 4:0, 6:0 | 40-42 | 4 | - | 2 |
| 18:2 < 16:0,18:0 / 18:2 ; 18:2 < 18:1 / 18:1 ; 18:2 < 18:1 / 18:2 | 52-54 | 4-5 | - | 6 |

a Carbon number of TGs
b Control milk fat
c 18:2-rich milk fat
d Number of double bonds per TG molecule
e Calculated from the data for the high, medium and low mol. wt. fractions
( )[f] Minor constituents

individual TG species appear likely to make an appreciable contribution to the total milk fat, with these TGs comprising only about 8 and 5% of the control and 18:2-rich milk fats respectively.

The cis - monoene TGs were of major importance in both milk fats with TGs containing 34 - 42 acyl carbons e.g. 18:1 - 16:0 - 4:0 comprising 15 and 12% of the control and 18:2-rich milk fats respectively and those containing 46 - 52 acyl carbons comprising 11 and 7% of the control and 18:2-rich milk fats respectively. The major individual species of TG, with a single cis double bond, likely to be contributing to the control and 18:2-rich milk fats are: 18:1 - 16:0 - 4:0, 18:1 - 14:0 - 4:0, 16:0 - 16:0 - 18:1, 18:0 - 14:0 - 18:1, 18:1 - 14:0 - 18:0, 18:0 - 16:0 - 18:1 and 18:1 - 16:0 - 18:0. On the other hand the individual species of monoene TGs containing trans 18:1 did not appear to be of quantitative importance in the total milk fat with these TGs in total contributing only 9 and 5% to the control and 18:2-rich milk fats respectively.

The diene TGs provided a greater proportion of the TGs of the total 18:2-rich milk fat than the cis - monoene TGs, with those containing 38 - 42 acyl carbons e.g. 18:0 - 18:2 - 4:0 comprising 14% of the total TGs and those containing 48 - 54 acyl carbons e.g. 16:0 - 18:2 - 18:0 providing 10% of the total TGs. In addition the diene TGs of the 18:2-rich milk fat also contained TGs comprised of two 18:1s and a saturated FA with the major species likely to be 18:1 - 18:1 - 4:0, 18:1 - 16:0 - 18:1 and 18:1 - 18:0 - 18:1. In the control milk fat the diene TGs containing 36 - 42 acyl carbons, with the major species likely to be 18:1 - 18:1 - 4:0 with lesser amounts of 18:2 - 14:0 - 4:0, 16:0 - 18:2 - 4:0 and 18:0 - 18:2 - 4:0, comprised 4% of the TGs, whereas those containing 48 - 54 acyl carbons e.g. 18:1 - 16:0 - 18:1, 18:1 - 14:0 - 18:1 and 18:1 - 18:0 - 18:1 comprised 6% of the TGs of the

control milk fat.

The triene TGs contributed only a minor proportion of the TGs of the control milk fat with those containing 4:0 or 6:0, such as 18:1 - 18:2 - 4:0 comprising only about 3% and those containing three long chain FAs e.g. 18:1 - 18:1 - 18:1 comprising about 4% of the TGs of the control milk fat. In contrast these triene TGs contributed nearly twice as much to the 18:2-rich milk fat with 4% in the form of low mol. wt. triene TGs ($C_{40}$ - $C_{42}$) and 8% as high mol. wt. triene TGs ($C_{50}$ - $C_{54}$), with major individual TG species likely to be 18:1 - 18:2 - 4:0, 18:1 - 18:2 - 6:0, 16:0 - 18:2 - 18:1, 18:1 - 16:0 - 18:2, 18:2 - 16:0 - 18:1, 18:0 - 18:2 - 18:1 and 18:1 - 18:2 - 18:0. In addition TGs containing 4 or 5 double bonds, such as 18:2 - 18:2 - 4:0 and 18:1 - 18:2 - 18:1 comprised another 9% of the 18:2-rich milk fat.

In summary, comparison of the contributions of the various TG species to the total milk fats indicated that in the 18:2-rich milk fat there was a much greater contribution from the diene and triene TGs with a corresponding decrease in the contribution from the saturated and monoene TGs. This trend occurred both from the TGs of low mol. wt. i.e. those containing 4:0 or 6:0 and from the TGs of high mol. wt.. The medium mol. wt. TGs contributed only minor amounts to each class of TG in the total milk fats, except in the case of the saturated TGs which contributed 9 and 5% of the total TGs of the control and 18:2-rich milk fats respectively.

## Section 3.7. Thermal analysis of the triacylglycerols of the control and 18:2-rich milk fats and their respective high, medium and low molecular weight fractions

### 3.7.1. Thermal analysis of the triacylglycerols of the fractions of high molecular weight

The high mol. wt. fraction of the control milk fat melted over the range -27 to 41°C with the bulk of the sample melting between -10 and 40°C (Figure 15), whereas the high mol. wt. fraction of the 18:2-rich milk fat melted between -38 and 38°C with the bulk of the sample melting between -9 and 34°C (Figure 16). The main melting peak of the control fraction, at 34 - 35°C was preceded by a dip in the thermogram, centred at 15°C. Tempering of the sample as in section 2.6.1. caused the partial removal of this dip without affecting the remainder of the thermogram, indicating the possibility of the dip being caused by a polymorphic transition. The high mol. wt. fraction of the 18:2-rich milk fat contained only one broad peak centred at 17°C.

### 3.7.2. Thermal analysis of the triacylglycerols of the fractions of medium molecular weight

The medium mol. wt. fraction of the control milk fat melted over the range -25 to 25°C with a single main melting peak at 19°C following a minor melting peak at 10°C (Figure 15). On the other hand in the 18:2-rich milk fat, the medium mol. wt. fraction contained two main melting peaks centred at -2 and 13°C, with the total sample melting between -42 and 22°C, although 75% of the sample melted between -9 and 22°C (Figure 16) (Figure 18). Tempering of the sample, at the temperature of the dip between the melting peaks caused the first peak to be shifted from -2°C to -10°C (Figure 17). This behaviour would be consistent with solid solution formation in the

medium mol. wt. fraction of the 18:2-rich milk fat. The position of the lower melting peak in the thermogram of the fraction of medium mol. wt. of the 18:2-rich milk fat corresponded quite closely to the melting peak at 0°C in the fraction of low mol. wt. of the 18:2-rich milk fat, whereas the second melting peak corresponded closely to the melting peak at 17°C in the fraction of high mol. wt. of the 18:2-rich milk fat. In addition the second melting peak also corresponded closely to the main melting peak of the medium mol. wt. fraction of the control milk fat.

### 3.7.3. Thermal analysis of the triacylglycerols of the fractions of low molecular weight

The thermogram of the low mol. wt. fraction of the control milk fat was very complex, with a large broad peak melting between -41 and 4°C followed by an exothermic transition at 6°C, a dip at 13°C and the main melting peak at 19°C (Figure 15). The melting range of the sample was -41 to 25°C. with 65% of the sample melting before the exothermic transition at 6°C (Figure 18). Tempering the sample at 6°C removed the exothermic transition at 6°C but not the dip at 13°C, indicating the presence of a polymorphic transition at 6°C (Figure 17). Whereas the low mol. wt. fraction of the control milk fat showed a complex thermal behaviour, that of the 18:2-rich milk fat exhibited one broad melting peak centred at 0°C (Figure 16). This sample melted over the range -49 to 18°C, with 96% of the sample melting between -42 and 11°C. (Figure 18).

The temperature of the melting peak of the low mol. wt. fraction of the 18:2-rich milk fat corresponded closely to the initial broad peak of the low mol. wt. fraction of the control milk fat, with the finish of melting for both of these peaks being at about 6°C.

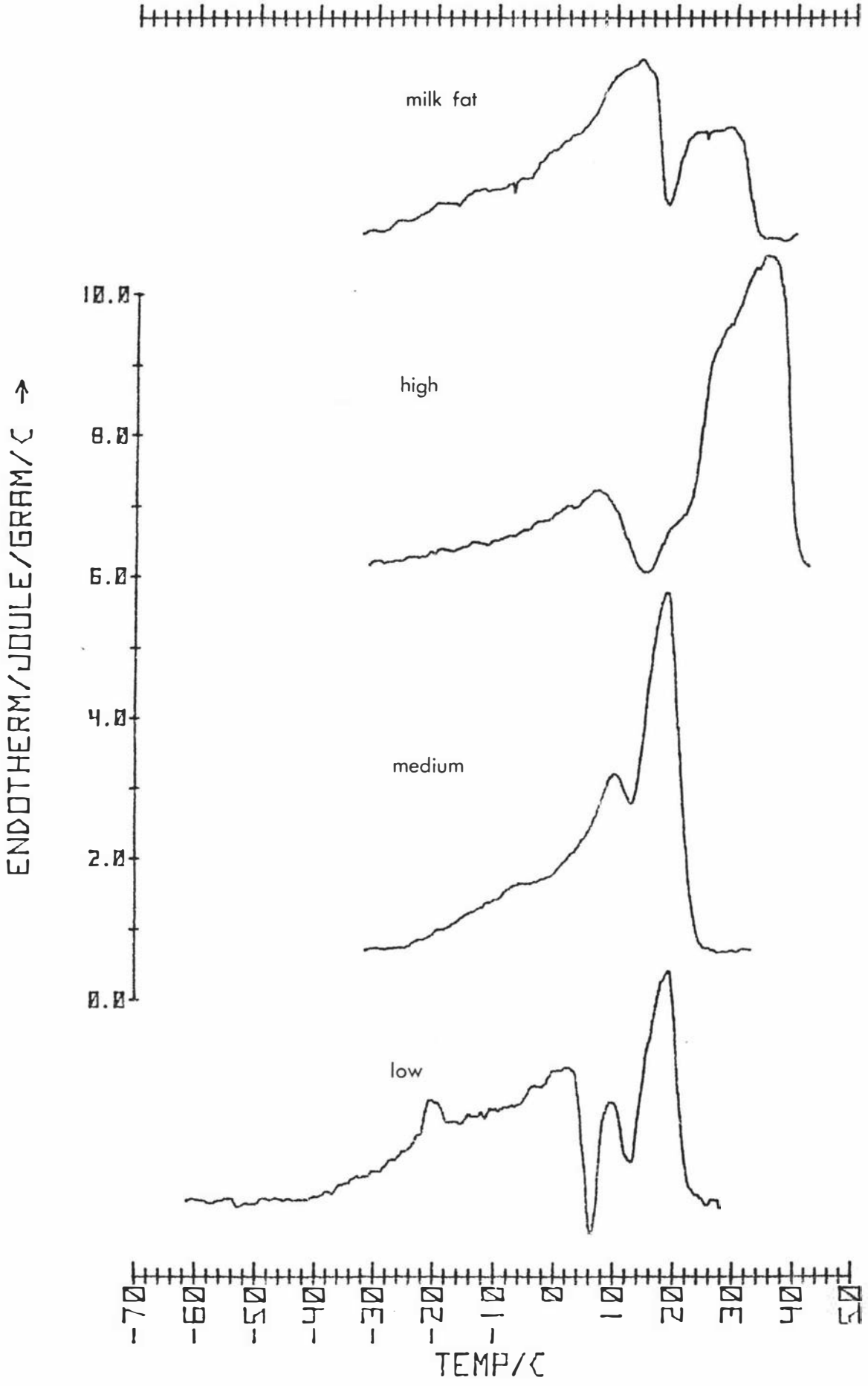



Figure 15. Heating thermograms of the control milk fat and its triacylglycerol fractions of high, medium and low molecular weight.

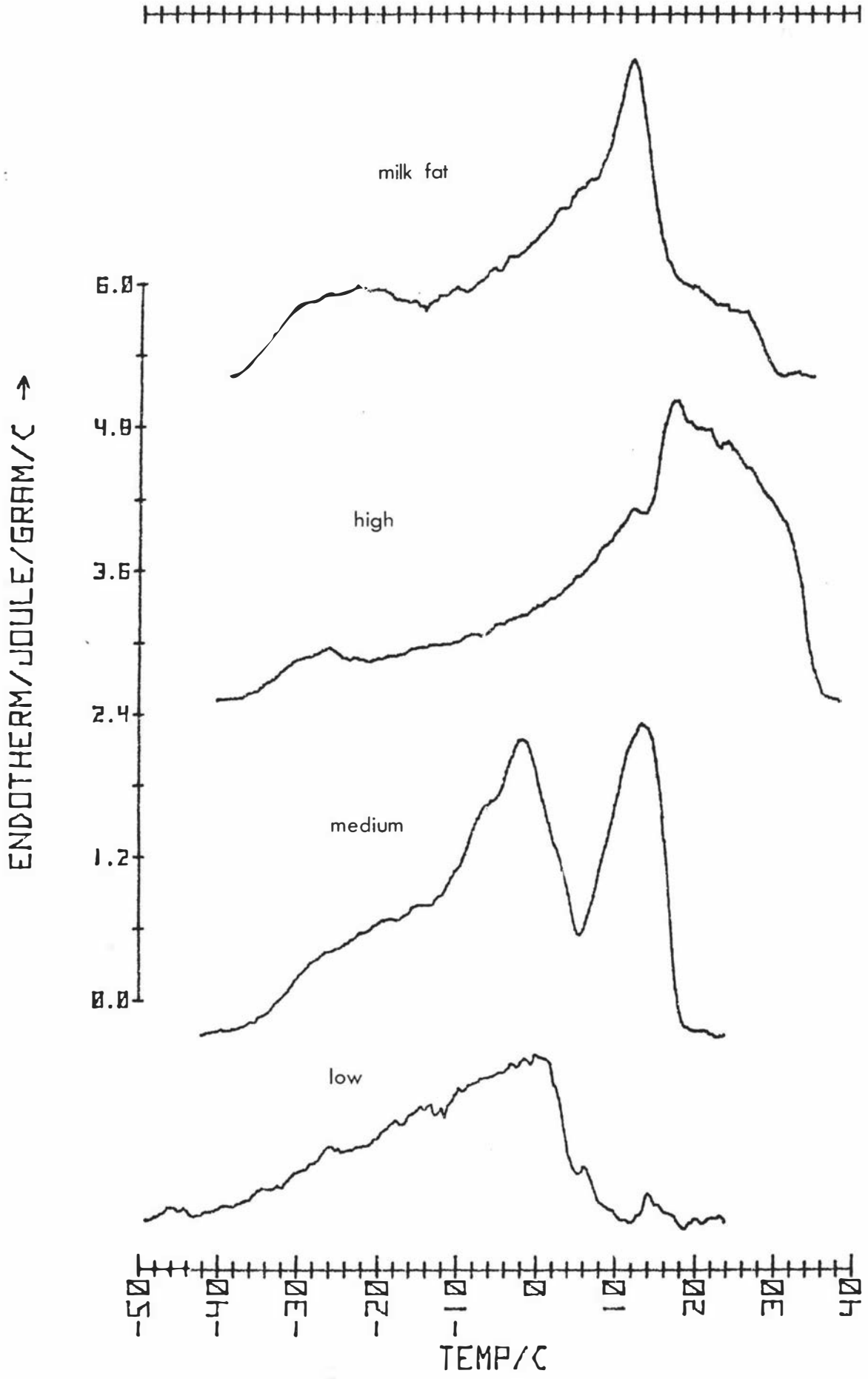



Figure 16. Heating thermograms of the 18:2-rich milk fat and its triacylglycerol fractions of high, medium and low molecular weight.

3.7.4. Thermal analysis of the triacylglycerols of the total milk fats

The control milk fat melted over the range -33 to 34°C, compared to -37 to 30°C for the 18:2-rich milk fat. Two major melting peaks, one at 14°C and another broader peak at 21 - 31°C, were present in the control milk fat: whereas in the 18:2-rich milk fat there was only one major peak, at 12°C, which corresponded to the melting peak of it's high mol. wt. fraction, together with a broad hump at -24°C and a small shoulder between 17 and 28°C. The major melting peak of the 18:2-rich milk fat occurred at a similar temperature (12-13°C) to the first major melting peak of the control milk fat (Figures 15 and 16).

A large dip occurred in the thermogram of the control milk fat at 18°C, but tempering of the sample failed to alter the thermogram indicating that a polymorphic transition was not occurring at that temperature.

3.7.5. Liquid fat content of control and 18:2-rich milk fats and their respective fractions of high, medium and low molecular weight

The liquid fat content of the control and 18:2-rich milk fats and their respective fractions was determined using the integral of the D.S.C. melting curve. Although this does not provide an absolute measurement of liquid fat content, it does provide a measure of the relative liquid fat contents (Figure 18).

The high mol. wt. fraction of the 18:2-rich milk fat melted at a much lower temperature than it's counterpart in the control milk fat, being 50% liquid at 16°C as compared with 29°C for the control fraction (Table 34). At 16°C only about 30% of the high mol. wt. fraction of the control milk fat had melted (Figure 18).

In the medium mol. wt. fraction, the 18:2-rich milk fat again melted at a much lower temperature with the sample being 50% liquid at

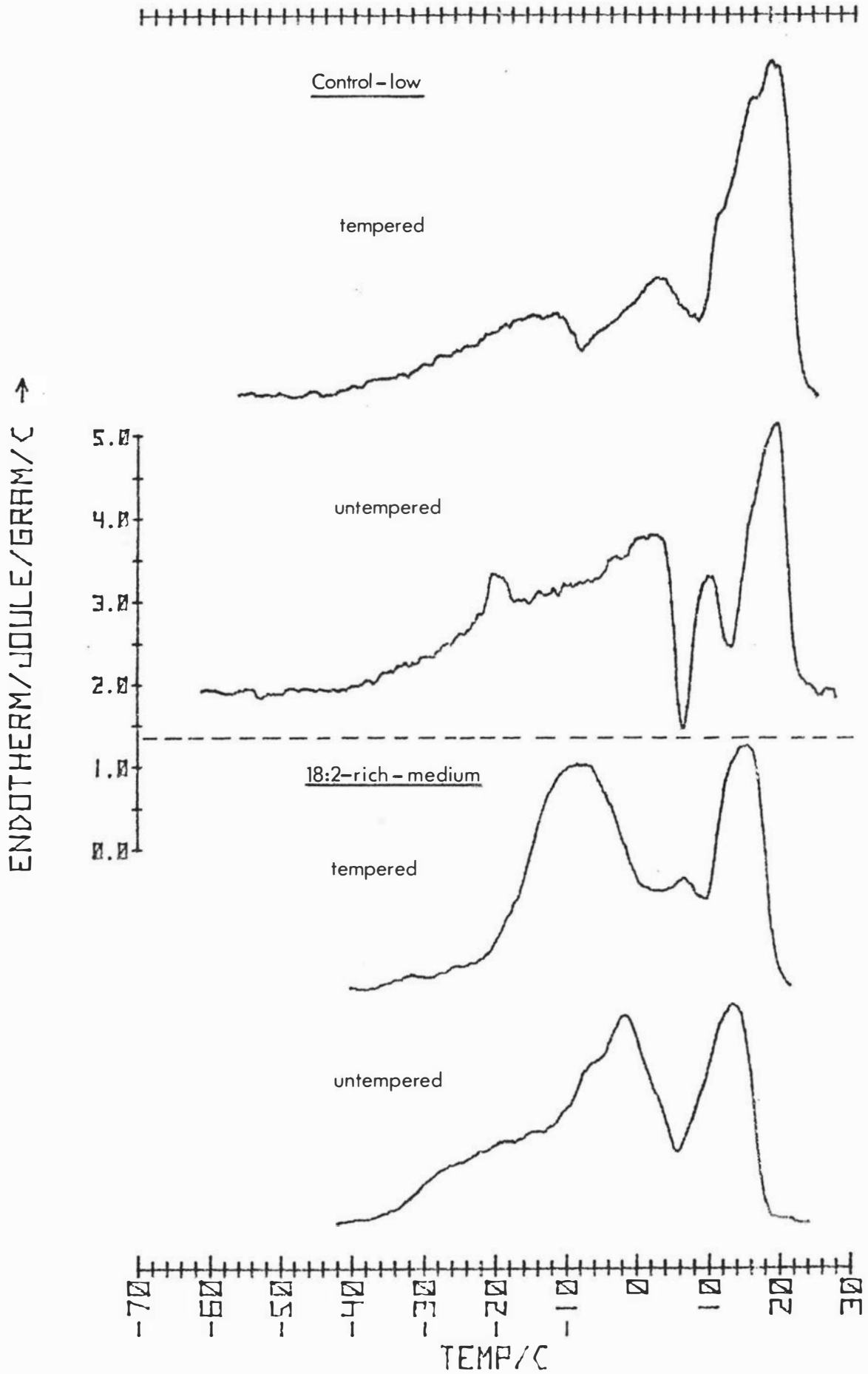



Figure 17. Heating thermograms of the low molecular weight fraction of the control milk fat and the medium molecular weight fraction of the 18:2-rich milk fat with and without the use of the tempering procedure.

