# Effect of Dietary Fat Supplementation on the Composition and Positional Distribution of Fatty Acids in Ruminant and Porcine Glycerides

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## ABSTRACT

Dietary fats which were protected from ruminal metabolism were fed to ruminants, and the constituent fatty acids subsequently appeared in the glycerides of tissues and secretory products. These dietary fat induced alterations in tissue lipid composition were particularly apparent when the fat source was enriched with linoleic acid. Similarly, when pigs were fed linoleic-enriched fats, the linoleic acid was incorporated into the adipose tissue triglycerides. Stereospecific analyses were carried out on triglycerides from various tissues and secretory products obtained from animals fed control or linoleate-enriched diets. The analysis of adipose tissue triglycerides showed that linoleate and oleate were preferentially esterified to positions 2 and 3 (cattle and sheep), and positions 1 and 3 (pigs). Of the other major adipose tissue fatty acids, palmitate was preferentially esterified at position 1 (ruminants) and position 2 (pigs), and stearate was preferentially esterified at positions 1 and 3 (ruminants), and position 1 (pigs). Stereospecific analysis of high mol wt milk triglycerides showed that linoleate was either evenly distributed on all three positions (goats), or predominantly on position 3 (cows). Furthermore, the incorporation of this linoleate did not markedly alter the positional specificity of the other major milk triglyceride fatty acids. Of these fatty acids, the short and medium chain length acids (butyrate-laurate) were mainly on position 3, myristate and palmitate on positions 1 and 2, and stearate and oleate evenly distributed. Thoracic duct lymph triglycerides from sheep tended to show preferential incorporation of linoleate at position 3, palmitate at position 2, and stearate at position 1 and 3; oleate, on the other hand, tended to be evenly distributed on all three positions of the lymph triglyceride. The stereospecific arrangement of fatty acids in sheep liver triglycerides was similar to that of lymph triglycerides, and this may reflect the uptake of intact or partially hydrolysed chylomicron and/or very low density lipoprotein triglycerides by the liver. There were also some analogies in the stereospecific arrangement of fatty acids on ruminant lymph and milk triglycerides and this may reflect an incomplete hydrolysis of chylomicron and/or very low density lipoprotein triglycerides prior to uptake by the mammary gland. An unusual feature of lymph from sheep fed linoleate was the presence of phospholipids which contained large amounts of linoleate in ca. equal proportion at both positions 1 and 2 of the phospholipid molecule.

## INTRODUCTION

The fatty acid composition of tissue triglycerides (TGs) from monogastric animal species is influenced by the composition of the dietary fat, and the component fatty acids within the tissue TGs of animal species generally show a characteristic positional distribution (1). This distribution is such that the saturated fatty acids are generally esterified to position 1 of the glyceride molecule, and the unsaturated fatty acids are generally esterified to position 2 and to a lesser extent to position 3. (The carbon atoms of the glycerol skeleton are numbered stereospecifically but for convenience the *Sn* prefix is omitted.) The pig is a notable exception in that the adipose tissue TGs from this species show the "reverse structure," i.e., the principal saturated fatty acid, palmitic acid (16:0), is located on position 2, while the unsaturated fatty acids are on positions 1 and 3 (1).

The concentrations of linoleic acid (18:2) in adipose tissue of monogastric species can be markedly increased by feeding this acid, and studies with mice and rats have shown that the 18:2 is preferentially esterified at position 2 of the TGs (2,3). These studies, however, were carried out using pancreatic lipase, and by this method alone it is not possible to differentiate between the fatty acids at positions 1 and 3 of

TABLE I

Fatty Acid Composition of Dietary Lipids

| Oil or Lipid supplement | Fatty acids (% by wt) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 10:0 | 12:0 | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | Others[a] |
| Palm oil | - | 1 | 1 | 43 | - | 4 | 41 | 10 | - | - |
| Tallow | - | - | 2 | 24 | 3 | 25 | 45 | 1 | - | - |
| Safflower oil | - | - | - | 7 | - | 2 | 14 | 77 | - | - |
| Sunflower oil | - | - | - | 6 | - | 3 | 31 | 60 | - | - |
| Safflower seeds[b] | - | - | - | 8 | - | 2 | 12 | 78 | - | - |
| Sunflower seeds[c] | - | - | - | 10 | - | 8 | 22 | 60 | - | - |
| Protected sunflower seed supplements[d] | - | - | 1 | 8 | - | 8 | 17 | 66 | - | - |
| Control cows' milk[e] | 3 | 4 | 11 | 26 | 4 | 15 | 26 | 5 | 3 | 3 |
| 18:2-enriched[e] cows' milk | 3 | 3 | 7 | 18 | 2 | 13 | 25 | 21 | 1 | 7 |

[a]Principally short chain fatty acids.
[b]Safflower seeds (40% oil).
[c]Sunflower seeds (50% oil).
[d]Protected sunflower seed supplements (45% oil) were prepared by using procedures previously reported (8).
[e]Control and 18:2-enriched cows' milk both contained ca. 4.5% fat.

the glyceride molecule.

Other techniques have been developed whereby the fatty acids at the 1 and 3 positions may be separated and quantified (4,5). One of these techniques, Brockerhoff's stereospecific analysis (4), has been used in the present studies to evaluate the effects of dietary fat supplementation on the positional distribution of fatty acids in the tissue TGs of two classes of animal, ruminants and pigs.