-2°C compared to 12°C for the control milk fat.

Table 34. Proportions of liquid fat in the control and 18:2-rich milk fats and their respective triacylglycerol fractions[b]

| | | Temperature for 25% melting (°C) | Temperature for 50% melting (°C) | Temperature for 75% melting (°C) |
|---|---|---|---|---|
| Milk fat | Control | 1 | 10 | 20 |
| | 18:2-rich | -10 | 5 | 12 |
| High mol. wt. | Control | 19 (12)[a] | 29 (27) | 34 (33) |
| | 18:2-rich | 3 | 16 | 24 |
| Medium mol. wt. | Control | 3 | 12 | 18 |
| | 18:2-rich | -11 | -2 | 9 |
| Low mol. wt. | Control | -13 (-10) | -1 (8) | 15 (16) |
| | 18:2-rich | -18 | -8 | -1 |

( )[a] fat percentages after tempering

[b] liquid fat contents obtained by integration of D.S.C. melting curves.

The low mol. wt. fraction of the 18:2-rich milk fat had a 50% liquid fat content at -8°C compared to -1°C for the control milk fat.

At 0°C. the high, medium and low mol. wt. fractions of the 18:2-rich milk fat contained 21%, 56% and 79% liquid fat respectively compared to 11%, 21% and 52% for the same fractions of the control milk fat (Table 35). At 20°C where the low and medium mol. wt. fractions of the 18:2-rich milk fat were completely in the liquid state and the high mol. wt. fraction was 64% liquid, the high mol. wt. fraction of the control milk fat was only 24% liquid and the low and medium mol. wt. fractions were 95% and 92% liquid respectively.

The same effect on the melting of the fat was also evident in the

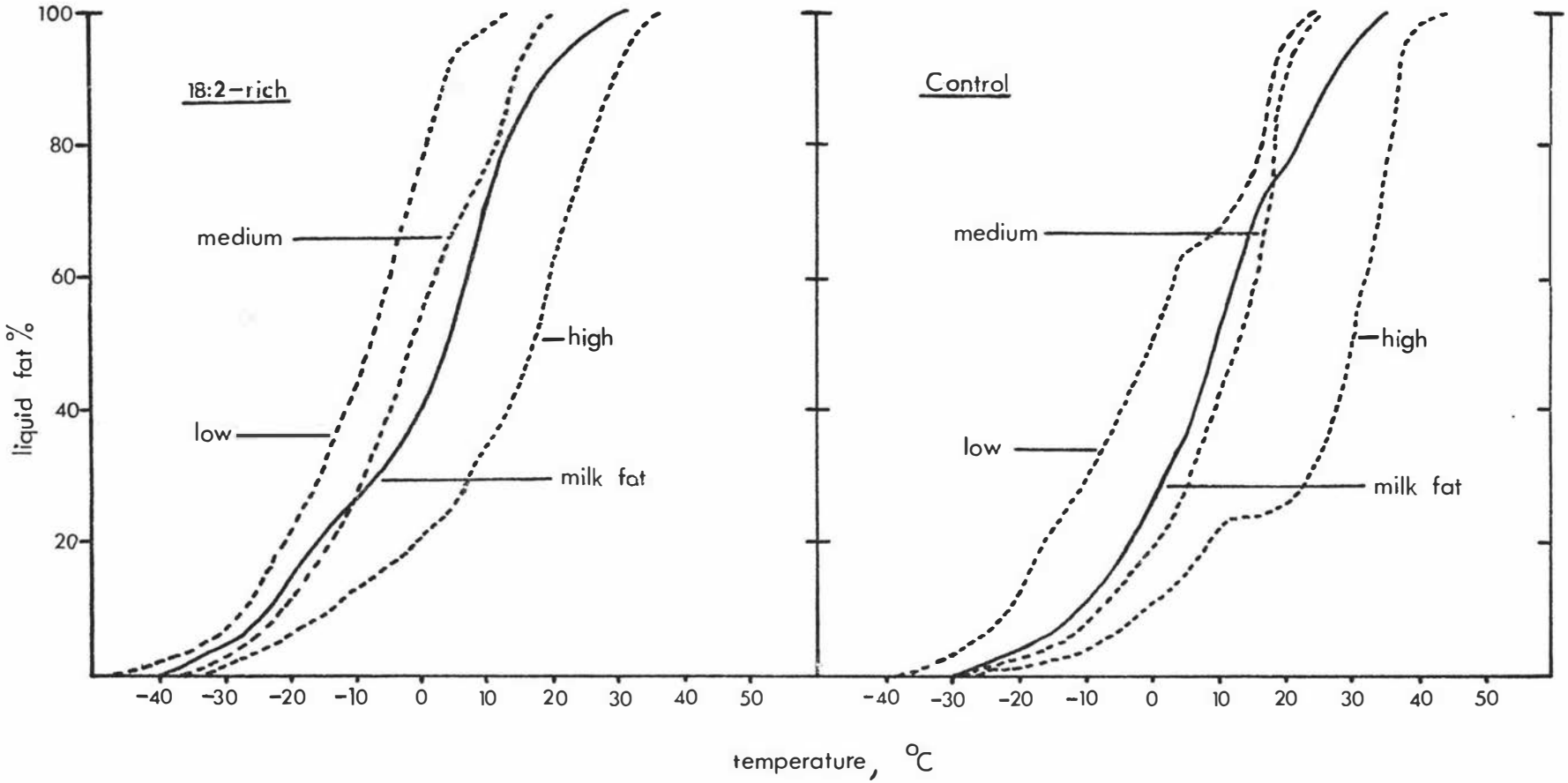



Figure 18. Liquid fat contents of the control and 18:2-rich milk fats and their triacylglycerol fractions of high, medium and low molecular weight, at different temperatures.

Table 35. Proportions of liquid fat in each sample of the control and 18:2-rich milk fats at specified temperatures[b]

| Temperature (°C) | % liquid fat | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | High mol. wt. | | Medium mol. wt. | | Low mol. wt. | | Milk fat | |
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| -30 | 0 (0)[a] | 2 | 0 | 3 (1) | 3 (5) | 7 | 0 | 4 |
| -20 | 1 (1) | 7 | 1 | 12 (5) | 12 (13) | 23 | 3 | 15 |
| -10 | 5 (3) | 13 | 8 | 28 (27) | 31 (25) | 46 | 11 | 25 |
| 0 | 11 (9) | 21 | 21 | 56 (55) | 52 (38) | 79 | 25 | 42 |
| 10 | 22 (23) | 36 | 44 | 76 (73) | 68 (53) | 97 | 50 | 66 |
| 20 | 24 (33) | 64 | 92 | 100 (100) | 95 (94) | 100 | 75 | 92 |
| 30 | 52 (63) | 92 | 100 | 100 (100) | 100 (100) | 100 | 96 | 100 |
| 40 | 100 (100) | 100 | 100 | 100 (100) | 100 (100) | 100 | 100 | 100 |

( )[a] Fat percentages after tempering

[b] Liquid fat contents obtained by integration of D.S.C. melting curves

total milk fat, with 50% of the 18:2-rich milk fat being liquid at 5°C compared to 10°C for the control milk fat.

## Section 3.8. Biosynthesis of triacylglycerols in freshly secreted milk

### 3.8.1. Biosynthesis of triacylglycerols in freshly secreted bovine milk

This study was initiated to determine the manner in which the intake of large amounts of 18:2 by ruminants was affecting TG biosynthesis in the mammary gland. As milk had been found to be a convenient source of enzymes capable of the synthesis of TGs (McCarthy and Patton, 1964; McCarthy et al, 1965; Christie, 1974 a; Kinsella, 1974), freshly secreted milk was used in the current study.

The first aim in these experiments was to obtain a sufficiently high and consistent level of incorporation of FAs into TGs by the enzymes of freshly secreted bovine milk. In the next stage, monozygous twin cows were to be used in paired feeding experiments in which both cows would start on a diet of pasture grass before one animal was fed a daily supplement of 1 kg protected sunflower oil. The rate and level of incorporation of FAs was to be determined in both cows before and after feeding the protected supplement, to obtain a comparison of the TG synthesis in freshly secreted milk collected from cows fed on a normal diet or on a diet high in 18:2, and attempt to relate this to the situation occurring in the mammary gland itself.

The results of a preliminary experiment to establish the presence of suitable enzyme activity in bovine milk are given in Table 36. The whole milk and the supernatant after centrifugation of the milk at 700 xg, incorporated 12.2% and 12.9% respectively of the $[1\text{-}^{14}C]$ palmitate into TGs. Difficulties were experienced in the analyses of

Table 36. Incorporation of [1-$^{14}$C] palmitate into triacylglycerols by freshly secreted bovine milk

| Enzyme source | Added cofactors | Incorporation into triacylglycerols per hour | | |
|---|---|---|---|---|
| | | % | Total (nmoles) | per ml (nmoles) |
| Whole milk | - | 12.2 | 3.7 | 0.12 |
| 700 xg supernatant[a] | - | 12.9 | 3.9 | 0.13 |
| Cream layer[b] | - | 7.3 | 2.2 | 0.07 |
| 25.000 xg supernatant[c] | - | 3.0 | 0.9 | 0.03 |
| 25.000 xg supernatant[c] | ATP (0.5 µmole), CoA (0.2 µmole), glycerol (20 µmole) | 5.8 | 1.7 | 0.06 |

Incubation conditions

(1) Substrate [1-$^{14}$C] palmitate 1.25 x $10^5$ dpm, 0.03 µmole dissolved in ethanol.

(2)[a] Milk centrifuged at 700 xg for 5 min.

(3)[c] Milk centrifuged first at 700 xg for 5 min then at 25,000 xg for 10 min.

(4)[b] Cream layer redispersed in 0.1 M-phosphate buffer, pH 7.5.

(5) Incubation at 37°C for 1 h - 30 ml milk.

both samples because of the presence of large quantities of fat. The level of incorporation achieved compared favourably with the approximately 13.5% incorporation of [1-$^{14}$C] palmitate into TGs obtained by McCarthy and Patton (1964). The redispersed cream layer and the 25,000 xg supernatant incorporated only 7% and 3% of the substrate respectively. The poor incorporation using the 25,000 xg supernatant was in direct contrast with the results of McCarthy and Patton (1964) who obtained good incorporation using a similar centrifugation speed. Some stimulation of the incorporation of [1-$^{14}$C] palmitate was obtained by the addition of the cofactors, ATP, CoA and glycerol.

In subsequent experiments the milk was centrifuged at the higher speed of 1000 xg for 10 min, to remove greater quantities of the fat.

(a) Effect of cofactors

The addition of ATP, CoA or glycerol-3-phosphate to the incubation media had been shown to have little effect on the incorporation of [1-$^{14}$C] palmitate (McCarthy and Patton, 1964; Christie, 1974) but Christie (1974) found a significant stimulation by glycerol. Addition of ATP, CoA and glycerol to the incubations in the present study stimulated incorporation of [1-$^{14}$C] palmitate more than three-fold (Table 37). About 50% of the [1-$^{14}$C] palmitate, which was equivalent to 0.5 nmoles/ml/h was incorporated into TGs in the presence of the cofactors. This level of incorporation was higher than the approximately 0.11 nmoles/ml/h obtained by McCarthy and Patton (1964) in the milk of goats or cows, but was much lower than the 4.4 nmoles/ml/h obtained in the milk of goats by Christie (1974).

Table 37. Incorporation of $[1\text{-}^{14}C]$ palmitate into triacylglycerols by freshly secreted bovine milk

| Enzyme source | Added cofactors | Incorporation into triacylglycerols per hour | | |
|---|---|---|---|---|
| | | % | Total (nmoles) | per ml milk (nmoles) |
| 1000 xg supernatant | - | 15.4 | 4.6 | 0.15 |
| 1000 xg supernatant | ATP, CoA, glycerol | 50.4 | 15.1 | 0.50 |

Incubation conditions

(1) Substrate $[1\text{-}^{14}C]$ palmitate 1.25 x $10^5$ dpm, 0.03 µmoles dissolved in ethanol.

(2) Milk centrifuged at 1000 xg for 10 min.

(3) Cofactors - CoA (0.9 µmole), ATP (3.0 µmole), glycerol (60 µmole)

(4) Incubation at 37°C for 1 h - 30 ml milk.

(b) Effect of substrate dispersion

The effect of two different methods of preparing the substrate are shown in Table 38. After one hour, 13% of the substrate was incorporated into TGs when it was added as a complex with bovine serum albumen [[$1\text{-}^{14}C$] palmitate (BSA)], whereas 8.3% was incorporated when the substrate was dissolved in ethanol [[$1\text{-}^{14}C$] palmitate (Eth.)]. This lower level of incorporation using [$1\text{-}^{14}C$] palmitate (Eth.) could possibly explain the low levels of incorporation achieved by McCarthy and Patton (1964).

Table 38. Effect of substrate dispersion on the incorporation of $[1\text{-}^{14}C]$ palmitate into TGs by freshly secreted bovine milk

| Enzyme source | Added Cofactors | Incorporation into triacylglycerols per hour | | |
|---|---|---|---|---|
| | | % | Total (nmoles) | per ml milk (nmoles) |
| 1000 xg supernatant[a] | ATP, CoA, glycerol | 13.0 | 6.5 | 0.43 |
| 1000 xg supernatant[b] | ATP, CoA, glycerol | 8.3 | 4.1 | 0.27 |

Table 38 (cont.)

Incubation conditions

(1) Substrates - [a] $[1\text{-}^{14}C]$ palmitate (BSA) 2.23 x$10^5$ dpm, 0.05 µmole
[b] $[1\text{-}^{14}C]$ palmitate (Eth.) 2.47 x $10^5$ dpm, 0.05 µmole

(2) Centrifugation of milk - 1000 xg for 10 min.

(3) Cofactors - ATP (1.5 µmoles), CoA (0.45 µmole), glycerol (30 µmole).

(4) Incubation at 37°C for 1 h - 15 ml milk.

(c) Incorporation of long chain fatty acids

Individual long chain FAs are incorporated into TGs by freshly secreted goats milk at different rates (Christie, 1974) with the relative rates being 18:2 > 18:1 > 14:0 > 12:0 > 10:0 > 16:0 > 18:0. To determine whether relative rates were similar in bovine milk fat, three long-chain FAs, $[1\text{-}^{14}C]$ myristate $[1\text{-}^{14}C]$ stearate and $[1\text{-}^{14}C]$ palmitate were incubated with freshly secreted bovine milk for 1 hour (Table 39). Myristate was incorporated into TGs to a higher level than palmitate but on the other hand the incorporation of stearate was only about half that of palmitate. Stearate seemed to give greater incorporation into DGs than either palmitate or myristate, which may indicate that the above difference may not be due to just the lower solubility of stearate. Therefore in summary the relative rates of incorporation into TGs by bovine milk fat were 14:0 > 16:0 > 18:0.

Table 39. Incorporation of long chain fatty acids into lipid classes by freshly secreted bovine milk

| Substrate | Incorporation into lipid classes per h | | | | | |
|---|---|---|---|---|---|---|
| | % | | Total (mmoles) | | per ml milk (mmoles) | |
| | TG | DG | TG | DG | TG | DG |
| $[1\text{-}^{14}C]$ palmitate (BSA) | 10.9 | 1.0 | 5.5 | 0.5 | 0.37 | 0.03 |
| $[1\text{-}^{14}C]$ myristate (BSA) | 13.7 | 1.8 | 6.9 | 0.9 | 0.46 | 0.06 |
| $[1\text{-}^{14}C]$ stearate (BSA) | 5.0 | 2.3 | 2.5 | 1.2 | 0.17 | 0.08 |

Table 39 (cont.)

Incubation conditions

(1) Substrates - $[1\text{-}^{14}C]$ palmitate (BSA) 2.23 x $10^5$ dpm, 0.05 µmoles; $[1\text{-}^{14}C]$ myristate (BSA) 1.80 x $10^5$ dpm. 0.05 µmoles: $[1\text{-}^{14}C]$ stearate (BSA) 2.11 x $10^5$ dpm, 0.05 µmole.

(2) Centrifugation of milk - 1000 xg for 10 min.

(3) Cofactors - ATP ( .5 µmole), CoA (0.45 µmole), glycerol (30 µmole).

(4) Incubation for 60 min at 37°C - 15 ml milk.

(d) Rate of incorporation of palmitate

The $[1\text{-}^{14}C]$ palmitate was rapidly incorporated into TGs over the first 30 min but then incorporation virtually ceased (Figure 19a) (Table 40). The initial rate of incorporation of 0.76 nmoles/ml milk/h was much lower than the initial rate (6 nmoles/ml milk/h) calculated from the results of Christie (1974) using goats milk. but the shape of the rate curves was very similar. Incorporation of palmitate into DGs was much less than into TGs, and reached a maximum after 20 min , then decreased. The initial rate of incorporation into DGs (0.42 nmoles/ml milk/h) over the first 20 min was much slower than the initial rate (6.6 nmoles/ml milk/h) found by Christie (1974), but once again the shape of the rate curve was similar.

Table 40. Rate of incorporation of $[1\text{-}^{14}C]$ palmitate into lipid classes by freshly secreted bovine milk

| Time (min) | Incorporation into lipid classes | | | | | |
|---|---|---|---|---|---|---|
| | % | | Total (nmoles) | | per ml milk (nmoles) | |
| | TG | DG | TG | DG | TG | DG |
| 10 | 6.5 | 2.7 | 3.3 | 1.4 | 0.22 | 0.09 |
| 20 | 9.1 | 4.3 | 4.5 | 2.1 | 0.30 | 0.14 |
| 30 | 11.3 | 3.7 | 5.7 | 1.9 | 0.38 | 0.12 |
| 60 | 13.0 | 3.8 | 6.5 | 1.9 | 0.43 | 0.13 |

Table 40 (cont.)

Incubation conditions

(1) Substrate - [1-$^{14}$C] palmitate (BSA) 2.47 x $10^5$ dpm, 0.05 μmole.

(2) Centrifugation - 1000 xg for 10 min.

(3) Cofactors - ATP (1.5 μmole), CoA (0.45 μmole), glycerol (30 μmole).

(4) Incubation at 37°C for 1 h - 15 ml milk.

(e) Incorporation of [1-$^{14}$C] palmitate into lipids by freshly secreted milk from a pair of monozygous twin cows

Milk samples were collected from a pair of monozygous twin cows and separately incubated with [1-$^{14}$C] palmitate. Rates of incorporation into TGs varied (Table 41) but the final level of incorporation attained by milk from both cows was similar (Figure 19a).

Table 41. Rates of incorporation of [1-$^{14}$C] palmitate into triacylglycerols by freshly secreted milk from a pair of monozygous twin cows

| Time (min) | Incorporation into TGs | | | | | |
|---|---|---|---|---|---|---|
| | % | | Total (nmoles) | | per ml milk (nmoles) | |
| | Twin 1 | Twin 2 | Twin 1 | Twin 2 | Twin 1 | Twin 2 |
| 5 | 3.7 | 4.7 | 1.9 | 2.4 | 0.13 | 0.16 |
| 10 | 9.1 | 6.0 | 4.6 | 3.0 | 0.31 | 0.20 |
| 25 | 11.2 | 10.8 | 5.6 | 5.4 | 0.37 | 0.36 |
| 40 | 11.4 | 11.8 | 5.7 | 5.9 | 0.38 | 0.39 |
| 60 | 11.6 | 11.0 | 5.8 | 5.5 | 0.39 | 0.37 |
| 90 | 12.9 | 11.8 | 6.5 | 5.9 | 0.43 | 0.39 |

Incubation conditions

(1) Substrate - [1-$^{14}$C] palmitate (BSA) 3.52 x $10^5$ dpm, 0.05 μmoles.

(2) Centrifugation - 1000 xg for 10 min.

(3) Cofactors - ATP (1.5 μmoles), CoA (0.45 μmoles), glycerol (30 μmoles).

(4) Incubation at 37°C - 15 ml milk.

These two cows were nearing the end of their lactation period, and although the above levels of incorporation were somewhat less than satisfactory it was decided to proceed with a preliminary experiment to determine the possibility of any effect of feeding protected sunflower oil on the characteristics of the synthesis of TGs in freshly secreted milk. One of the cows of the twin pair was fed the protected sunflower oil as a daily supplement to pasture, while the other member of the pair continued on pasture. Unfortunately before a suitable level < 18:2 in the milk could be achieved, i.e. a level considered high enough to affect TG biosynthesis in the mammary gland, the milk yields of the two cows, especially the animal receiving the protected sunflower oil, had declined to the point where insufficient milk was available for the experiment.

(f) Attempts to improve the levels of incorporation

At the beginning of the next dairying season, freshly lactating cows (1 - 2 months) were used in a further attempt to achieve greater and more constant levels of incorporation (Table 42). Noticeably improved incorporations of palmitate were obtained by (1) the addition of glycerol-3-phosphate and $MgCl_2$ as additional cofactors, (2) hand milking. Unfortunately variations in incorporation were experienced between experiments, irrespective of whether the milk was collected from the same cow or a different cow. For example the levels of incorporation in two experiments, under seemingly identical conditions were 1.09 nmoles/ml milk/h and 0.62 nmoles/ml milk/h. Overall the incorporation varied between 0.12 and 1.09 nmoles/ml milk/h. This contrasts markedly with the results of Christie (1974) using goats, who achieved very consistent results between experiments with standard deviations being less than 10 - 12% of the mean value. Only one experiment, in the present study, gave incorporation in the range

Table 42. Incorporation of [1-$^{14}$C] palmitate into TGs by freshly secreted bovine milk

| Enzyme source | Cofactors | Incorporation into TGs | |
|---|---|---|---|
| | | % | per ml milk (nmoles) |
| 1000 xg supernatant * | CoA, glycerol, ATP | 2.6 ± 0.1 | 0.22 ± 0.03 |
| 1000 xg supernatant * | CoA, glycerol, ATP, Glycerol-3-phosphate | 7.3 ± 2.5 | 0.62 ± 0.21 |
| 1000 xg supernatant * | CoA, glycerol, ATP, $MgCl_2$ | 3.1 ± 0.3 | 0.26 ± 0.03 |
| 1000 xg supernatant * | CoA, glycerol, ATP, DTT | 1.6 ± 0.3 | 0.13 ± 0.03 |
| 1000 xg supernatant * | CoA, glycerol, ATP, NaF | 2.4 ± 0.5 | 0.20 ± 0.04 |
| 1000 xg supernatant * | CoA, glycerol, ATP, EDTA | 1.5 ± 0.3 | 0.12 ± 0.03 |
| 1000 xg supernatant * | CoA, glycerol, ATP, $MgCl_2$, DTT | 2.6 ± 0.2 | 0.22 ± 0.02 |
| 1000 xg supernatant * | CoA, glycerol, ATP, DTT, NaF, EDTA | 2.4 ± 0.8 | 0.20 ± 0.07 |
| 1000 xg supernatant + c | CoA, glycerol, ATP, Glycerol-3-phosphate | 1.9 ± 0.3 | 0.16 ± 0.03 |
| 1000 xg supernatant + d | CoA, glycerol, ATP, Glycerol-3-phosphate | 3.0 ± 0.9 | 0.25 ± 0.08 |
| 1000 xg supernatant[a] | CoA, glycerol, ATP, Glycerol-3-phosphate | 32.6 ± 2.2 | 1.09 ± 0.07 |
| Buttermilk[ae] | CoA, glycerol, ATP, Glycerol-3-phosphate | 1.1 ± 0.3 | 0.04 ± 0.01 |
| 1000 xg supernatant (5 min)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 2.7 ± 1.3 | 0.27 ± 0.13 |
| 1000 xg supernatant (3 min)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 3.5 ± 1.2 | 0.35 ± 0.12 |
| 1000 xg supernatant (1½ min)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 4.4 ± 1.0 | 0.44 ± 0.10 |
| 1000 xg supernatant (10 sec)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 4.2 ± 0.6 | 0.42 ± 0.06 |
| 700 xg supernatant (3 min)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 5.0 ± 0.9 | 0.50 ± 0.09 |
| 700 xg supernatant (10 sec)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 4.4 ± 0.5 | 0.44 ± 0.05 |
| 1500 xg supernatant (3 min)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 4.8 ± 0.9 | 0.48 ± 0.09 |
| 1500 xg supernatant (10 sec)[bf] | CoA, glycerol, ATP, Glycerol-3-phosphate | 4.3 ± 0.5 | 0.43 ± 0.05 |

(To be continued)

Table 42. Incorporation of $[1\text{-}^{14}C]$ palmitate into TGs by freshly secreted bovine milk (cont.)

Incubation conditions

(1) Substrates * $[1\text{-}^{14}C]$ palmitate (BSA) $1.82 \times 10^5$ dpm, 0.1 µmoles

\+ $[1\text{-}^{14}C]$ palmitate (BSA) $2.02 \times 10^5$ dpm, 0.1 µmoles

[a] $[1\text{-}^{14}C]$ palmitate (BSA) $3.82 \times 10^5$ dpm, 0.05 µmoles

[b] $[1\text{-}^{14}C]$ palmitate (BSA) $3.50 \times 10^5$ dpm, 0.05 µmoles

(2) Cofactors (per 10 ml milk), ATP (1 µmole), glycerol (20 µmole), glycerol-3-phosphate (10 µmole), $MgCl_2$ (0.5 µmole), NaF (5 µmole), EDTA (5 µmole), DTT (0.5 µmole), CoA (0.3 µmole)

(3) Centrifugation of milk - 1000 xg for 10 min unless otherwise stated.

(4) Incubation - 60 min at 37°C

(5) [c] Milk collected from single machine milking.

[d] Milk collected from hand milking after previously machine milking.

(6) [e] Cream layer redispersed in 0.1 M-phosphate buffer then recentrifuged at 1000 xg.

(7) [f] Samples centrifuged using different times for the attainment of the final speed.

(1 to 2 nmoles/ml milk/h) which was considered to be sufficient to permit comparison of milk from different cows.

3.8.2. Biosynthesis of triacylglycerols in freshly secreted goats milk

As Christie (1974) had achieved rapid incorporation of fatty acids into TGs using goats milk preliminary investigations were carried out to determine whether goats might be more suitable animals for use in comparative experiments.

Preliminary experiments using freshly secreted milk from goats confirmed the presence of an active system for incorporation of $[1\text{-}^{14}C]$ palmitate into TGs (Table 43). This incorporation was stimulated by the addition of CoA, ATP and glycerol to the incubation media. After a 1 h incubation, 31% of the $[1\text{-}^{14}C]$palmitate (31.1 nmoles) was incorporated into lipids, with 88% going into TGs, 6.4% into DGs, and the remainder into phospholipids.

More comprehensive experiments were carried out to determine the rate of incorporation of palmitate and linoleate into TGs, and the effect of different cofactors on the incorporation of 16:0 (Tables 44 and 45) (Figures 19b and 19c). Incorporation into TGs was rapid in all instances. The greatest rate of incorporation of palmitate occurred in the presence of glycerol, ATP, CoA, $Mg^{2+}$ and glycerol-3-phosphate, and in the presence of glycerol alone (Table 44) (Figure 19b). With these cofactors, incorporation was still taking place after 120 min, although at a much slower rate than initially. In the absence of cofactors, the rate of incorporation was slower, and with the level of incorporation being 25 - 30% lower. Glycerol-3-phosphate alone appeared to be slightly inhibitory. Linoleate, with glycerol added as cofactor, was incorporated into TGs up to a level 10% higher than palmitate, and with a very similar initial rate (Table 45) (Figure 19c).

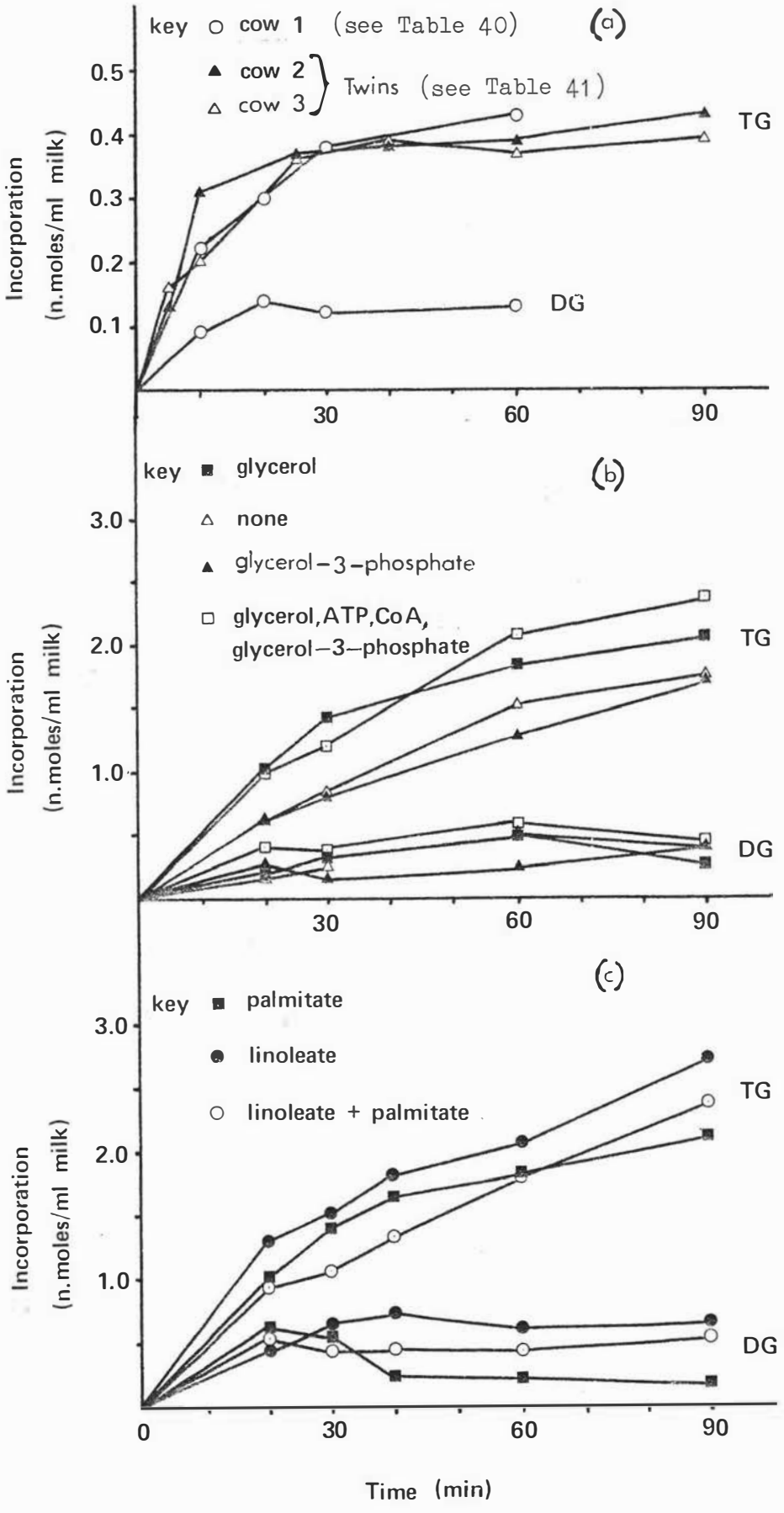



Figure 19. Rate of incorporation of fatty acids into triacylglycerols and diacylglycerols by freshly secreted bovine and goats milk.

Table 43. Incorporation of $[1\text{-}^{14}C]$ palmitate into lipids by freshly secreted goats milk

| Time of incubation (min) | Added Cofactors | Incorporation into lipids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % Incorporation | | | Total (nmoles) | | | per ml milk (nmoles) | | |
| | | Total | TG | DG | Total | TG | DG | Total | TG | DG |
| 30 | ATP, glycerol, CoA | 20.8 | 15.7 | 3.1 | 20.8 | 15.7 | 3.1 | 1.73 | 1.31 | 0.26 |
| 60 | | 31.1(31.4)[a] | 27.5(28.5) | 2.0(1.8) | 31.1(31.4) | 27.5(28.5) | 2.0(1.8) | 2.59(2.62) | 2.29(2.37) | 0.17(0.15) |
| 30 | - | 12.3 | 9.4 | 1.6 | 12.3 | 9.4 | 1.6 | 1.02 | 0.78 | 0.13 |
| 60 | | 24.1(24.6)[a] | 21.4(21.5) | 1.2(1.3) | 24.1(24.6) | 21.4(21.5) | 1.2(1.3) | 2.01(2.05) | 1.78(1.79) | 0.10(0.11) |

Incubation conditions

(1) Substrate - $[1\text{-}^{14}C]$ palmitate (BSA) 2.10 x$10^5$ dpm, 0.1 µmoles

( )[a] $[1\text{-}^{14}C]$ palmitate (BSA) 3.52 x$10^5$ dpm, 0.1 µmoles

(2) Cofactors - ATP (1.0 µmoles), CoA (0.3 µmoles), glycerol (20 µmoles)

(3) Centrifugation of milk - 1000 xg for 10 min.

(4) Incubation at 37°C - 12 ml milk.

Table 44. Incorporation of [1-$^{14}$C] palmitate into lipids by freshly secreted goats milk

| Time of incubation (min) | Added cofactors | Incorporation into lipids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % Incorporation | | | Total (nmoles) | | | per ml milk (nmoles) | | |
| | | Total | TG | DG | Total | TG | DG | Total | TG | DG |
| 20 | - | 8.9 | 5.6 | 1.3 | 9.8 | 6.2 | 1.4 | 0.98 | 0.62 | 0.14 |
| 30 | - | 13.3 | 7.7 | 2.2 | 14.7 | 8.4 | 2.4 | 1.47 | 0.84 | 0.24 |
| 60 | - | 23.2 | 13.8 | 4.7 | 25.5 | 15.2 | 5.1 | 2.55 | 1.52 | 0.51 |
| 90 | - | 23.8 | 15.9 | 3.5 | 26.2 | 17.5 | 3.8 | 2.62 | 1.75 | 0.38 |
| 20 | glycerol | 14.3 | 9.3 | 1.5 | 15.7 | 10.2 | 1.7 | 1.57 | 1.02 | 0.17 |
| 30 | glycerol | 20.0 | 13.1 | 2.8 | 22.0 | 14.4 | 3.1 | 2.20 | 1.44 | 0.31 |
| 60 | glycerol | 26.5 | 16.7 | 4.4 | 29.2 | 18.4 | 4.8 | 2.92 | 1.84 | 0.48 |
| 90 | glycerol | 26.4 | 18.5 | 2.3 | 29.0 | 20.4 | 2.5 | 2.90 | 2.04 | 0.25 |
| 20 | glycerol-3-phosphate | 9.5 | 5.6 | 2.5 | 10.4 | 6.1 | 2.8 | 1.04 | 0.61 | 0.28 |
| 30 | glycerol-3-phosphate | 11.0 | 7.1 | 1.4 | 12.1 | 7.8 | 1.5 | 1.21 | 0.78 | 0.15 |
| 60 | glycerol-3-phosphate | 17.2 | 11.5 | 2.1 | 18.9 | 12.7 | 2.3 | 1.89 | 1.27 | 0.23 |
| 90 | glycerol-3-phosphate | 21.3 | 15.4 | 3.5 | 23.5 | 17.0 | 3.9 | 2.35 | 1.70 | 0.39 |
| 20 | ATP, CoA | 17.5 | 9.0 | 3.7 | 19.3 | 9.9 | 4.0 | 1.93 | 0.99 | 0.40 |
| 30 | glycerol | 17.8 | 10.8 | 3.4 | 19.6 | 11.9 | 3.7 | 1.96 | 1.19 | 0.37 |
| 60 | glycerol-3-phosphate | 29.5 | 18.8 | 5.4 | 32.5 | 20.7 | 5.9 | 3.25 | 2.07 | 0.59 |
| 90 | $MgCl_2$ | 30.3 | 21.4 | 4.0 | 33.3 | 23.6 | 4.3 | 3.33 | 2.36 | 0.43 |

Incubation conditions

(1) Substrate - [1-$^{14}$C] palmitate (BSA) 1.73 x $10^5$ dpm, 0.11 µmoles.

(2) Cofactors - ATP (1 µmoles), CoA (0.3 µmoles), glycerol (20 µmoles), glycerol-3-phosphate (10 µmoles), $MgCl_2$ (0.5 µmoles).

(3) Centrifugation of milk - 1000 xg for 10 min.

(4) Incubation at 37°C - 10 ml milk.

Table 45. Incorporation of [1-$^{14}$C] palmitate and [1-$^{14}$C] linoleate into lipids by freshly secreted goats milk

| Time of incubation (min) | Substrate | Incorporation into lipids | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | % Incorporation | | | Total (nmoles) | | | per ml milk (nmoles) | | |
| | | Total | TG | DG | Total | TG | DG | Total | TG | DG |
| 20 | [1-$^{14}$C] palmitate[a] | 11.9 | 6.1 | 3.7 | 23.8 | 12.2 | 7.4 | 1.98 | 1.01 | 0.61 |
| 30 | | 12.8 | 8.5 | 3.3 | 25.6 | 17.0 | 6.6 | 2.13 | 1.41 | 0.55 |
| 40 | | 14.1 | 10.1 | 1.5 | 28.2 | 20.2 | 3.0 | 2.35 | 1.66 | 0.25 |
| 60 | | 15.3 | 11.1 | 1.3 | 30.6 | 22.2 | 2.6 | 2.55 | 1.83 | 0.21 |
| 90 | | 16.4 | 12.6 | 1.1 | 32.8 | 25.2 | 2.2 | 2.73 | 2.10 | 0.18 |
| 20 | [1-$^{14}$C] linoleate[a] | 16.5 | 7.7 | 2.6 | 33.0 | 15.4 | 5.2 | 2.75 | 1.30 | 0.43 |
| 30 | | 21.0 | 9.1 | 3.9 | 42.0 | 18.2 | 7.8 | 3.50 | 1.52 | 0.65 |
| 40 | | 22.6 | 10.9 | 4.4 | 45.2 | 21.8 | 8.8 | 3.93 | 1.82 | 0.75 |
| 60 | | 22.2 | 13.4 | 3.5 | 44.4 | 24.8 | 7.0 | 3.70 | 2.07 | 0.60 |
| 90 | | 25.7 | 16.3 | 3.7 | 51.4 | 32.6 | 7.4 | 4.29 | 2.72 | 0.63 |
| 20 | [1-$^{14}$C] palmitate[b] | 13.8 | 5.6 | 3.2 | 27.6 | 11.2 | 6.2 | 2.30 | 0.93 | 0.52 |
| 30 | | 13.2 | 6.3 | 2.6 | 26.4 | 12.6 | 5.2 | 2.20 | 1.05 | 0.43 |
| 40 | | 15.4 | 7.9 | 2.7 | 30.8 | 15.8 | 5.4 | 2.57 | 1.32 | 0.45 |
| 60 | [1-$^{14}$C] linoleate[b] | 18.0 | 10.8 | 2.5 | 36.0 | 21.6 | 5.0 | 3.00 | 1.80 | 0.42 |
| 90 | | 22.5 | 14.2 | 3.1 | 45.0 | 28.4 | 6.2 | 3.75 | 2.37 | 0.52 |

Incubation conditions

(1) Substrates - [1-$^{14}$C] palmitate[a] (BSA) - 5.44 x $10^5$ dpm, 0.2 µmoles [1-$^{14}$C] palmitate[b] (BSA) - 2.06 x $10^5$ dpm, 0.1 µmoles
[1-$^{14}$C] linoleate[a] (BSA) - 4.49 x $10^5$ dpm, 0.2 µmoles [1-$^{14}$C] linoleate[b] (BSA) - 2.65 x $10^5$ dpm, 0.1 µmoles

(2) Cofactor - glycerol (24 µmoles).

(3) Centrifugation of milk - 1000 xg for 10 min.

(4) Incubation at 37°C - 12 ml milk.

Incubation with palmitate and linoleate gave a rate of incorporation only slightly below the individual rates of incorporation of palmitate and linoleate.

The rate of incorporation of fatty acids into TGs by goats milk was greater than by bovine milk, with 2.1 nmoles palmitate incorporated/ml milk/h compared to only 0.5 nmoles/ml milk/h for bovine milk. With goats milk, there was a greater consistency between experiments, with the incorporation varying between 2.04 and 2.37 nmoles/ml milk/h, compared with a variation between 0.62 and 1.09 nmoles/ml milk/h for bovine milk. The rate of incorporation obtained using goats milk was still somewhat lower than the rate of 4.4 nmoles/ml milk/h achieved by Christie (1974).

### 3.8.3. Positional analysis of the triacylglycerols synthesized by freshly secreted goats milk

The proportions of 16:0 and 18:2 in position 2 of the triacylglycerols formed by incubation with these two FAs were determined by hydrolysis of the TGs with pancreatic lipase (Section 2.5.1.b.).

Palmitate was found to be preferentially located in position 2 (58% of the 16:0), whereas linoleate was almost evenly distributed between the primary and secondary positions of the TG, but with a slight preference for the primary positions (Table 46). This was very similar to the results of Christie (1974) who found 53% of the 16:0 in position 2 and 30% of 18:2 in position 2. As concluded by Christie (1974) it is obvious that FAs enter all three positions of the TG and that positional specificities do exist in this TG biosynthesizing system.

When radicactive TGs containing both $[1\text{-}^{14}C]$ 16:0 and $[1\text{-}^{14}C]$ 18:2 were analysed, 16:0 was found to be concentrated in position 2 (86% of the 16:0) whereas 18:2 showed the same distribution as when incubated

Table 46. Positional analysis of triacylglycerols synthesized by freshly secreted goats milk

| Substrate | Relative no. of moles of FA in TG [c] | Relative no. of moles of FA in MG [c] | Counts per min/ mole of FA in TG | Counts per min/ mole of FA in MG | Proportion of radioactivity in position 2 |
|---|---|---|---|---|---|
| [1-$^{14}$C] palmitate[a] | 1.00 | 0.17 | $1.91 \times 10^4$ | $1.10 \times 10^4$ | 57.7% |
| [1-$^{14}$C] linoleate | 1.00 | 0.24 | $5.16 \times 10^3$ | $1.46 \times 10^3$ | 28.3% |
| [1-$^{14}$C] palmitate[b] | 1.00 | 0.21 | $6.87 \times 10^3$ | $5.92 \times 10^3$ | 86.2% |
| [1-$^{14}$C] linoleate | 1.00 | 0.21 | $5.92 \times 10^3$ | $1.33 \times 10^3$ | 22.5% |

Incubation conditions

(1) Substrate - [1-$^{14}$C] palmitate[a](BSA) $5.44 \times 10^5$ dpm, 0.2 µmoles
[1-$^{14}$C] linoleate[a](BSA) $4.49 \times 10^5$ dpm, 0.2 µmoles
[1-$^{14}$C] palmitate[b](BSA) $2.06 \times 10^5$ dpm, 0.1 µmoles
[1-$^{14}$C] linoleate[b](BSA) $2.65 \times 10^5$ dpm, 0.1 µmoles

(2) Cofactors - glycerol (24 µmoles).

(3) Incubation - 12 ml milk for 90 min at 37°C.

(4)[c] Relative no. of moles of FA in TG and MG determined by adding internal standard (17:0) before g.l.c. as FA methyl esters.

alone. Therefore it would appear that the presence of added linoleate (0.1 μmole) was affecting the esterification of palmitate. The significance of such a result was difficult to assess because of several factors, one of which was how much of the unesterified fatty acid already in the milk (Christie, 1974) was available to the synthetase, another was whether or not the synthetase system in the freshly secreted milk was capable of utilising FAs in a similar manner to that occurring in vivo.

# Chapter 4

# DISCUSSION

## Section 4.1. General

The availability of high levels of linoleic acid for uptake by the tissues of non-ruminants (Privett et al, 1965) and ruminants (Scott et al, 1971) has been observed to raise the level of 18:2 in the TGs of both milk and depot fats. The effect of the elevated levels of 18:2 on the positional distribution of FAs in the TGs has been determined in the depot fat of the rat (Privett et al, 1965) and in the depot fat of the sheep (Scott and Cook, 1975; Hawke et al, 1976), and the current study was undertaken to determine the effect of elevated levels of 18:2 on the positional distribution in bovine milk fat. In addition the effect on the composition of the TG classes of differing degrees of unsaturation was determined, and used to assist in assessing the effect on the abundance of individual TG species. This information was then related to the thermal properties of the milk fat.

In this discussion the FA and TG composition, the levels of the TG classes of differing levels of unsaturation, the positional distribution of FAs in the TGs and the thermal properties of a milk fat containing elevated levels of 18:2 will be compared with the composition and properties of milk fat containing normal levels of 18:2. In addition the effects of the elevated levels of 18:2 on the composition and properties of the milk fat will be compared with the effect of elevated 18:2 on the composition and properties of several depot fats. Also a comparison will be made with the composition of animal depot fats, e.g. dog, and seed fats, e.g. sunflower seeds, naturally containing higher levels of 18:2.

Section 4.2. The effect of elevated levels of linoleic acid on the composition of fats and oils

(a) Fatty acid composition of milk fat

Feeding the protected supplement of sunflower oil at the rate of 3 kg per day gave milk fat containing a level of 15.8 mole % 18:2 (equivalent to 18.7 wt. % 18:2) after 7 days. This compared favourably with a level of approximately 25% 18:2 (by wt.) obtained by Cook et al (1972) on feeding 1 kg per day of a protected supplement of safflower oil for about 13 days. Concurrently the yield of fat in the milk increased from 560 to 690 g with the feeding of the oil seed supplement which was in agreement with the observations of Pan et al (1972). The increased level of 18:2 in the milk fat was accompanied by a decrease in the levels of 14:0 and 16:0 as observed by Cook et al (1972).

(b) Triacylglycerol composition of milk fat

The major effect of the feeding of protected supplement on the TG composition of milk fat, was to increase the proportions of $C_{54}$ and to a lesser extent $C_{40}$ TGs. In contrast Edmondson et al (1974) observed that milk fat containing 30% 18:2 had a higher proportion of $C_{54}$ TGs but a normal level of $C_{40}$ TGs when compared to normal milk fats.

As would be expected with the replacement of 14:0 and 16:0 by 18:2, the observed increases in the levels of $C_{40}$ and $C_{54}$ TGs were accompanied by decreases in the levels of $C_{36}$, $C_{38}$ and $C_{50}$ TGs. $C_{52}$ showed a slight increase.

(c) Fractionation of milk fat

Separation of milk fat into fractions by adsorption chromatography gives a reasonable degree of constancy in the relative levels of high, medium and low mol. wt. TGs, even when the milk is from different sources (Breckenridge and Kuksis, 1968b, 1969; Taylor and Hawke, 1975a;

Section 3.2.1.), with the high mol. wt. fraction comprising 36 - 41% of the milk fat, the medium mol. wt. fraction 16 - 20% and the low mol. wt. fraction 42 - 45%.

However in the milk fat high in 18:2 the proportions of the fractions of high, medium and low mol. wt. were 43.0, 19.5 and 37.5% respectively, indicating a higher proportion of high mol. wt. TGs and a lower proportion of low mol. wt. TGs in the 18:2-rich milk fat than in normal milk fat. The FA composition of the three fractions of the 18:2-rich milk fat showed the trends as the total milk fat with higher proportions of 18:2 and lower proportions of 14:0 and 16:0 than in the corresponding fractions of the control milk fat. Similarly the TG composition of TG fractions of the 18:2-rich milk fat showed the same trends relative to the respective fractions of control milk fat as the total 18:2-rich milk fat exhibited relative to the total control milk fat, having higher proportions of: $C_{40}$, in the low mol. wt. fraction, $C_{40}$ in the medium mol. wt. fraction and $C_{54}$ in the high mol. wt. fraction.

(d) Composition of the triacylglycerol classes of differing levels of unsaturation

The separation of TGs into groups which differ in the average unsaturation, using argentation t.l.c., has been used extensively to study the TG composition of fats and oils (Gunstone and Qureshi, 1965; Privett et al, 1965; Breckenridge and Kuksis, 1968b, 1969; Gold, 1968; Bottino et al, 1970; Christie and Moore, 1970; Taylor and Hawke, 1975a; Hawke et al, 1976). When the TG fractions of high, medium and low mol. wt. obtained from the control milk fat were separated using this technique, the TG fractions contained similar proportions of the different TG classes to those obtained by Taylor and Hawke (1975a) in samples of New Zealand milk fat (Table 48). Corresponding TG classes

Table 48. Proportions of the triacylglycerol classes of milk fat of differing levels of unsaturation

| TG class | 18:2-rich (mole %) | | Control (mole %) | | Ref.1[a] (mole %) | | |
|---|---|---|---|---|---|---|---|
| | observed | predicted[b] | observed | predicted[b] | Sept. | Jan. | March |
| **TG fraction of high mol. wt.** | | | | | | | |
| Saturated TGs | 20.0 | 15.4 | 29.5 | 40.0 | 28.5 | 33.8 | 31.7 |
| Monoene TGs | 24.6 | 25.8 | 44.0 | 42.3 | 39.3 | 41.1 | 41.1 |
| Diene TGs | 27.5 | 27.6 | 17.5 | 19.0 | 21.7 | 17.5 | 17.4 |
| Triene TGs | 15.6 | 18.5 | 8.9 | 3.2 | 10.5 | 7.6 | 9.8 |
| Tetraene TGs | 12.1 | 8.4 | - | - | - | - | - |
| **TG fraction of medium mol. wt.** | | | | | | | |
| Saturated TGs | 28.0 | 31.7 | 46.9 | 47.8 | 45.4 | 50.5 | 49.1 |
| Monoene TGs | 32.0 | 25.1 | 31.7 | 35.9 | 37.5 | 30.2 | 32.9 |
| Diene TGs | 22.5 | 22.1 | 13.2 | 11.7 | 10.3 | 11.2 | 10.0 |
| Triene TGs | 9.0 | 8.7 | 8.1 | 3.2 | 6.8 | 8.1 | 8.0 |
| Tetraene TGs | 8.5 | 3.6 | - | - | - | - | - |
| **TG fraction of low mol. wt.** | | | | | | | |
| Saturated TGs | 31.2 | 36.2 | 47.2 | 56.1 | 46.6 | 54.3 | 53.4 |
| Monoene TGs | 25.1 | 24.5 | 37.3 | 32.4 | 37.2 | 32.7 | 32.0 |
| Diene TGs | 27.7 | 26.1 | 9.2 | 8.4 | 9.9 | 8.4 | 9.0 |
| Triene TGs | 9.1 | 8.7 | 6.3 | 2.4 | 6.3 | 4.6 | 5.6 |
| Tetraene TGs | 6.9 | 3.3 | - | - | - | - | - |

[a] Taylor and Hawke (1975a)

[b] Predicted from 1-random, 2-random, 3-random hypothesis

also had similar FA and TG compositions. Conversely all fractions of the 18:2-rich milk fat contained lower proportions of saturated and monoene TGs, and higher proportions of the more unsaturated TG classes, namely the diene, triene and tetraene TG classes (Table 48), when compared to the control milk fat. In summary, the major changes in the total 18:2-rich milk fat as compared to the total control milk fat were the lower proportions of 000 (25.8% compared to 40.7%) (without regard to positional specificities) and 001 (26.3% compared to 38.6%) which were compensated by much increased proportions of TGs such as 002 (18.8% compared to 1.8%), 012 (11.0% compared to 2.8%) and 022 (9.5% compared to 0%).

Comparison of the proportions of the different TG classes present in the 18:2-rich milk fat with those in seed oils containing similar or higher levels of 18:2 indicated some similarities. In oil from Madhuca latifolia which contained 14.4% 18:2 and 37.6% 18:1, 001 (35%), 011 (21%), 002 (17%) and 012 (15%) were the most abundant TG species (Gunstone and Qureshi, 1965) which was very similar to the proportions of the major TG species in the 18:2-rich milk fat. On the other hand in sunflower oil, which contained 59.5% 18:2 and 30.0% 18:1, the major TG species were more unsaturated and consisted mainly of 012 (11%), 112 (17%), 022 (12%), 122 (32%) and 222 (20%) (Gunstone and Qureshi, 1965).

In animal species only limited work has been carried out on the comparison of the altered TG composition of fat after feeding diets high in 18:2. In depot fats the major change observed was an increase in the proportions of 18:2 at the expense of 16:0, the major saturated FA, and 18:1, the major unsaturated FA in normal depot fat. This led to increases in the levels of triene, tetraene and pentaene TGs and decreases in monoene TG species (Privett et al, 1965; Hawke et al, 1976). For example in the rat (Privett et al, 1965), proportions of

16:0 - 18:2 - 18:1, 18:1 - 18:2 - 18:1, 16:0 - 18:2 - 18:2 and 18:1 - 18:2 - 18:2 were increased and proportions of 16:0 - 18:1 - 16:0 and 16:0 - 18:1 - 18:1 were decreased on feeding corn oil. In addition, in lambs, where higher levels of saturated FAs were present than in the rat, the proportion of the TG species 002 was also increased on feeding protected supplements high in 18:2 (Hawke et al, 1976). No previous work has been reported in the literature concerning the effect of elevated 18:2 levels in the diet on the TG composition of milk fat, but similar changes to those reported for rat and sheep depot fats were observed in the 18:2-rich milk fat in the present study.

In a depot fat naturally high in 18:2 e.g. the dog (14 - 16% 18:2) (Gold, 1968) the TG species 001, 011, 111 and 012 were found to predominate.

The proportions of the TG classes of many plant and animal fats can be adequately predicted by either the 1,3-random, 2-random hypothesis (Vander Wal, 1960; Litchfield, 1972) or the 1-random, 2-random, 3-random hypothesis (Litchfield, 1972) for the esterification of fatty acids. For example the results of Gunstone and Qureshi (1965) and Weber et al (1971) for seed fats and the results of Privett et al (1965) and Christie and Moore (1970) for animal depot fats show considerable resemblance to those predicted by these distribution hypothesis. The proportions of the different TG classes of the control and 18:2-rich milk fat predicted by the 1-random, 2-random, 3-random hypothesis gave somewhat limited agreement with the observed values (Table 48). The predicted values were comparable for the monoene and diene TGs but in general the levels of saturated TGs were actually slightly lower than those predicted, and the levels of triene and tetraene somewhat higher than those predicted.

Therefore it would appear that knowing the proportions of the FAs

in each position in the TGs one can to some extent make a prediction about the proportions of the various TG classes. Conversely it can be said the proportions of the various TG classes depend to some extent directly on the proportions of FAs available for esterification and on the preferences of these FAs for esterification in the three positions of the TGs.

## Section 4.3. The effect of elevated levels of linoleic acid on the positional distribution of fatty acids in the triacylglycerols of fats and oils

### (a) Positional distribution of fatty acids in the triacylglycerols of fats and oils containing low levels of linoleic acid

In the depot fats of mammals, the distribution of FAs in the TGs has been found to be reasonably predictable, with position 1 most likely to be occupied by predominantly saturated FAs, position 2 by unsaturated and shorter chain FAs and position 3 by a more random distribution of FAs with a preference for longer chain FAs (Brockerhoff et al, 1966; Brockerhoff, 1966). A notable exception is the pig where 16:0 shows a greater preference for position 2 than in most other animal fats. Numerous analyses of a variety of species have confirmed these generalizations (Brockerhoff et al, 1966; Brockerhoff, 1966; Christie and Moore, 1970, 1971; Kuksis et al, 1973). The positional distribution of FAs in the TGs of the depot fat of the bovine, sheep and goat (Kuksis et al, 1973) holds to the distribution rules proposed by Brockerhoff (1966).

On the other hand in the milk fats so far examined i.e. bovine (Breckenridge and Kuksis, 1968a, 1969; Taylor and Hawke, 1975b; Section 3.5.2.), sheep and goat (Kuksis et al, 1973), the distribution pattern is complicated by the presence of short chain FAs. For

example 4:0 comprises up to 30% of the FAs in position 3 of the TGs of bovine milk fat. Comparison of the distribution of FAs in the total milk fats of the bovine (Section 3.5.2.), sheep and goat (Kuksis et al, 1973) (Figure 20) demonstrates that similarities exist in the positional distribution of FAs in the TGs of these milk fats, with the only significant differences between these three fats being the slightly greater preference of 16:0 for position 1 and the greater preference of 18:1 for position 3 in the milk fat of the goat and sheep. In the milk fat of the bovine, goat and sheep the notable features are that (a) 4:0 and 6:0 are esterified almost exclusively in position 3 (b) 14:0 shows a preference for position 2 (c) 16:0 is esterified in positions 1 and 2 in preference to position 3 (d) 18:0 is preferentially esterified in position 1 (e) 18:1 prefers position 1 and 3 as in the bovine or position 3 as in the goat and sheep.

The stereospecific distribution of FAs in TG fractions of high, medium and low mol. wt. of the control milk fat in the present study was similar to that obtained by Taylor and Hawke (1975b) in TG fractions of high, medium and low mol. wt. from samples of New Zealand milk fat collected at various times of the year. Moreover the overall pattern of positional distribution of FAs in the high mol. wt. fraction was similar to that observed by Breckenridge and Kuksis (1969) in a molecular distillate of bovine milk TGs of high mol. wt., and furthermore the same similarity of distribution existed between the low mol. wt. fraction of the control milk fat and a molecular distillate of low mol. wt. (Breckenridge and Kuksis, 1968a).

The positional distribution of minor FAs e.g. 16:1 and 18:2 can not be accurately determined by stereospecific analysis because of the errors involved in calculation. Consequently this may explain the variation in results reported in the literature for the positional

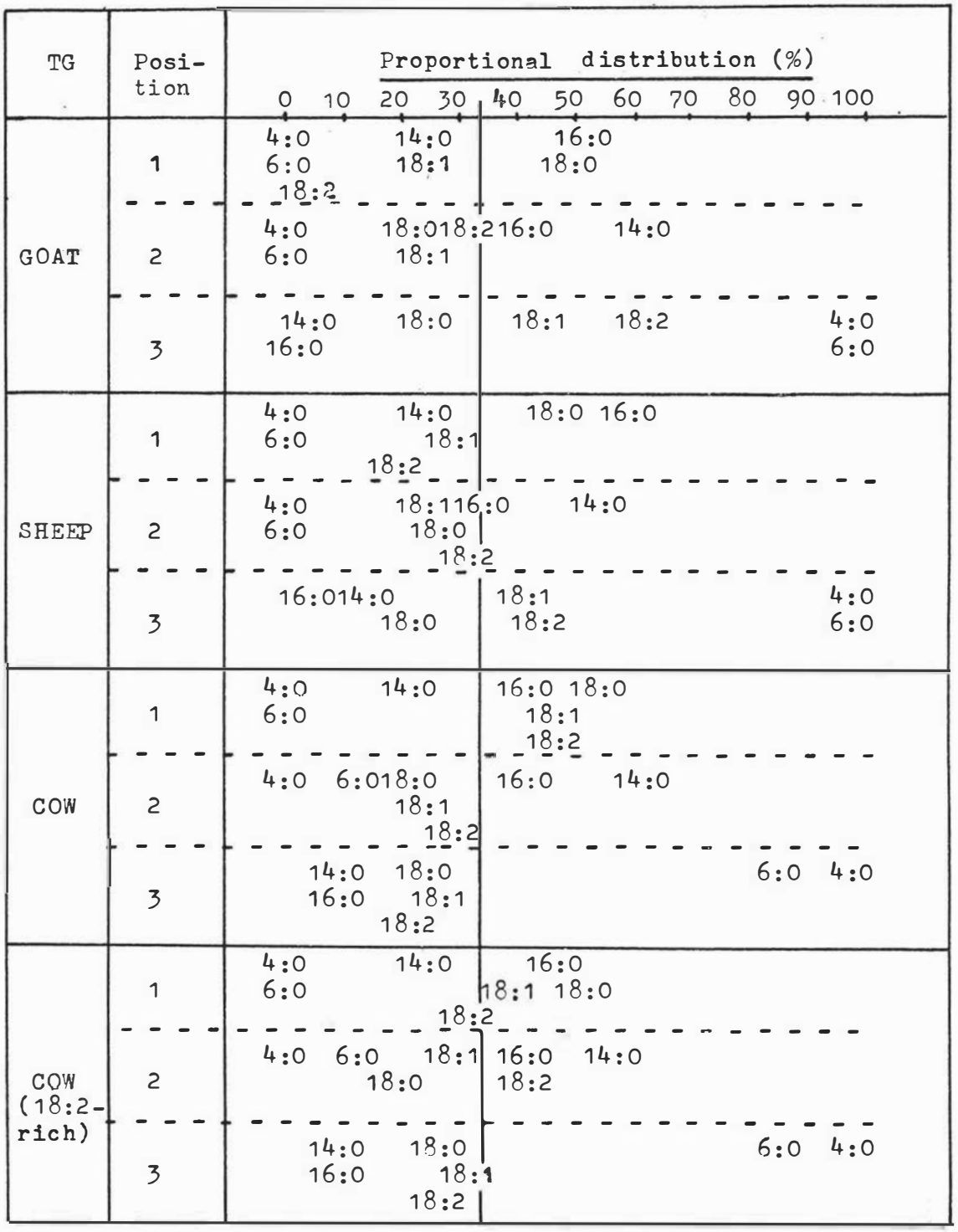



Figure 20. Proportional distribution of major fatty acids in the triacylglycerols of ruminant milk fats.

distribution of 18:2 in bovine milk fat (Breckenridge and Kuksis, 1969, Taylor and Hawke, 1975b) where the only consistent feature was the slight preference of 18:2 for position 2 in all fractions of milk fat. This contrasted with the distribution in the control milk fat in the present study where 18:2 showed a similar distribution to 18:1 but with more 18:2 in positions 1 and 2 than position 3 of the high mol. wt. fraction and a preference for position 1 in the medium and low mol. wt. fractions.

(b) Positional distribution in fats and oils containing high levels of linoleic acid

The positional distribution of FAs between positions 1, 2 and 3 of the TGs of the 18:2-rich milk fat (Figure 20) and it's respective TG fractions was very similar to that found for the major FAs of normal milk fat. The high levels of 18:2 enabled the distribution of 18:2 to be more accurately determined. 18:2 showed a preference for position 2 in the total milk fat and for positions 1 and 2 in the medium and low mol. wt. fractions but a preference for positions 2 and 3 in the high mol. wt. fraction.

These results would indicate, that when high levels of 18:2 are available for esterification, the positional distribution of the FAs appears to remain virtually unaltered and that the FAs remain distributed within the TGs of bovine milk fat in a very characteristic manner (Figure 20).

Comparison of the positional distribution of FAs in the TGs of seed fats high in 18:2 with that in milk fats high in 18:2 demonstrated that similarities do exist, such as the preference of 18:2 for position 2, but in contrast to the seed fats e.g. soybean, corn and maize oils, where the positional distribution of 18:2 and other FAs was largely symmetrical (Brockerhoff and Yurkowski. 1966: Weber et al, 1971), the

distribution of 18:2 and other FAs in the 18:2-rich milk fat was distinctly asymmetrical.

As predicted by the rules of Brockerhoff (1966), 18:2 being an unsaturated FA is esterified in position 2 of depot fats containing normal levels of 18:2 (Privett et al, 1965, Brockerhoff et al, 1966; Christie and Moore, 1971). When the level of 18:2 in depot fat is elevated, a large proportion of the 18:2 becomes esterified in position 2 as before but preferential esterification also occurs into position 3 (Privett et al, 1965; Christie et al, 1974; Scott and Cook, 1975; Hawke et al, 1976) presumably because of the chain length of this FA (Brockerhoff, 1966). This situation is similar to that occurring in the high mol. wt. fraction of the 18:2-rich milk fat where position 3 contained higher proportions of 18:2 than position 2.

The overall pattern to emerge is that when 18:2 levels in milk or depot fats are elevated, 18:2 becomes esterified preferentially in positions 2 and 3 except in the low and medium mol. wt. fractions of milk fat where competition from the short chain FAs, e.g. 4:0, 6:0 which are almost totally esterified in position 3, causes 18:2 to be preferentially esterified only in position 2. Also it becomes evident that when 18:2 is blocked from position 3 as in the low and medium mol. wt. fractions of the 18:2-rich milk fat, the proportions of 18:2 in position 1 become elevated almost up to the levels in position 2, rather than further increasing the levels in position 2 to any great extent. Conversely this could mean that once a certain level of 18:2 is attained in position 2. e.g. 15 - 20%, 18:2 becomes esterified in positions 1 and 3.

Section 4.4. The effect of elevated levels of linoleic acid on the molecular species of triacylglycerol in bovine milk fat

The molecular species of TG can be assessed from the compositions of the TG classes of differing levels of unsaturation and from the positional distribution of FAs in the TGs, but the positional distribution of FAs in the total milk fat does not always reflect the distribution in the individual TG classes of differing mol. wt. and unsaturation. For example, the positional distribution of 18:2 in the 18:2-rich milk fat would be different in the individual TG fractions and their respective TG classes, with the majority of the diene TGs, containing 18:2, in the low and medium mol. wt. fractions, having 18:2 in position 1 or 2, whereas those of high mol. wt. are likely to have 18:2 in position 2 or position 3. Since there is a preference of 18:1 for the primary positions of the TGs the majority of the triene TGs of high mol. wt. probably have 18:2 in position 2. Furthermore in the tetraene TGs of low and medium mol. wt., 18:2 is likely to fill positions 1 and 2 but in the tetraene TGs of high mol. wt. 18:2 presumably occupies mainly positions 2 and 3.

In summary, the 18:2-rich milk fat appeared to contain the same range of molecular species of TG as milk fats of normal origin, but the quantities of certain TGs were considerably altered. For example the TG species, 020 or 002 which were of minor importance in the control milk fat, were present in large amounts in the 18:2-rich milk fat. In addition 022 and 220 which would be present in the control milk in negligible amounts were readily observable in the 18:2-rich milk fat. Taking into account that the stereospecific distribution of FAs was virtually unchanged by the presence of elevated levels of 18:2, it would appear that the molecular species of TG that were present in the control milk fat would still be present in the 18:2-rich milk fat.

Section 4.5. Effects of the availability of elevated levels of linoleic acid on the biosynthesis of milk fat in the cow

The weight yields, in the milk fat, of short chain FAs (4:0 - 12:0) appear to remain relatively unaltered after feeding diets of protected supplement (Mattos and Palmquist, 1974; Section 3.1.1.), although a slight decrease in the yield of 6:0 to 12:0 and an increase in the yield of 4:0 was observed by Mattos and Palmquist (1974). In addition the acetate/propionate ratio and the level of acetate in the rumen also appear to be not affected (Mattos and Palmquist, 1974). On the other hand there is an increase in the weight yields of 18 carbon FAs and a significant decrease in the weight yields of 14:0 and 16:0. Notably 14:0 and 16:0 originate mainly from two sources (Storry, 1970), namely, biosynthesis from acetate and β-hydroxybutyrate within the mammary gland, and from the diet, which contains greatly increased levels of 18 carbon FAs and only relatively low levels of 14:0 and 16:0. One possible explanation for the decreased weight yield of 14:0 and 16:0 in the milk fat is suggested from the results of Gooden and Lascelles (1973), who observed a three-fold decrease in the uptake of 16:0 by the mammary gland during supplement feeding, although the actual arterial concentration of 16:0 was slightly increased. Mattos and Palmquist (1974) put forward the suggestion that the increased uptake of long chain FAs may be inhibiting de novo synthesis of FAs within the mammary gland probably by feedback inhibition of the long chain acyl CoA esters on acetyl CoA carboxylase. This suggestion is in agreement with observations in adipose tissue of sheep where feeding protected lipids was found, both *in vivo* and *in vitro* to decrease lipogenesis (Cook and Scott, 1975; Hood *et al*, 1975).

On feeding protected supplements the weight yields of all 18 carbon FAs, even 18:0, were raised. As the supplements contain very little

18:0, two possible reasons for the increase in 18:0 could be: (a) biohydrogenation of an unprotected component of the supplement (b) inhibiting of the desaturation of 18:0 tc 18:1 in the mammary gland, which is a major source of 18:1 in normal milk fats (Kinsella, 1972a)

The minimal effect of 18:2 on the distribution of other FAs in the milk fat would indicate that in bovine milk fat, the FAs are distributed between the three positions in the TG in constant proportions even when the composition of the fats may vary considerably. This is in agreement with observations in rabbit adipose tissue (Christie et al, 1974). This would suggest that the acyltransferases in these tissues use FAs at constant rates from a common FA pool.

Comparison of the FA compositions of the 1,2-DGs, calculated from the composition of positions 1 and 2 of the TGs; of molecular distillates of high and low mol. wt. (Breckenridge and Kuksis, 1969) and of fractions of high, medium and low mol. wt. of milk fat (Taylor and Hawke, 1975b), with those obtained from the control milk fat in the present study, would indicate that certain similarities exist (Table 49). This suggests that these 1,2-DGs, from the fractions of differing mol. wt., are derived from a common pool during biosynthesis of the milk fat. The acyltransferases specific for 4:0 will presumably only esterify on to position 3 e.g. a requirement for a diacylglycerol acyl acceptor or alternatively the acyltransferases esterifying on to position 3 use FAs from a distinct FA pool not available to the acyltransferases esterifying on to positions 1 and 2. Furthermore in view of the similarities in the positional distribution of FAs in the TGs of the milk fat of different ruminants, the acyltransferases in these different mammary tissues would appear to exhibit similar FA specificities.

Table 49. Fatty acid composition of the 1,2-diacylglycerols of the fractions of high, medium and low molecular weight of bovine milk fat

| | Fatty acid composition (mole %) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | High mol. wt. | | | | Medium mol. wt. | | | Low mol. wt. | | | |
| FA | Control | 18:2-rich | Ref 1.[a] | Ref 2.[b] | Control | 18:2-rich | Ref 2.[b] | Control | 18:2-rich | Ref 1.[a] | Ref 2.[b] |
| 4:0 | - | - | - | - | - | - | - | 0.1 | - | - | - |
| 6:0 | - | - | - | - | 0.9 | 0.7 | 1.3 | 1.6 | 2.8 | - | 1.0 |
| 8:0 | 1.1 | 0.9 | tr. | 0.2 | 1.8 | 2.6 | 2.5 | 3.0 | 3.9 | 0.4 | 2.0 |
| 10:0 | 3.3 | 3.0 | 2.0 | 2.0 | 3.5 | 4.3 | 6.4 | 5.1 | 5.9 | 2.6 | 5.6 |
| 10:1 | - | 0.1 | - | - | 0.1 | 0.1 | 0.7 | 0.4 | 0.3 | - | 0.3 |
| 12:0 | 4.9 | 4.4 | 2.8 | 3.3 | 5.1 | 4.1 | 6.6 | 6.0 | 5.5 | 4.8 | 5.1 |
| 14:0 | 16.3 | 12.2 | 11.1 | 13.1 | 16.2 | 11.0 | 15.3 | 16.3 | 11.6 | 16.8 | 16.7 |
| 14:1 | 0.7 | 0.3 | tr. | 0.6 | 0.6 | 0.3 | 0.7 | 0.8 | 0.5 | 1.4 | 1.0 |
| 15:0 | 1.9 | 0.9 | 2.8 | 1.3 | 1.0 | 0.9 | 1.7 | 1.9 | 1.5 | 3.3 | 2.4 |
| 16:0 | 33.0 | 22.6 | 38.3 | 31.9 | 32.2 | 20.0 | 28.7 | 30.0 | 18.5 | 39.1 | 29.8 |
| 16:1 | 2.0 | 2.0 | 3.3 | 1.3 | 1.5 | 1.4 | 1.3 | 1.6 | 1.8 | 2.5 | 1.4 |
| 17:0 | 0.6 | 0.9 | 1.2 | 0.5 | 0.4 | 0.7 | 0.6 | 0.5 | 0.4 | 2.3 | 0.4 |
| 18:0 | 13.1 | 15.9 | 13.7 | 17.5 | 13.5 | 20.1 | 13.3 | 12.3 | 12.4 | 9.1 | 12.8 |
| 18:1 | 20.8 | 22.1 | 22.8 | 27.8 | 19.2 | 19.5 | 20.7 | 17.7 | 16.7 | 15.8 | 21.1 |
| 18:2 | 1.4 | 13.9 | 1.3 | 0.8 | 1.7 | 13.3 | 0.4 | 1.6 | 17.0 | 1.1 | 0.4 |
| 18:3 | 0.9 | 0.9 | - | - | 1.3 | 0.8 | - | 0.8 | 1.1 | - | 0.2 |
| 20:? | - | - | 0.7 | - | 0.8 | 0.2 | - | 0.4 | - | 0.8 | - |

[a] Breckenridge and Kuksis (1969)

[b] Calculated from Taylor and Hawke (1975b)

Section 4.6. The effect of elevated levels of linoleic acid on the thermal characteristics of bovine milk fat

As noted in the preceding discussion the 18:2-rich milk fat and its composite fractions contained lower proportions of saturated and monounsaturated TGs, and higher proportions of the more unsaturated TGs containing 18:2. This would tend to lower the melting point of the milk fat.

The control milk fat and the fractions of high, medium and low mol. wt. showed melting characteristics similar to those obtained by Taylor (1973). The bulk of the high mol. wt. fraction melted between -10 and 40°C, with the main melting peak between 18 and 40°C. The saturated TGs of the high mol. wt. fraction melt between 35 and 45°C (Taylor, 1973), with the unsaturated TGs melting between -23 and 29°C. Therefore the main melting peak would be expected to largely arise from the melting of the saturated TGs, with a small portion of the unsaturated TGs providing the lower melting glycerides of this main melting peak. The small hump, at 6°C, preceding the main melting peak could result from the melting of the remaining unsaturated TGs.

The medium mol. wt. fraction of the control milk fat provided TGs melting predominantly between -15 and 22°C.

The bulk of the fraction of low mol. wt. melted between -26 and 22°C. The saturated components of these TGs which melt between 14 and 30°C (Taylor, 1973), contributed the main melting peak of the low mol. wt. fraction. The unsaturated TGs which melt between -57 and 2°C (Taylor, 1973), contributed the broad peak preceding the main melting peak. It should be noted that very little overlap occurred between the melting curves of the saturated and unsaturated TGs of either the low or high mol. wt. fraction, indicating the presence of only minimal solid solution effects.

The melting curves of the two total milk fats can be divided into three regions: (1) start of melting to -13°C (2) -13 to 17°C (3) 17°C to finish of melting (Section 3.6.). Very similar proportions of the two milk fats melted between -13 and 17°C i.e. 65 - 67% of the milk fat, but 31% of the 18:2-rich milk fat melted below -13°C and only 11% above 17°C, whereas only 17% of the control milk fat melted below -13°C and 26% above 17°C. Another interesting feature was that 21% of the 18:2-rich milk fat melted below -15°C, whereas only 6% of the control milk fat melted below this temperature. Therefore it would appear that high melting glycerides (>17°C) in the 18:2-rich milk fat were replaced by low melting glycerides (<13°C).

As found by Edmondson et al (1974), increasing the unsaturation of the milk fat diminished the high melting shoulder in the total milk fat, as well as moving the main melting peak to a slightly lower temperature i.e. 14°C to 12°C and lowering the temperature for the start (-33°C to -38°C) and the finish of melting (34°C to 30°C). The liquid fat content of the 18:2-rich milk fat at all temperatures between -30 and 30°C was greater than the control milk fat, which agrees with similar results obtained by Edmondson et al (1974).

The reduction in the proportions of the saturated TGs of high mol. wt. in the 18:2-rich milk fat was accompanied by the lowering, from 41°C to 38°C, of the temperature for the finish of melting. This was consistent with the observation that the saturated TGs of high mol. wt. melt between 35 and 45°C (Taylor, 1973). The melting peak for the high mol. wt. fraction of the 18:2-rich milk fat was broad, in contrast to the two melting peaks, one minor, the other major, present in this fraction of the control milk fat. The minor peak in the high mol. wt. fraction of the control milk fat was most likely to be comprised of diene TGs, from interpretation of the results of Taylor (1973).

Without taking into account the possible effects of the presence of high levels of 18:2 on the TG packing in the fat crystals, the lack of division of the high mol. wt. of the 18:2-rich milk fat into two melting peaks could possibly be explained by the large amounts (14%) of 002 and 020 in this fraction. The TG, 002 e.g. 16:0 - 16:0 - 18:2, melts at a higher temperature than 101 e.g. 18:1 - 16:0 - 18:1 (Hilditch, 1949). In addition, Berger and Akehurst (1966) making use of D.T.A. cooling curves, observed that 002 and 012 solidified at a similar or even slightly higher temperature than 011. It could perhaps be extrapolated from these results that the same relative temperatures would exist for the melting of these TGs. If this were so, it would provide further explanation for the broad melting peak of the high mol. wt. fraction of the 18:2-rich milk fat. The TGs in this fraction melting below-20°C would most likely be the very unsaturated TG species e.g. 18:1 - 18:2 - 18:1 and 18:1 - 18:2 - 18:2.

The medium mol. wt. TGs of the 18:2-rich milk fat melted between -20 and 20°C which was very similar to the melting range of the bulk of the low mol. wt. fraction of the control milk fat. Of the two melting peaks of the medium mol. wt. fraction, one corresponded closely to the melting peak, at 17°C, of the high mol. wt. fraction of the 18:2-rich milk fat and the other to the melting peak, at 0°C, of the low mol. wt. fraction of the 18:2-rich milk fat. The lower melting peak would presumably result from the melting of the more unsaturated TGs of medium mol. wt., namely the diene, triene and tetraene TGs which comprised about 40% of the medium mol. wt. fraction. The solid solution effects observed must be the result of some interaction between these glycerides i.e. the TG species present must exhibit sufficient structural similarities to permit the formation of mixed crystals. The higher melting peak of the medium mol. wt. fraction was

probably formed from the melting of the monoene and saturated TGs which comprised 60% of the medium mol. wt. fraction.

The major portion of the fraction of low mol. wt. of the 18:2-rich milk fat melted between -42 and 11°C which was very similar to the range over which the unsaturated TGs of low mol. wt. of normal milk fat melted (Taylor, 1973). A small portion of the low mol. wt. fraction melted between 11 and 18°C which was the temperature range for the melting of the saturated TGs of low mol. wt. (Taylor, 1973), but this was insufficient to account for the quantity of saturated TGs present in this fraction. This was the only TG fraction of the 18:2-rich milk fat where the contribution from the saturated TGs was not readily evident. Possible explanations for this could be the ability of this fat to accommodate the reduced level of saturated TGs in a lower melting peak i.e. mixed crystal formation, or that diene and triene TGs containing 18:2 mainly in position 2 can better accommodate the saturated TGs than can the monoene TGs containing 18:1 primarily in position 1, or the diene TGs containing 18:1 in positions 1 and 2.

In summary, the fractions of the 18:2-rich milk fat appeared to exhibit significant mixed crystal formation between the saturated and unsaturated TGs, which gave rise to very broad peaks in contrast to the fractions of the control milk fat where only limited interaction appeared to be occurring between the respective saturated and unsaturated TGs (Taylor, 1973). These characteristics of the two milk fats were not readily observable in the total milk fats.

The suitability of milk fats containing elevated levels of 18:2 for use in the manufacture of spreadable butter can best be judged by comparison of the melting characteristics of these milk fats at normal working temperatures with those of milk fats of normal 18:2 content. Normal butter was considered to have a satisfactory spreadability

between 18 and 25°C (Norris R., personal communication) which was equivalent to liquid phase contents between 73 and 84% as measured by D.S.C.. According to this criteria the 18:2-rich milk fat is likely to have a satisfactory spreadability in the narrow range between 12 and 15°C. Therefore at refrigerator temperatures (2 - 5°C), the 18:2-rich milk fat being 45 - 51% liquid phase would be almost spreadable, whereas the control milk fat being only 29 - 34% liquid phase would not be spreadable at this temperature. At room temperature, e.g. 20°C, the 18:2-rich and control milk fats being 92% and 75% liquid phase respectively, are both likely to be readily spreadable.

Hardness measurements of butter which was manufactured from milk fat containing 17 - 22% polyunsaturated FAs, and measured according to the sectility method of Taylor et al (1971), showed that this polyunsaturated butter had hardness values (arbitrary units) of 200 - 450 at 5°C and 110 - 190 at 12.5°C. whereas normal butter had hardness values of 800 - 1600 at 12.5°C (Dolby R.M., personal communication). For comparison, a polyunsaturated margarine had a hardness value between 130 - 270 at 5°C and 85 - 150 at 12.5°C. A product spreadable at refrigerator temperatures was considered to have a hardness value of <250 at 5°C (Norris R., personal communication), therefore the polyunsaturated butter could be considered readily spreadable almost directly from the refrigerator whereas normal butter would still be unspreadable at 12.5°C.

The problem associated with these 18:2-rich milk fats is their very narrow spreadable range as a result of the fat greatly favouring the liquid state at temperatures above 20°C, e.g. at 20°C the liquid phase content of 18:2-rich milk fat, as measured by D.S.C. was greater than 90%. This was consistent with the oiling off at 20°C observed by Wood et al (1975) in butter made from milk fat containing elevated levels of 18:2.

Section 4.6. Summary

1. The fatty acids most affected by increasing the proportion of linoleic acid in the milk fat from 1.8 to 15.5 mole % were myristic acid (14:0) and palmitic acid (16:0) which were present in lower proportions in the 18:2-rich milk fat. As a consequence, triacylglycerols with 40 and 54 acyl carbons became major contributors to the low and high mol. wt. fractions respectively, and to the total milk fat, with reductions in the proportions of TGs containing 36, 38, and 50 acyl carbons.

2. The positional distribution of each individual FA in the TGs of the high, medium and low mol. wt. fractions and in the total milk fat was little affected by the presence of high levels of 18:2 for esterification.

3. Linoleic acid was preferentially esterified at position 1 and 2 of the low and medium mol. wt. TGs and at positions 2 and 3 of the high mol. wt. TGs. In the total 18:2-rich milk fat, 18:2 was esterified in the three positions of the TGs in the order of preference 2 > 1 > 3.

4. The 18:2-rich milk fat contained lower proportions of saturated TGs, especially those containing 14:0 and 16:0 e.g. 16:0 - 14:0 - 4:0 and 16:0 - 14:0 - 18:0, monoene TGs e.g. 18:1 - 16:0 - 4:0 and 16:0 - 16:0 - 18:1, and diene TGs containing 18:1 e.g. 18:1 - 18:1 - 4:0 and 18:1 - 16:0 - 18:1. The above decreases were compensated for, by increases in the proportions of diene TGs such as 18:0 - 18:2 - 4:0 and 18:0 - 16:0 - 18:2, triene TGs e.g. 18:1 - 18:2 - 4:0 and 18:1 - 18:2 - 18:0, and tetraene TGs e.g. 18:2 - 18:2 - 4:0 and 18:0 - 18:2 - 18:2.

5. The TG fractions of the 18:2-rich milk fat generally melted at lower temperatures than the corresponding TG fractions of the control

milk fat. The total 18:2-rich milk fat also melted at a lower temperature than the total control milk fat. It was anticipated that the 18:2-rich milk fat would have a narrow spreadable range (≈12 to 15°C) whereas the control milk fat would have a wider spreadable range (≈18 to 25°C).

6. The TG fractions of the 18:2-rich milk fat appeared to exhibit significant mixed crystal formation of saturated and unsaturated TGs which gave rise to very broad melting peaks. In contrast the TG fractions of the normal milk fat exhibit only limited interaction of the saturated and unsaturated TGs (Taylor, 1973).

7. Freshly secreted bovine or goats milk was active in the synthesis of triacylglycerols using fatty acid substrates. Goats milk was more active in this aspect than bovine milk. Incubation of 18:2 and 16:0 with freshly secreted goats milk demonstrated almost no change in the level of incorporation of either fatty acid but 16:0 was differently distributed from when incubated individually, with a greater concentration being esterified in position 2.

Appendix 1. Fatty acid composition of milk fats from the pair of monozygous twin cows fed on the control and experimental diets.

| | Fatty acid composition (mole %) | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-experimental period | | | | | | | | Experimental period | | | | | | | | | | | | | |
| Day | 5 | | 6 | | 7 | | Average[c] | | 10 | | 12 | | 14 | | 15 | | 16 | | 18 | | 19[d] | |
| Cow TG | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] | 1[a] | 2[b] |
| 4:0 | 10.5 | 10.7 | 11.3 | 11.5 | 11.8 | 11.0 | 11.3 | 11.1 | 11.4 | 11.0 | 11.1 | 10.0 | 11.1 | 10.2 | 12.1 | 9.8 | 11.4 | 11.3 | 11.9 | 10.9 | 11.9 | 10.4 |
| 6:0 | 4.9 | 4.6 | 5.3 | 4.9 | 5.2 | 5.7 | 5.1 | 5.1 | 5.8 | 5.9 | 5.1 | 5.4 | 5.1 | 4.9 | 5.4 | 4.7 | 5.4 | 5.2 | 5.1 | 5.1 | 5.3 | 5.4 |
| 8:0 | 2.1 | 1.9 | 2.5 | 2.2 | 1.9 | 2.5 | 2.2 | 2.2 | 2.5 | 2.4 | 2.1 | 2.5 | 2.3 | 2.1 | 2.4 | 2.4 | 2.3 | 2.1 | 2.2 | 2.3 | 2.3 | 2.1 |
| 10:0 | 3.4 | 3.2 | 4.2 | 4.0 | 3.1 | 4.4 | 3.6 | 3.9 | 4.7 | 4.3 | 4.0 | 4.7 | 3.9 | 3.7 | 4.2 | 4.1 | 4.1 | 3.5 | 3.8 | 3.3 | 4.1 | 3.2 |
| 10:1 | 0.2 | 0.1 | 0.3 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.3 | 0.3 | 0.2 | 0.3 | 0.2 | 0.2 | 0.1 | 0.3 | 0.2 | 0.3 | 0.2 |
| 12:0 | 3.5 | 3.3 | 3.8 | 4.0 | 3.1 | 3.8 | 3.5 | 3.7 | 4.4 | 3.7 | 4.2 | 4.9 | 3.9 | 3.3 | 3.7 | 3.8 | 3.5 | 2.5 | 3.4 | 2.9 | 3.5 | 2.6 |
| 14:0 | 10.2 | 8.9 | 10.4 | 9.8 | 8.7 | 10.1 | 9.7 | 9.6 | 11.9 | 9.6 | 10.1 | 9.7 | 11.2 | 8.7 | 11.0 | 8.5 | 9.8 | 7.2 | 11.6 | 7.2 | 11.9 | 7.7 |
| 14:1 | 0.9 | 0.7 | 0.9 | 0.8 | 0.8 | 0.9 | 0.9 | 0.8 | 1.1 | 0.6 | 0.8 | 0.6 | 0.8 | 0.5 | 1.0 | 0.5 | 0.8 | 0.2 | 0.8 | 0.3 | 1.0 | 0.5 |
| 15:0 | 1.1 | 0.9 | 0.8 | 0.8 | 0.8 | 1.0 | 0.9 | 0.9 | 1.0 | 0.6 | 1.0 | 0.8 | 0.6 | 0.8 | 1.1 | 0.8 | 0.8 | 0.7 | 1.0 | 0.5 | 1.0 | 0.7 |
| 16:0 | 21.7 | 22.0 | 21.2 | 19.8 | 23.0 | 19.6 | 22.0 | 20.5 | 21.0 | 17.1 | 22.5 | 17.6 | 20.1 | 16.2 | 21.2 | 14.6 | 20.0 | 12.1 | 20.9 | 12.9 | 22.1 | 14.1 |
| 16:1 | 1.4 | 1.8 | 1.5 | 1.9 | 2.6 | 1.7 | 1.8 | 1.8 | 2.0 | 1.4 | 1.5 | 1.1 | 1.7 | 1.6 | 2.1 | 1.7 | 2.0 | 1.4 | 1.8 | 1.3 | 2.0 | 1.1 |
| 17:0 | 0.9 | 0.8 | 0.6 | 0.4 | 0.7 | 0.5 | 0.7 | 0.6 | 0.7 | 0.6 | 0.8 | 0.7 | 0.9 | 0.7 | 0.7 | 0.6 | 0.8 | 0.7 | 1.0 | 0.5 | 0.9 | 0.5 |
| 18:0 | 13.8 | 13.7 | 12.2 | 14.3 | 12.2 | 13.9 | 12.7 | 14.0 | 11.8 | 12.6 | 13.5 | 12.3 | 12.9 | 13.4 | 12.3 | 14.3 | 14.0 | 14.7 | 13.4 | 13.8 | 11.9 | 14.1 |
| 18:1 | 21.1 | 23.7 | 20.8 | 22.1 | 20.9 | 20.8 | 20.9 | 22.2 | 18.3 | 19.5 | 20.6 | 19.0 | 22.1 | 20.6 | 18.7 | 19.7 | 21.6 | 20.7 | 19.8 | 20.5 | 18.6 | 20.6 |
| 18:2 | 1.5 | 2.0 | 2.0 | 1.1 | 1.9 | 1.7 | 1.8 | 1.6 | 1.6 | 7.5 | 1.1 | 8.9 | 1.4 | 11.5 | 1.7 | 12.4 | 1.9 | 15.8 | 1.4 | 16.5 | 1.8 | 15.5 |
| 18:3 | 1.0 | 0.8 | 1.1 | 1.1 | 1.9 | 1.2 | 1.3 | 1.0 | 0.7 | 1.6 | 1.2 | 0.9 | 0.9 | 0.8 | 0.9 | 0.7 | 0.8 | 0.6 | 0.7 | 0.7 | 0.8 | 0.7 |
| 20:? | 0.8 | 0.6 | 1.0 | 0.8 | 1.2 | 0.9 | 1.0 | 0.8 | 0.8 | 1.2 | 1.2 | 0.9 | 0.8 | 0.9 | 1.1 | 1.2 | 0.7 | 1.0 | 0.9 | 1.0 | 0.5 | 0.7 |

[a] Milk fat from the control cow

[b] Milk fat from the experimental cow

[c] Average FA composition in the pre-experimental period

[d] Milk fat selected for comparison of the control and experimental milk fats

Appendix 2. Milk and milk fat yields of the control and experimental cows over the trial period.[a]

| | Control milk fat | | | Experimental milk fat | | |
|---|---|---|---|---|---|---|
| | Milk yield (Kg/day) | Fat % | Fat yield (g/day) | Milk yield (Kg/day) | Fat % | Fat yield (g/day) |
| Day | | | | | | |
| 5 | 13.64 | 4.73 | 645.2 | 12.36 | 4.89 | 604.4 |
| 6 | 12.18 | 4.75 | 578.6 | 14.41 | 4.55 | 655.7 |
| | Average | | 611.9 | | | 630.1 |
| 12 | 15.36 | 3.64 | 559.1 | 15.41 | 4.97 | 765.9 |
| 14 | 13.41 | 4.32 | 579.3 | 15.55 | 4.74 | 737.1 |
| 15 | 13.50 | 4.76 | 642.6 | 12.77 | 5.50 | 702.4 |
| 16 | 13.86 | 4.17 | 578.0 | 14.14 | 4.84 | 684.3 |
| 18 | 12.86 | 4.06 | 522.1 | 13.05 | 5.26 | 686.4 |
| 19 | 12.18 | 4.59 | 559.1 | 13.23 | 5.27 | 697.2 |
| | Average | | 553.1 | | | 689.3 |

[a] Milk fat yields were determined on an infra-red milk analyser (IRMA)

Appendix 3. Stereospecific analysis of the triacylglycerols in the fraction of high molecular weight of the control milk fat

| | Fatty acid composition (mole %) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 1[a] | 2[a] | 3[a] | |
| FA | TGs | | 1,2(2,3)-DGs | | 1,3-DGs | | 2,3-PLs | 1-PLs | 2-MGs | 3[e] | 3[f] |
| | Orig. | Recalc.[b] | Exp. | Calc.[c] | Exp. | Calc.[d] | | | | | |
| 4:0 | - | - | - | - | - | - | - | - | - | - | - |
| 6:0 | 0.4 | 0.7 | 0.2 | 0.3 | 0.3 | 0.6 | 1.1 | - | - | 2.2 | 1.2 |
| 8:0 | 1.1 | 1.6 | 1.4 | 1.0 | 0.8 | 1.3 | 1.6 | 1.7 | 0.6 | 2.6 | 1.0 |
| 10:0 | 3.8 | 4.7 | 4.3 | 3.6 | 3.3 | 4.2 | 5.3 | 3.6 | 3.0 | 7.6 | 4.8 |
| 10:1 | - | - | - | - | 0.6 | - | - | - | - | - | - |
| 12:0 | 4.0 | 4.9 | 4.3 | 4.4 | 3.0 | 3.0 | 5.3 | 4.0 | 5.9 | 4.7 | 2.1 |
| 14:0 | 13.3 | 12.9 | 16.1 | 16.1 | 9.2 | 7.8 | 15.3 | 8.2 | 24.4 | 6.2 | 7.3 |
| 14:1 | 0.7 | 1.5 | 1.3 | 1.0 | 2.3 | 0.6 | 2.0 | 0.4 | 1.0 | 3.0 | 0.7 |
| 15:0 | 1.3 | 1.8 | 1.6 | 1.3 | 1.1 | 1.0 | 1.8 | 1.9 | 1.9 | 1.7 | 0.1 |
| 16:0 | 27.3 | 27.2 | 28.0 | 29.7 | 22.0 | 22.5 | 26.2 | 29.1 | 36.9 | 15.5 | 15.9 |
| 16:1 | 1.9 | 1.6 | 2.0 | 2.0 | 1.6 | 1.7 | 1.6 | 1.7 | 2.3 | 0.9 | 1.7 |
| 17:0 | 0.9 | 0.6 | 1.3 | 0.8 | 2.7 | 1.1 | 0.6 | 0.6 | 0.6 | 0.6 | 1.5 |
| 18:0 | 16.4 | 14.9 | 14.2 | 14.1 | 19.8 | 21.0 | 12.9 | 18.9 | 7.2 | 18.6 | 23.1 |
| 18:1 | 26.5 | 25.3 | 23.3 | 23.5 | 31.2 | 32.5 | 24.3 | 27.2 | 14.4 | 34.2 | 37.9 |
| 18:2 | 1.7 | 1.2 | 1.3 | 1.6 | 1.6 | 2.0 | 1.0 | 1.5 | 1.2 | 0.8 | 2.4 |
| 18:3 | 0.7 | 1.1 | 0.7 | 0.7 | 0.6 | 0.8 | 1.0 | 1.1 | 0.6 | 1.4 | 0.4 |
| 20:? | - | - | - | - | - | - | - | - | - | - | - |

[a] Positions relative to sn-glycerol-3-phosphate

[b] $[2 \times (2,3\text{-PLs}) + (1\text{-PLs})]/3$

[c] $[3 \times (\text{TGs}) + (2\text{-MGs})]/4$

[d] $[3 \times (\text{TGs}) - (2\text{-MGs})]/2$

[e] $2 \times (2,3\text{-PLs}) - (2\text{-MGs})$

[f] $3 \times (\text{TGs}) - (1\text{-PLs}) - (2\text{-MGs})$

(-) Not detected

Appendix 4. Stereospecific analysis of the triacylglycerols in the fraction of high molecular weight of the 18:2-rich milk fat

| | Fatty acid composition (mole %) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 1[a] | 2[a] | 3[a] | |
| FA | TGs | | 1,2(2,3)-DGs | | 1,3-DGs | | 2,3-PLs | 1-PLs | 2-MGs | 3[e] | 3[f] |
| | Orig. | Recalc.[b] | Exp. | Calc.[c] | Exp. | Calc.[d] | | | | | |
| 4:0 | - | - | - | - | 0.1 | - | - | - | - | - | - |
| 6:0 | 1.1 | 0.9 | 0.9 | 0.8 | 0.4 | 1.7 | 1.4 | - | - | 2.8 | 3.3 |
| 8:0 | 1.5 | 1.2 | 1.8 | 1.5 | 1.1 | 1.5 | 1.6 | 0.3 | 1.6 | 1.6 | 2.6 |
| 10:0 | 3.5 | 2.6 | 3.8 | 3.8 | 2.8 | 2.9 | 3.2 | 1.3 | 4.7 | 1.7 | 4.5 |
| 10:1 | - | 0.1 | 0.1 | - | 0.1 | - | 0.1 | 0.1 | - | 0.2 | -0.1 |
| 12:0 | 3.2 | 3.4 | 3.6 | 4.0 | 2.6 | 1.7 | 3.9 | 2.5 | 6.2 | 1.6 | 0.9 |
| 14:0 | 8.8 | 9.1 | 10.4 | 10.7 | 6.1 | 5.0 | 9.6 | 8.0 | 16.4 | 2.8 | 2.0 |
| 14:1 | 0.6 | 0.3 | 1.1 | 0.5 | 0.3 | 0.5 | 0.3 | 0.4 | 0.2 | 0.4 | 1.2 |
| 15:0 | 0.7 | 1.3 | 1.0 | 0.7 | 0.8 | 0.7 | 1.3 | 1.3 | 0.6 | 2.0 | 0.2 |
| 16:0 | 16.2 | 17.4 | 17.7 | 18.0 | 14.6 | 12.7 | 15.2 | 21.8 | 23.3 | 7.1 | 3.5 |
| 16:1 | 1.4 | 1.9 | 1.7 | 1.5 | 1.6 | 1.3 | 1.6 | 2.4 | 1.7 | 1.5 | 0.1 |
| 17:0 | 0.7 | 1.0 | 0.8 | 0.6 | 2.5 | 0.9 | 0.8 | 1.5 | 0.3 | 1.3 | 0.3 |
| 18:0 | 18.6 | 17.6 | 16.0 | 16.4 | 22.1 | 23.0 | 15.4 | 22.0 | 9.8 | 21.0 | 24.0 |
| 18:1 | 26.5 | 25.9 | 23.8 | 24.5 | 29.0 | 30.6 | 26.0 | 25.7 | 18.4 | 33.6 | 35.4 |
| 18:2 | 16.1 | 15.4 | 16.0 | 16.1 | 15.7 | 16.2 | 17.3 | 11.7 | 16.0 | 18.6 | 20.6 |
| 18:3 | 1.1 | 1.9 | 1.4 | 1.0 | 0.2 | 1.3 | 2.3 | 1.1 | 0.8 | 3.8 | 1.4 |
| 20:? | - | - | - | - | - | - | - | - | - | - | - |

a Positions relative to sn-glycerol-3-phosphate
b [2 x (2,3-PLs) + (1-PLs)] /3
c [3 x (TGs) + (2-MGs)] /4
d [3 x (TGs) - (2-MGs)] /2
e 2 x (2,3-PLs) - (2-MGs)
f 3 x (TGs) - (1-PLs) - (2-MGs)
(-) Not detected

Appendix 5. Stereospecific analysis of the triacylglycerols in the fraction of medium molecular weight of the control milk fat

| | Fatty acid composition (mole %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 1[a] | 2[a] | 3[a] | |
| FA | TGs | | 1,2(2,3)-DGs | | 2,3-PLs | 1-PLs | 2-MGs | 3[e] | 3[f] |
| | Orig. | Recalc.[b] | Exp. | Calc.[c] | | | | | |
| 4:0 | 5.3 | 6.3 | 4.6 | 4.0 | 9.5 | - | - | 19.0 | 15.9 |
| 6:0 | 10.4 | 10.7 | 10.0 | 8.3 | 16.0 | - | 1.8 | 30.2 | 29.4 |
| 8:0 | 4.3 | 3.6 | 3.2 | 4.0 | 5.0 | 0.7 | 3.0 | 7.0 | 9.2 |
| 10:0 | 5.9 | 4.1 | 5.1 | 6.0 | 5.7 | 0.9 | 6.1 | 5.3 | 10.7 |
| 10:1 | 0.5 | 0.2 | 0.4 | 0.4 | 0.3 | - | 0.3 | 0.3 | 1.2 |
| 12:0 | 4.5 | 4.2 | 4.9 | 5.2 | 4.8 | 2.9 | 7.3 | 2.3 | 3.3 |
| 14:0 | 11.8 | 11.4 | 13.5 | 14.5 | 12.2 | 9.8 | 22.6 | 1.8 | 3.0 |
| 14:1 | 1.4 | 0.7 | 0.8 | 1.3 | 1.0 | - | 1.2 | 0.8 | 3.0 |
| 15:0 | 1.0 | 0.8 | 1.1 | 1.1 | 1.0 | 0.4 | 1.6 | 0.4 | 1.0 |
| 16:0 | 23.6 | 24.8 | 26.2 | 25.7 | 21.1 | 32.3 | 32.1 | 10.1 | 6.4 |
| 16:1 | 2.1 | 1.8 | 2.7 | 2.0 | 2.0 | 1.5 | 1.6 | 2.4 | 3.2 |
| 17:0 | 0.8 | 0.4 | 0.8 | 0.7 | 0.5 | 0.3 | 0.5 | 0.5 | 1.6 |
| 18:0 | 11.1 | 12.2 | 10.6 | 10.1 | 8.4 | 19.8 | 7.2 | 9.6 | 6.3 |
| 18:1 | 15.3 | 15.6 | 14.3 | 14.7 | 10.5 | 25.7 | 12.7 | 8.3 | 7.5 |
| 18:2 | 1.0 | 1.5 | 1.0 | 1.1 | 1.2 | 2.1 | 1.3 | 1.1 | -0.4 |
| 18:3 | 0.8 | 1.1 | 0.7 | 0.8 | 0.7 | 1.9 | 0.7 | 0.7 | -0.2 |
| 20:? | 0.2 | 0.5 | 0.2 | 0.2 | - | 1.6 | - | 0.0 | -1.0 |

[a] Positions relative to sn-glycerol-3-phosphate
[b] [2 x (2,3-PLs) + (1-PLs)] /3
[c] [3 x (TGs) + (2-MGs)] /4
[e] 2 x (2,3-PLs) - (2-MGs)
[f] 3 x (TGs) - (1-PLs) - (2-MGs)
(-) Not detected

Appendix 6. Stereospecific analysis of the triacylglycerols in the fraction of medium molecular weight of the 18:2-rich milk fat

| FA | Fatty acid composition (mole %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TGs | | 1,2(2,3)-DGs | | 2,3-PLs | 1[a] 1-PLs | 2[a] 2-MGs | 3[a] 3[e] | 3[a] 3[f] |
| | Orig. | Recalc.[b] | Exp. | Calc.[c] | | | | | |
| 4:0 | 8.2 | 8.1 | 7.0 | 6.1 | 12.1 | - | - | 24.2 | 24.6 |
| 6:0 | 12.3 | 12.5 | 11.9 | 9.6 | 18.7 | - | 1.4 | 36.0 | 35.5 |
| 8:0 | 3.8 | 2.9 | 3.3 | 4.0 | 4.2 | 0.4 | 4.8 | 3.6 | 6.2 |
| 10:0 | 4.5 | 3.8 | 4.7 | 5.0 | 4.8 | 1.9 | 6.7 | 2.9 | 4.9 |
| 10:1 | 0.3 | 0.1 | 0.2 | 0.3 | 0.2 | - | 0.3 | 0.1 | 0.6 |
| 12:0 | 2.6 | 3.2 | 3.6 | 3.3 | 3.4 | 2.8 | 5.3 | 1.5 | -0.3 |
| 14:0 | 7.3 | 7.8 | 8.8 | 9.0 | 7.9 | 7.6 | 14.3 | 1.5 | 0.0 |
| 14:1 | 0.4 | 0.3 | 0.8 | 0.5 | 0.4 | - | 0.7 | 0.1 | 0.5 |
| 15:0 | 0.6 | 0.8 | 0.7 | 0.7 | 0.7 | 1.0 | 0.9 | 0.5 | -0.1 |
| 16:0 | 14.0 | 16.2 | 15.3 | 15.6 | 14.5 | 19.5 | 20.4 | 8.6 | 2.1 |
| 16:1 | 0.9 | 1.1 | 1.5 | 1.0 | 0.9 | 1.6 | 1.3 | 0.5 | -0.2 |
| 17:0 | 0.6 | 0.6 | 0.5 | 0.6 | 0.4 | 0.9 | 0.5 | 0.3 | 0.4 |
| 18:0 | 15.3 | 16.1 | 13.8 | 14.4 | 9.9 | 28.5 | 11.6 | 8.2 | 5.8 |
| 18:1 | 15.9 | 15.7 | 16.5 | 16.3 | 12.8 | 21.4 | 17.5 | 8.1 | 8.8 |
| 18:2 | 11.6 | 10.1 | 10.5 | 12.0 | 8.5 | 13.4 | 13.1 | 3.9 | 8.3 |
| 18:3 | 0.7 | 0.6 | 0.5 | 0.7 | 0.5 | 0.8 | 0.9 | 0.1 | 0.4 |
| 20:? | 1.0 | 0.1 | 0.5 | 0.8 | 0.1 | 0.2 | 0.3 | -0.1 | 2.5 |

[a] Positions relative to sn-glycerol-3-phosphate
[b] $[2 \times (2,3\text{-PLs}) + (1\text{-PLs})] / 3$
[c] $[3 \times (\text{TGs}) + (2\text{-MGs})] / 4$
[e] $2 \times (2,3\text{-PLs}) - (2\text{-MGs})$
[f] $3 \times (\text{TGs}) - (1\text{-PLs}) - (2\text{-MGs})$
(-) Not detected

Appendix 7. Stereospecific analysis of the triacylglycerols in the fraction of low molecular weight of the control milk fat

| FA | Fatty acid composition (mole %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TGs | | 1,2(2,3)-DGs | | 2,3-PLs | 1[a] 1-PLs | 2[a] 2-MGs | 3[a] 3[e] | 3[a] 3[f] |
| | Orig. | Recalc.[b] | Exp. | Calc.[c] | | | | | |
| 4:0 | 25.0 | 24.6 | 22.5 | 18.8 | 36.9 | - | 0.1 | 73.7 | 75.0 |
| 6:0 | 6.2 | 6.1 | 5.9 | 5.5 | 9.1 | - | 3.2 | 15.0 | 15.4 |
| 8:0 | 2.3 | 1.7 | 2.4 | 3.1 | 2.4 | 0.4 | 5.6 | -0.8 | 0.9 |
| 10:0 | 3.6 | 3.1 | 4.5 | 4.9 | 4.1 | 1.2 | 8.9 | -0.7 | 0.7 |
| 10:1 | 0.5 | 0.3 | 0.7 | 0.6 | 0.4 | 0.1 | 0.8 | 0.0 | 0.6 |
| 12:0 | 3.8 | 4.2 | 5.2 | 5.1 | 4.9 | 2.9 | 9.0 | 0.8 | -0.5 |
| 14:0 | 11.5 | 11.8 | 16.1 | 14.2 | 12.5 | 10.3 | 22.3 | 2.7 | 1.9 |
| 14:1 | 0.9 | 0.5 | 1.0 | 1.0 | 0.7 | 0.2 | 1.4 | 0.0 | 1.1 |
| 15:0 | 0.9 | 1.2 | 1.0 | 1.2 | 0.9 | 1.7 | 2.2 | -0.4 | -1.2 |
| 16:0 | 20.2 | 22.1 | 21.0 | 21.5 | 16.0 | 34.4 | 25.5 | 6.5 | 0.7 |
| 16:1 | 1.4 | 1.5 | 1.3 | 1.5 | 1.5 | 1.6 | 1.6 | 1.4 | 1.0 |
| 17:0 | 0.4 | 0.4 | 0.1 | 0.4 | 0.2 | 0.7 | 0.3 | 0.1 | 0.2 |
| 18:0 | 7.6 | 8.9 | 5.9 | 6.9 | 3.4 | 19.8 | 4.8 | 2.0 | -1.8 |
| 18:1 | 12.6 | 11.4 | 10.3 | 12.4 | 5.4 | 23.5 | 11.8 | -1.0 | 2.5 |
| 18:2 | 1.2 | 1.3 | 1.0 | 1.2 | 0.9 | 2.1 | 1.1 | 0.7 | 0.4 |
| 18:3 | 1.2 | 0.7 | 0.7 | 1.1 | 0.6 | 0.8 | 0.9 | 0.3 | 1.5 |
| 20:? | 0.8 | 0.1 | 0.3 | 0.7 | - | 0.4 | 0.5 | -0.5 | 1.5 |

[a] Positions relative to sn-glycerol-3-phosphate
[b] [2 x (2,3-PLs) + (1-PLs)] /3
[c] [3 x (TGs) + (2-MGs)] /4
[e] 2 x (2,3-PLs) - (2-MGs)
[f] 3 x (TGs) - (1-PLs) - (2-MGs)
(-) Not detected

Appendix 8. Stereospecific analysis of the triacylglycerols in the fraction of low molecular weight of the 18:2-rich milk fat

| FA | Fatty acid composition (mole %) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | TGs | | 1,2(2,3)-DGs | | 2.3-PLs | 1[a] 1-PLs | 2[a] 2-MGs | 3[a] 3[e] | 3[a] 3[f] |
| | Orig. | Recalc.[b] | Exp. | Calc.[c] | | | | | |
| 4:0 | 24.3 | 23.7 | 22.0 | 18.2 | 35.5 | - | - | 71.0 | 72.9 |
| 6:0 | 5.9 | 4.9 | 4.6 | 5.8 | 7.3 | - | 5.6 | 9.0 | 12.1 |
| 8:0 | 2.3 | 1.9 | 2.3 | 3.3 | 2.1 | 1.5 | 6.3 | -2.1 | -0.9 |
| 10:0 | 3.7 | 3.4 | 3.9 | 5.1 | 3.8 | 2.6 | 9.2 | -1.6 | -0.7 |
| 10:1 | 0.3 | 0.2 | 0.2 | 0.4 | 0.2 | 0.1 | 0.6 | -0.2 | 0.1 |
| 12:0 | 3.4 | 3.9 | 4.2 | 4.4 | 4.2 | 3.4 | 7.5 | 0.9 | -0.7 |
| 14:0 | 9.1 | 8.8 | 9.5 | 10.5 | 8.9 | 8.7 | 14.5 | 3.3 | 4.1 |
| 14:1 | 0.6 | 0.4 | 0.4 | 0.7 | 0.4 | 0.3 | 0.8 | 0.0 | 0.7 |
| 15:0 | 0.6 | 1.2 | 0.7 | 0.7 | 0.8 | 2.1 | 1.0 | 0.6 | -1.3 |
| 16:0 | 13.1 | 14.8 | 13.6 | 13.3 | 10.6 | 23.3 | 13.7 | 7.5 | 2.3 |
| 16:1 | 1.0 | 1.6 | 1.5 | 1.1 | 1.4 | 2.1 | 1.5 | 1.3 | -0.6 |
| 17:0 | 0.2 | 0.5 | 0.5 | 0.2 | 0.4 | 0.6 | 0.3 | 0.5 | -0.3 |
| 18:0 | 8.0 | 9.4 | 8.3 | 7.5 | 4.7 | 18.9 | 5.8 | 3.6 | -0.7 |
| 18:1 | 13.2 | 12.4 | 13.4 | 13.3 | 8.8 | 19.6 | 13.7 | 3.9 | 6.3 |
| 18:2 | 13.5 | 12.2 | 14.4 | 14.8 | 10.5 | 15.5 | 18.5 | 2.5 | 6.5 |
| 18:3 | 0.8 | 0.7 | 0.6 | 0.9 | 0.4 | 1.3 | 1.0 | -0.2 | 0.1 |
| 20:? | - | - | - | - | - | - | - | - | - |

[a] Positions relative to sn-glycerol-3-phosphate
[b] [2 x (2,3-PLs) + (1-PLs)] /3
[c] [3 x (TGs) + (2-MGs)] /4
[e] 2 x (2,3-PLs) - (2-MGs)
[f] 3 x (TGs) - (1-PLs) - (2-MGs)
(-) Not detected

Appendix 9. Molecular species of triacylglycerol in the high molecular weight fraction of the control and 18:2-rich milk fats

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| **Saturated TGs** | | | | | | |
| 42* 16:0-12:0-16:0 | 14.0 | 20.1 | 4.1 | 4.0 | 1.5 | 1.7 |
| 44 14:0-14:0-16:0 | 19.8 | 18.7 | 5.8 | 3.7 | 2.1 | 1.6 |
| 16:0-10:0-18:0 | | | | | | |
| 46 16:0-14:0-16:0 | 21.0 | 15.5 | 6.2 | 3.1 | 2.2 | 1.3 |
| 16:0-12:0-18:0 | | | | | | |
| 48 16:0-14:0-18:0 | 19.5 | 13.5 | 5.8 | 2.7 | 2.1 | 1.2 |
| 50 18:0-14:0-18:0 | 14.9 | 13.0 | 4.4 | 2.6 | 1.6 | 1.1 |
| 16:0-16:0-18:0 | | | | | | |
| 52 18:0-16:0-18:0 | 6.8 | 7.4 | 2.0 | 1.5 | 0.7 | 0.6 |
| **Trans - monoene TGs** | | | | | | |
| 46 16:0-12:0-18:1 | 9.2 | 7.6 | 0.9 | 0.3 | 0.3 | 0.1 |
| 48 16:0-14:0-18:1 | 21.6 | 12.1 | 2.1 | 0.4 | 0.8 | 0.2 |
| 50 16:0-16:0-18:1 | 35.2 | 22.1 | 3.5 | 0.8 | 1.3 | 0.3 |
| 18:0-14:0-18:1 | | | | | | |
| 18:1-14:0-18:0 | | | | | | |
| 52 18:0-16:0-18:1 | 25.0 | 24.3 | 2.5 | 0.9 | 0.9 | 0.4 |
| 18:1-16:0-18:0 | | | | | | |
| 54 18:0-18:0-18:1 | 5.3 | 13.3 | 0.5 | 0.5 | 0.2 | 0.2 |
| 18:1-18:0-18:0 | | | | | | |
| **Cis - monoene TGs** | | | | | | |
| 46 16:0-12:0-18:1 | 10.7 | 12.1 | 3.6 | 2.6 | 1.3 | 1.1 |
| 48 16:0-14:0-18:1 | 22.7 | 16.4 | 7.7 | 3.5 | 2.8 | 1.5 |
| 50 16:0-16:0-18:1 | 33.1 | 24.6 | 11.3 | 5.2 | 4.1 | 2.2 |
| 18:0-14:0-18:1 | | | | | | |
| 18:1-14:0-18:0 | | | | | | |

(To be cont.)

Appendix 9. (cont.)

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| Cis - monoene TGs (cont.) | | | | | | |
| 52 18:0-16:0-18:1<br>18:1-16:0-18:0 | 23.2 | 22.6 | 7.9 | 4.8 | 2.9 | 2.1 |
| 54 18:0-18:0-18:1<br>18:1-18:0-18:0 | 6.0 | 11.0 | 2.0 | 2.3 | 0.7 | 1.0 |
| Diene TGs | | | | | | |
| 46 16:0-12:0-18:2<br>18:1-10:0-18:1<br>16:0-14:0-18:2 | - | 8.9 | - | 2.4 | - | 1.1 |
| 48 18:1-12:0-18:1 | 6.9 | 11.4 | 1.2 | 3.1 | 0.4 | 1.3 |
| 50 18:1-14:0-18:1<br>18:0-14:0-18:2<br>18:2-14:0-18:0<br>16:0-16:0-18:2 | 22.4 | 20.5 | 3.9 | 5.6 | 1.4 | 2.4 |
| 52 18:1-16:0-18:1<br>18:0-16:0-18:2<br>16:0-18:2-18:0<br>18:2-16:0-18:0 | 41.7 | 26.5 | 7.3 | 7.3 | 2.6 | 3.1 |
| 54 18:1-18:0-18:1<br>18:0-18:2-18:0 | 21.8 | 25.9 | 3.8 | 7.1 | 1.4 | 3.1 |
| Triene TGs | | | | | | |
| 50 18:2-14:0-18:1<br>18:1-14:0-18:2 | 16.5 | 15.1 | 1.5 | 2.4 | 0.5 | 1.0 |
| 52 16:0-18:2-18:1<br>18:2-16:0-18:1<br>18:1-16:0-18:2<br>16:0-18:3-18:0<br>18:0-16:0-18:3 | 28.6 | 29.4 | 2.6 | 4.6 | 0.9 | 2.0 |

(To be cont.)

Appendix 9. (cont.)

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| **Triene TGs (cont.)** | | | | | | |
| 54 18:0-18:2-18:1 | 48.0 | 45.9 | 4.3 | 7.2 | 1.6 | 3.1 |
| 18:1-18:2-18:0 | | | | | | |
| 18:1-18:1-18:1 | | | | | | |
| 18:0-18:3-18:0 | | | | | | |
| **Tetraene TGs** | | | | | | |
| 52 16:0-18:2-18:2 | - | 16.9 | - | 2.0 | - | 0.9 |
| 18:2-16:0-18:2 | | | | | | |
| 54 18:0-18:2-18:2 | - | 73.0 | - | 8.8 | - | 3.8 |
| 18:2-18:2-18:0 | | | | | | |
| 18:1-18:2-18:1 | | | | | | |
| 18:1-18:2-18:2 | | | | | | |

* Carbon number of triacylglycerols

Appendix 10. Molecular species of triacylglycerol in the medium molecular weight fraction of the control and 18:2-rich milk fats

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| **Saturated TGs** | | | | | | |
| 34* 16:0-14:0-4:0 | 3.1 | 8.1 | 1.5 | 2.3 | 0.3 | 0.4 |
| 36 16:0-16:0-4:0 | 17.0 | 26.1 | 8.0 | 7.3 | 1.6 | 1.4 |
| 16:0-14:0-6:0 | | | | | | |
| 38 16:0-16:0-6:0 | | | | | | |
| 18:0-14:0-6:0 | 33.5 | 36.7 | 15.7 | 10.3 | 3.1 | 2.0 |
| 18:0-16:0-4:0 | | | | | | |
| 40 18:0-16:0-6:0 | 24.8 | 20.9 | 11.6 | 5.9 | 2.3 | 1.1 |
| 18:0-18:0-4:0 | | | | | | |
| 42 18:0-18:0-6:0 | 12.3 | 5.0 | 5.8 | 1.4 | 1.1 | 0.3 |
| **Trans - monoene TGs** | | | | | | |
| 38 18:1-14:0-6:0 | 19.3 | 23.0 | 1.5 | 1.8 | 0.3 | 0.4 |
| 18:1-16:0-4:0 | | | | | | |
| 40 18:1-18:0-4:0 | 31.7 | 41.8 | 2.5 | 3.3 | 0.5 | 0.6 |
| 18:1-16:0-6:0 | | | | | | |
| 42 18:1-18:0-6:0 | 20.3 | 20.1 | 1.6 | 1.6 | 0.3 | 0.3 |
| **Cis - monoene TGs** | | | | | | |
| 38 18:1-14:0-6:0 | 15.0 | 23.6 | 3.6 | 5.7 | 0.7 | 1.1 |
| 18:1-16:0-4:0 | | | | | | |
| 40 18:1-18:0-4:0 | 32.1 | 44.7 | 7.7 | 10.8 | 1.5 | 2.1 |
| 18:1-16:0-6:0 | | | | | | |
| 42 18:1-18:0-6:0 | 23.4 | 20.2 | 5.6 | 4.9 | 1.1 | 1.0 |
| 44 18:1-18:0-8:0 | 13.5 | 4.6 | 3.2 | 1.1 | 0.6 | 0.2 |

(To be cont.)

Appendix 10.(cont.)

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| **Diene TGs** | | | | | | |
| 40 18:1-18:1-4:0 | 11.5 | 34.5 | 1.5 | 7.8 | 0.3 | 1.5 |
| 18:0-18:2-4:0 | | | | | | |
| 18:2-18:0-4:0 | | | | | | |
| 42 18:1-18:1-6:0 | 21.1 | 36.4 | 2.8 | 8.2 | 0.6 | 1.6 |
| 18:0-18:2-6:0 | | | | | | |
| 18:2-18:0-6:0 | | | | | | |
| 44 18:1-18:1-8:0 | 18.2 | 12.0 | 2.4 | 2.7 | 0.5 | 0.5 |
| 18:0-18:2-8:0 | | | | | | |
| 18:2-18:0-8:0 | | | | | | |
| 46 18:1-18:1-10:0 | 16.7 | 6.3 | 2.2 | 1.4 | 0.4 | 0.3 |
| 18:0-18:2-10:0 | | | | | | |
| 18:2-18:0-10:0 | | | | | | |
| **Triene TGs** | | | | | | |
| 42 18:2-18:1-6:0 | 6.5 | 40.2 | 0.5 | 3.6 | 0.1 | 0.7 |
| 18:1-18:2-6:0 | | | | | | |
| 18:0-18:3-6:0 | | | | | | |
| 44 18:1-18:2-8:0 | 7.8 | 19.4 | 0.6 | 1.7 | 0.1 | 0.3 |
| 18:2-18:1-8:0 | | | | | | |
| 18:0-18:3-8:0 | | | | | | |
| 46 18:0-18:3-10:0 | 12.7 | 17.5 | 1.0 | 1.6 | 0.2 | 0.3 |
| 18:2-18:1-10:0 | | | | | | |
| 18:1-18:2-10:0 | | | | | | |
| 48 16:0-14:0-18:3 | 12.9 | 5.5 | 1.0 | 0.5 | 0.2 | 0.1 |
| 18:2-12:0-18:1 | | | | | | |
| 50 16:0-16:0-18:3 | 18.3 | 6.1 | 1.5 | 0.5 | 0.3 | 0.1 |
| 18:0-14:0-18:3 | | | | | | |

(To be cont.)

Appendix 10. (cont.)

| | Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|---|
| | | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| | Triene TGs (cont.) | | | | | | |
| | 18:2-14:0-18:1 | | | | | | |
| | 18:1-14:0-18:2 | | | | | | |
| 52 | 18:1-16:0-18:3 | 16.7 | 4.3 | 1.4 | 0.4 | 0.3 | 0.1 |
| | 18:3-16:0-18:0 | | | | | | |
| | 18:2-16:0-18:1 | | | | | | |
| | 16:0-18:2-18:1 | | | | | | |
| 54 | 18:1-18:1-18:1 | 12.1 | 4.2 | 1.0 | 0.4 | 0.2 | 0.1 |
| | 18:0-18:2-18:1 | | | | | | |
| | Tetraene TGs | | | | | | |
| 46 | 18:2-10:0-18:2 | - | 12.9 | - | 1.1 | - | 0.2 |
| 50 | 18:2-14:0-18:2 | - | 11.1 | - | 0.9 | - | 0.2 |
| 52 | 18:2-16:0-18:2 | - | 13.3 | - | 1.1 | - | 0.2 |
| | 16:0-18:2-18:2 | | | | | | |
| 54 | 18:2-18:2-18:0 | - | 39.0 | - | 3.3 | - | 0.6 |
| | 18:0-18:2-18:2 | | | | | | |
| | 18:1-18:2-18:1 | | | | | | |
| | 18:1-18:2-18:2 | | | | | | |
| | 18:2-18:2-18:1 | | | | | | |

* Carbon number of triacylglycerols

Appendix 11. Molecular species of triacylglycerol in the low molecular weight fraction of the control and 18:2-rich milk fats

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| **Saturated TGs** | | | | | | |
| 30* 14:0-12:0-4:0 | 5.7 | 9.7 | 2.7 | 3.0 | 1.2 | 1.1 |
| 16:0-10:0-4:0 | | | | | | |
| 32 14:0-14:0-4:0 | 13.9 | 16.7 | 6.6 | 5.2 | 2.9 | 2.0 |
| 16:0-12:0-4:0 | | | | | | |
| 34 16:0-14:0-4:0 | 30.2 | 27.4 | 14.3 | 8.5 | 6.3 | 3.2 |
| 36 16:0-16:0-4:0 | 35.2 | 28.8 | 16.6 | 9.0 | 7.3 | 3.4 |
| 18:0-14:0-4:0 | | | | | | |
| 38 18:0-16:0-4:0 | 12.4 | 11.8 | 5.9 | 3.7 | 2.6 | 1.4 |
| **Trans - monoene TGs** | | | | | | |
| 34 18:1-12:0-4:0 | 14.0 | 9.2 | 1.3 | 0.5 | 0.6 | 0.2 |
| 36 18:1-14:0-4:0 | 27.5 | 28.8 | 2.6 | 1.4 | 1.1 | 0.5 |
| 38 18:1-16:0-4:0 | 47.0 | 40.0 | 4.4 | 2.0 | 1.9 | 0.8 |
| 40 18:1-18:0-4:0 | 11.1 | 19.8 | 1.0 | 1.0 | 0.5 | 0.4 |
| 18:0-18:1-4:0 | | | | | | |
| **Cis - monoene TGs** | | | | | | |
| 34 18:1-12:0-4:0 | 9.2 | 11.2 | 2.6 | 2.3 | 1.1 | 0.9 |
| 36 18:1-14:0-4:0 | 29.4 | 27.1 | 8.2 | 5.5 | 3.6 | 2.1 |
| 38 18:1-16:0-4:0 | 45.6 | 37.7 | 12.8 | 7.6 | 5.6 | 2.9 |
| 40 18:1-18:0-4:0 | 12.0 | 18.0 | 3.4 | 3.6 | 1.5 | 1.4 |
| 18:0-18:1-4:0 | | | | | | |
| **Diene TGs** | | | | | | |
| 32 18:2-10:0-4:0 | 8.2 | 0.8 | 0.8 | 0.2 | 0.3 | 0.1 |
| 34 18:2-12:0-4:0 | 12.0 | 3.2 | 1.1 | 0.9 | 0.5 | 0.3 |
| 36 18:2-14:0-4:0 | 10.5 | 11.6 | 1.0 | 3.2 | 0.4 | 1.2 |

(To be cont.)

Appendix 11. (cont.)

| Likely major molecular species of triacylglycerol | % TG class | | % Fraction | | % Total | |
|---|---|---|---|---|---|---|
| | Control | 18:2-rich | Control | 18:2-rich | Control | 18:2-rich |
| Diene TGs (cont.) | | | | | | |
| 38 16:0-18:2-4:0 | 15.4 | 35.2 | 1.4 | 9.8 | 0.6 | 3.7 |
| 18:2-16:0-4:0 | | | | | | |
| 40 18:1-18:1-4:0 | 50.1 | 45.5 | 4.6 | 12.6 | 2.0 | 4.7 |
| 18:2-18:0-4:0 | | | | | | |
| 18:0-18:2-4:0 | | | | | | |
| Triene TGs | | | | | | |
| 36 18:3-14:0-4:0 | 12.5 | 2.1 | 0.8 | 0.2 | 0.3 | 0.1 |
| 38 18:3-16:0-4:0 | 25.5 | 4.1 | 1.6 | 0.4 | 0.7 | 0.1 |
| 40 18:1-18:2-4:0 | 47.2 | 76.2 | 3.0 | 6.9 | 1.3 | 2.6 |
| 18:0-18:3-4:0 | | | | | | |
| 42 18:1-18:2-6:0 | 6.8 | 15.4 | 0.4 | 1.4 | 0.2 | 0.5 |
| 18:0-18:3-6:0 | | | | | | |
| Tetraene TGs | | | | | | |
| 40 18:2-18:2-4:0 | - | 64.9 | - | 4.5 | - | 1.7 |
| 42 18:2-18:2-6:0 | - | 20.8 | - | 1.4 | - | 0.5 |

* Carbon number of triacylglycerols

# BIBLIOGRAPHY

Ackman, R.G. and Sipos, J.C. (1964) J. Am. Oil Chem. Soc. 41, 377-378

Annison, E.F., Linzell, J.L., Fazakerly, S. and Nichols, B.W. (1967). Biochem. J. 102, 637 - 647

Askew, E.W., Emery, R.S., and Thomas, J.W. (1971a). Lipids 6, 326 - 331

Askew, E.W., Emery, R.S., and Thomas, J.W. (1971b). Lipids 6, 777 - 782

Barbano, D.M. and Sherbon, J.W. (1974). J. Dairy Sci. 58, 1 - 8

Barry, J.M., Bartley, W., Linzell, J.L. and Robinson, D.S. (1963). Biochem. J. 89, 6 - 11

Bauman, D.E., Ingle, D.L., Mellenberger, R.W. and Davis, C.L. (1973). J. Dairy Sci. 56, 1520 - 1525

Bauman, D.E. and Davis, C.L. (1974). In Lactation A Comprehensive Treatise (Larson, B.L. and Smith, V.R., eds.), vol. II, pp 31 - 75, Academic Press, New York and London

Becker, M.E. and Kumar, S. (1965). Biochemistry 4, 1839 - 1843

Berger, K.G. and Akehurst, E.E. (1966). J. Fd Technol. 1, 237 - 247

Bickerstaffe, R., Linzell, J.L., Morris, L.J. and Annison, E.F. (1970). Biochem. J. 117, 39P

Bickerstaffe, R. and Annison, E.F. (1970). Comp. Biochem. Physiol. 35, 653 - 665

Bickerstaffe, R. (1971). In Lactation (Falconer, I.R., ed.), pp 317 - 332, Butterworths, London

Bickerstaffe, R. and Annison, E.F. (1971a). Proc. Nutr. Soc. 30, 28 - 29A

Bickerstaffe, R. and Annison, E.F. (1971b). Int. J. Biochem. 2, 153 - 162

Bitman, J., Dryden, L.P., Goering, H.K., Wrenn, T.R., Yoncoskie, R.A. and Edmondson, L.F. (1973). J. Am. Oil Chem. Soc. 50, 93 - 98

Blank, M.L. and Privett, O.S. (1964). J. Dairy Sci. 47, 481 - 487

Bottino, N.R., Anderson, R.E. and Reiser, R. (1970). Lipids 5, 165 - 170

Breckenridge, W.C. and Kuksis, A. (1968a). J. Lipid Res. 9, 388 - 393

Breckenridge, W.C. and Kuksis, A. (1968b). Lipids 3, 291 - 300

Breckenridge, W.C. and Kuksis, A. (1969). Lipids 4, 197 - 204

Brockerhoff, H. (1965). J. Lipid Res. 6, 10 - 15

Brockerhoff, H. and Yurkowski, M. (1966). J. Lipid Res. 7, 62 - 64

Brockerhoff, H., Hoyle, R.J. and Wolmark, N. (1966). Biochim. Biophys. Acta 116, 67 - 72

Brockerhoff, H. (1966). Comp. Biochem. Physiol. 19, 1 - 12

Buchanan, R.A., Lawrence, A.J. and Loftus Hills, G. (1970). Proc. XVIII Int. Dairy Congr., Sydney 1 E 511

Carey, E.M. and Dils, R. (1970). Biochim. Biophys. Acta 210, 388 - 399

Chandler, N.J., Robinson, I.B., Ripper, I.C. and Fowler, P. (1973). Aust. J. Dairy Technol. 28, 179

Christie, W.W. and Moore, J.H. (1969). Biochim. Biophys. Acta 176, 445 - 452

Christie, W.W. and Moore, J.H. (1970). Biochim. Biophys. Acta 210, 46 - 56

Christie, W.W. and Moore, J.H. (1971). J. Sci. Fd Agric. 22, 120 - 124

Christie, W.W. (1974). Lipids 9, 876 - 882

Christie, W.W. (1973). Lipid Analysis, Pergamon Press, Oxford, New

York, Toronto, Sydney and Braunschweig

Christie, W.W., Moore, J.H., and Gottenbos, J.J. (1974). Lipids 9, 201 - 207

Christopherson, S.W. and Glass, R.L. (1969). J. Dairy Sci. 52, 1289 - 1290

Cook, L.J., Scott, T.W., Ferguson, K.A. and McDonald, I.W. (1970). Nature, London 228, 178 - 179

Cook, L.J., Scott, T.W. and Pan, Y.S. (1972). J. Dairy Res. 39, 211 - 218

Cook, L.J. and Scott, T.W. (1975). Proc. Aust. Biochem. Soc. 8, 46

Cunningham, H.M. and Leat, W.M.F. (1969). Can. J. Biochem. 47, 1013 - 1020

Dawson, R.M.C. and Kemp, P. (1970). In Physiology of Digestion and Metabolism in the Ruminant (Phillipson A.T., ed.) pp. 504 - 518 Oriel Press, Newcastle-upon-Tyne

de Man, J.M. and Wood, F.W. (1959). J. Dairy Res. 26, 17 - 23

de Man, J.M. (1961). J. Dairy Res. 28, 81 - 86

Dimick, P.S., McCarthy, R.D. and Patton, S. (1965). J. Dairy Sci. 48, 735 - 737

Dimick, P.S., McCarthy, R.D. and Patton, S. (1966). Biochim. Biophys. Acta 116, 159 - 162

Dimick, P.S., McCarthy, R.D. and Patton, S. (1970). In Physiology of Digestion and Metabolism in the Ruminant (Phillipson A.T., ed.) pp 529 - 541 Oriel Press, Newcastle-upon-Tyne

Dryden, F.D., Marchello, J.A., Adams, G.H. and Hale, W.H. (1971). J. Animal Sci. 32, 1016 - 1029

Dryden, F.D., Marchello, J.A., Cuitin, L.L. and Hale, W.H. (1975). J. Animal Sci. 40, 697 - 705

Edmondson, L.F., Yoncoskie, R.A., Rainey, N.H., Douglas, F.W. Jr. and Bitman, J. (1974). J. Am. Oil Chem. Soc. 51, 72 - 76

Emery, R.S. (1973). J. Dairy Sci. 56, 1187 - 1195

Faichney, G.J., Davies, H.L., Scott, T.W. and Cook, L.J. (1972). Aust. J. Biol. Sci. 25, 205 - 212

Faruque, A.J.M.O., Jarvis, B.D.W. and Hawke, J.C. (1974). J. Sci. Fd. Agric. 25, 439 - 449

Ferguson, K.A., Hemsley, J.A. and Reis, P.J. (1967). Aust. J. Sci. 30 215 - 217

Ganguly, J. (1960). Biochim. Biophys. Acta 40, 110 - 118

Glascock, R.F., Welch, V.A., Bishop, C., Davies, T., Wright, E.W. and Noble, R.C. (1966). Biochem. J. 98, 149 - 156

Gold, M. (1968). Lipids 3, 539 - 544

Gooden, J.M. and Lascelles, A.K. (1973). Aust. J. Biol. Sci. 26, 1201 - 1210

Goodman, D.S. (1957). Science 125, 1296 - 1297

Gray, I.K. (1973). J. Dairy Res. 40, 207 - 214

Gunstone, F.D. and Qureshi, M.I. (1965). J. Am. Oil Chem. Soc. 42, 961 - 965

Hartmann, P.E. and Lascelles, A.K. (1965). Aust. J. Biol. Sci. 18, 114 - 123

Hartmann, P.E. and Lascelles, A.K. (1966). J. Physiol. 184, 193 - 202

Hawke, J.C. and Silcock, W.R. (1970). Biochim. Biophys. Acta 218, 201 - 212

Hawke, J.C. (1973). In Chemistry and Biochemistry of Herbage (Butler,

G.W. and Bailey, R.W., eds.) vol. I, pp. 213 - 263, Academic Press, London and New York

Hawke, J.C., Morrison, I.M. and Wood, P.R. (1976). Submitted J. Sci. Fd Agric., July, 1976

Hilditch, T.P. (1949). The Chemical Constitution of Natural Fats Chapman and Hall, London

Hilditch, T.P. and Williams, P.N. (1964). The Chemical Constitution of Natural Fats, Chapman and Hall, London

Hill, E.E., Husbands, D.R. and Lands, W.E.M. (1968). J. Biol. Chem. 243, 4440 - 4451

Hood, R.L., Cook, L.J. and Scott, T.W. (1975). Proc. Aust. Biochem. Soc. 8, 47

Hübscher, G. (1970). In Lipid Metabolism (Wakil, S.J., ed.) pp. 279 - 370, Academic Press, New York and London

Jack, E.L., Freeman, C.P., Smith, L.M. and Mickle, J.B. (1963). J. Dairy Sci. 46, 284 - 290

Jensen, R.C. (1973). J. Am. Oil Chem. Soc. 50, 186 - 192

Johnston, J.M. (1970). In Comprehensive Biochemistry (Florkin, M. and Stotz, E.H., eds.) vol. 18, pp 1 - 18, Elsevier Publishing Co., Amsterdam, London and New York

Kinsella, J.E. (1968). Biochim. Biophys. Acta 164, 540 - 549

Kinsella, J.E. (1970a). J. Dairy Sci. 53, 1757 - 1765

Kinsella, J.E. (1970b). Lipids 5, 892 - 895

Kinsella, J.E. (1972a). Lipids 7, 349 - 355

Kinsella, J.E. (1972b). Int. J. Biochem. 3, 89 - 92

Kinsella, J.E. and Gross, M. (1973). Biochim. Biophys. Acta 316, 109 - 113

Kinsella, J.E., Bruns, D. and Infante, J.P. (1975). Lipids 10, 227 - 237

Knoop, E. and Samhammer, E. (1962). XVI Int. Dairy Congr. B p 135

Kuksis, A. and Breckenridge, W.C. (1966). J. Lipid Res. 7, 576 - 579

Kuksis, A., Marai, L. and Myher, J.J. (1973). J. Am. Oil Chem. Soc. 50, 193 - 201

Linzell, J.L. (1968). Proc. Nutr. Soc. 27, 44 - 52

Litchfield, C. (1972). Analysis of Triglycerides Academic Press, New York and London

Marai, L., Breckenridge, W.C. and Kuksis, A. (1969). Lipids 4, 562 - 570

Massart-Leën, A., Florescu, S., Verbeke, R. and Peeters, G. (1970). J. Dairy Res. 37, 373 - 387

Mattos, W. and Palmquist, D.L. (1974). J. Dairy Sci. 57, 1050 - 1054

McCarthy, R.D. and Patton, S. (1964). Nature 202, 347 - 349

McCarthy, R.D., Ghiardi, F.L.A. and Patton, S. (1965). Biochim. Biophys Acta 98, 216 - 217

McCarthy, S. and Smith, G.H. (1972). Biochim. Biophys. Acta 260, 185 - 196

McDowall, F.H. and Patchell, M.R. (1958). J. Dairy Res. 25, 159 - 180

McNair, H.M. and Bonelli, E.J. (1968). Basic Gas Chromatography pp 64 - 66 Varian-Aerograph Publication

Moore, J.H. (1974). In Industrial Aspects of Biochemistry (Spencer, B. ed.) vol. 30, part II, pp. 835 - 863, North-Holland Publishing Company, Amsterdam and London

Mulder, H. (1953). Neth. Milk Dairy J. 7, 149 - 174

Nandedkar, A.K.N. and Kumar, S. (1969). Arch. Biochem. Biophys. 134, 563 - 571

Norris, G.E., Gray. I.K. and Dolby, R.M. (1973). J. Dairy Res. 40, 311 - 321

Norris, R., Gray, I.K., McDowell, A.K.R. and Dolby, R.M. (1971). J. Dairy Res. 38, 179 - 191

Nutter, L.J. and Privett, O.S. (1967). J. Dairy Sci. 50, 1194 - 1199

Ogilvie, B.M., McClymont, G.L. and Shorland, F.B. (1961). Nature Lond. 190, 725 - 726

Pan, Y.S., Cook. L.J. and Scott, T.W. (1972). J. Dairy Res. 39, 203 - 210

Patchell, M.R. (1956). N.Z. J. Sci. Technol. 38, A 1 - 14

Patton, S., McCarthy, R.D. and Dimick. P.S. (1965). J. Dairy Sci. 48, 1389 - 1391

Pitas, R.E., Sampugna, J. and Jensen, R.G. (1967). J. Dairy Sci. 50, 1332 - 1336

Plowman, R.D., Bitman, J., Gordon, C.H., Dryden, L.P., Goering, H.K., Wrenn, T.R., Edmondson, L.F., Yoncoskie, R.A. and Douglas, F.W. Jr. (1972). J. Dairy Sci. 55, 204 - 207

Privett, O.S., Blank, M.L. and Verdino, B. (1965). J. Nutr. 85, 187 195

Pynadath, T.I. and Kumar, S. (1964). Biochim. Biophys. Acta 84, 251 - 263

Raphael, B.C., Dimick, P.S. and Puppione, D.L. (1973). J. Dairy Sci.

56, 1025 - 1032

Rindsig, R.B. and Schultz, L.H. (1974). J. Dairy Sci. 57, 1459 - 1466

Sampugna, J., Quinn, J.G., Pitas, R.E., Carpenter, D.L. and Jensen, R.G. (1967). Lipids 2, 397 - 402

Sampugna, J. and Jensen, R.G. (1969). Lipids 4, 444 - 449

Scott, T.W., Cook, L.J., Ferguson, K.A., McDonald, I.W., Buchanan, R.A. and Loftus Hills, G. (1970). Aust. J. Sci. 32, 291 - 293

Scott, T.W., Cook, L.J. and Mills, S.C. (1971). J. Am. Oil Chem. Soc. 48, 358 - 364

Scott, T.W. and Cook, L.J. (1975). In Digestion and Metabolism in the Ruminant (McDonald, I.W. and Warner, A.C.I., eds.) pp 510 - 523 University of New England Publishing Unit, Armidale, Australia

Senior, J.R. (1964). J. Lipid Res. 5, 495 - 521

Shehata, A.A.Y., de Man, J.M. and Alexander, J.C. (1970). Can. Inst. Fd Technol. J. 3, 85 - 89

Shehata, A.A.Y., de Man, J.M. and Alexander, J.C. (1971). Can. Inst. Fd Technol. J. 4, 61 - 67

Shehata, A.A.Y., de Man, J.M. and Alexander, J.C. (1972). Can. Inst. Fd Technol. J. 5, 13 - 21

Sherbon, J.W. and Coulter, S.T. (1966). J. Dairy Sci. 49, 1126 - 1131

Smith, G.H. and McCarthy, S. (1969). Biochim. Biophys. Acta 176, 664 - 666

Smith, G.H., McCarthy, S. and Rook, J.A.F. (1974). J. Dairy Res. 41, 175 - 191

Smith, L.M., Freeman, C.P. and Jack, E.L. (1965). J. Dairy Sci. 48, 531 - 536

Smith, S. and Dils, R. (1966). Biochim. Biophys. Acta 116, 23 - 40

Smith, S. and Abraham, S. (1970). J. Biol. Chem. 245, 3209 - 3217

Smith, S. and Abraham, S. (1971). J. Biol. Chem. 246, 2537 - 2542

Storry, J.E., Tuckley. B. and Hall, A.J. (1969). Br. J. Nutr. 23, 157 - 172

Storry, J.E. (1970). J. Dairy Res. 37, 139 - 164

Storry, J.E. (1972). J. Soc. Dairy Technol. 25, 40 - 46

Storry, J.E., Brumby, P.E., Hall, A.J. and Johnson, V.W. (1974). J. Dairy Res. 41, 165 - 173

Taylor, M.W., Dolby, R.M. and Russell, R.W. (1971). N.Z. J. Dairy Sci. Technol. 6, 172 - 176

Taylor, M.W. (1973). Ph.D. thesis, "A Study of the Structure of Triacylglycerols of Bovine Milk Fat", Massey University, New Zealand

Taylor, M.W. and Hawke. J.C. (1975a). N.Z. J. Dairy Sci. Technol. 10, 40 - 48

Taylor, M.W. and Hawke. J.C. (1975b). N.Z. J. Dairy Sci. Technol. 10, 49 - 57

Tove, S.B. and Mochrie. R.D. (1963). J. Dairy Sci. 46, 686 - 689

Van Beresteyn, E.C.H. (1972). Neth. Milk Dairy J. 26, 117 - 130

Vander Wal, R.J. (1960). J. Am. Oil Chem. Soc. 37, 18 - 20

Van Wijngaarden, D. (1967). Anal. Chem. 39, 848 - 849

Vaskovsky, V.E. and Kostetsky, E.Y. (1968). J. Lipid Res. 9, 396

Viviani, R. (1970). Adv. Lipid Res. 8, 267 - 346

Weber, E.J., De La Roche I.A. and Alexander, D.E. (1971). Lipids 6, 525 - 530

Wendlandt, R.M. and Davis, C.L. (1973). J. Dairy Sci. 56, 337 - 339

West, C.E., Bickerstaffe, R., Annison, E.F. and Linzell, J.L. (1972). Biochem. J. 126, 477 - 490

Wood, F.W., Murphy, M.F. and Dunkley, W.L. (1975). J. Dairy Sci. 58, 839 - 845

Woodrow, I.L. and de Man, J.M. (1968). J. Dairy Sci. 51, 996 - 1000

Yamdagni, S. and Schultz, L.H. (1970). J. Dairy Sci. 53, 1046 - 1050

Yoncoski, R.A., Holsinger, V.H., Posati, L.P. and Pallansch, M.J. (1969). J. Am. Oil Chem. Soc. 46, 489 - 492