Because of the microbial metabolism and hydrogenation of dietary fatty acids in the stomach of ruminants (6), it has been necessary with these animals to feed "protected" lipid supplements. These lipid supplements are effectively protected from ruminal metabolism, are digested and absorbed from the small intestine, and the constituent fatty acids subsequently appear in milk and tissue glycerides (7,8). Because of the particular role these protected lipid supplements may play in ruminant and human nutrition (8,9), we have considered it necessary to examine in some detail the effects of varying the nature of the protected dietary fatty acid on the fatty acid composition and positional distribution of fatty acids in a variety of ruminant organs, tissues, and secretory products.

## MATERIALS AND METHODS

### Lipid Supplements

The fatty acid compositions of the lipid supplements fed to ruminants or pigs are given in Table I. Other details pertaining to diets, type of animal, period of feeding, etc., are described along with the relevant results. The fat content of basal hay and grain diets was ca. 2-3% in all studies.

### Collection of Samples and Extraction of Lipids

Lymph (20 ml) was collected from sheep by cannulation of the thoracic duct and cellular material was removed by centrifugation at 2000 x g for 10 min. Bile (5 ml) was obtained from the gall bladder of sheep, and adipose tissue and liver samples were removed from sheep, cattle, and pigs at slaughter. The lymph, bile, and tissues were extracted with chloroform:methanol (2:1, v/v) (10). Milk samples (100 ml) were obtained after morning milking of cows and goats, and lipids were extracted (11).

Phospholipids were removed from the total lipid extracts by shaking the lipid (400 mg) in chloroform (10 ml) for 10 min with silicic acid (500 mg). The silicic acid was removed by filtration in a sintered glass funnel and was washed with chloroform. The filtrate and

washings (containing the neutral lipids) were evaporated to dryness in vacuo. When necessary, the phospholipids were recovered from the silicic acid by elution with methanol. The purity of each preparation was checked by thin layer chromatography (TLC). The fatty acid composition of the samples was determined by gas liquid chromatography after methylation of the free fatty acid (12) except for milk TG, which was analyzed after preparation of butyl esters using the procedure of Parodi (13).

#### Stereospecific Analysis of Triglyceride

*Tissue and lymph triglyceride:* The intrapositional distribution of fatty acids on the TG from adipose tissue, liver, and lymph was determined using the Grignard reagent method described by Brockerhoff (4) as modified by Christie and Moore (14).

*Milk triglyceride:* It proved difficult to separate the resultant diglycerides (DGs) when milk TGs were directly reacted with the Grignard reagent (15). A modified procedure (16) was therefore used to isolate the higher mol wt TGs from milk and then to use this fraction for stereospecific analysis.

The milk TGs (150-300 mg) were partitioned into fractions of different mol wt by passing the samples through a glass column (300 x 30 mm ID) containing Sephadex LH 20 (Pharmacia Fine Chemicals, Inc., (Uppsala, Sweden) suspended in chloroform (16). The TGs were eluted with chloroform using a flow rate of 0.15-0.20 ml/min and the fatty acid composition was determined on portions of the eluate (13). On the basis of the fatty acid composition, the eluates were pooled to form three major fractions (fractions I, II, and III in order of decreasing mol wt) (Fig. 1). The TG from fraction I was subjected to stereospecific analysis (4,14). The TGs from fractions II and III were also subjected to the Grignard reagent, but the resultant 1,2 and 1,3 DGs were not resolved effectively by TLC. These lower mol wt TGs were subsequently treated with pancreatic lipase (17) to ascertain the distribution of fatty acids on position 2.

*Lymph and biliary phospholipids:* The positional distributions of fatty acids in the lymph and biliary phospholipids were determined after reaction with phospholipase $A_2$ (EC 3.1.1.4) (18).

## RESULTS

### *RUMINANTS*

#### Adipose Tissue Triglyceride

*Fatty acid composition:* The feeding to

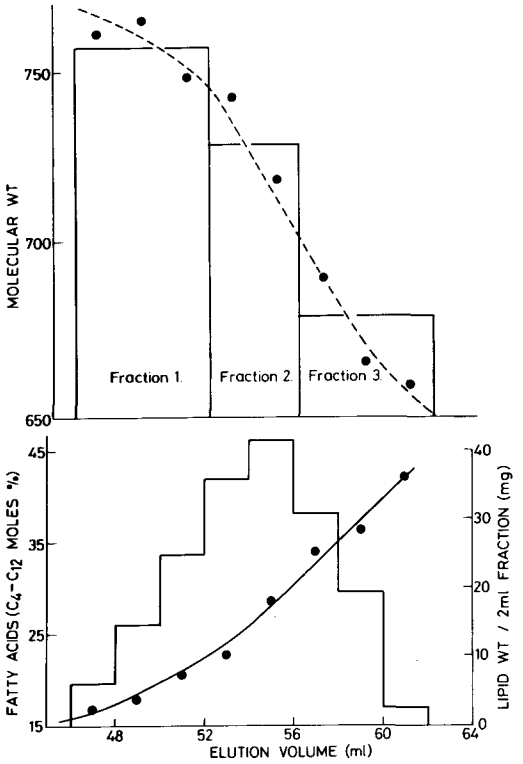


FIG. 1. Sephadex LH-20 separation of goats' milk triglycerides into three arbitrary mol wt fractions. The lower graph shows the relationship between elution volume, wt of lipid (histogram), and the proportion of $C_4$-$C_{12}$ fatty acids (•—•). The upper graph shows the relationship between elution volume, arbitrary fractions (histogram) and mol wt of triglycerides (•- - -•). Chloroform was the eluting solvent; other details of the method are given in the text.

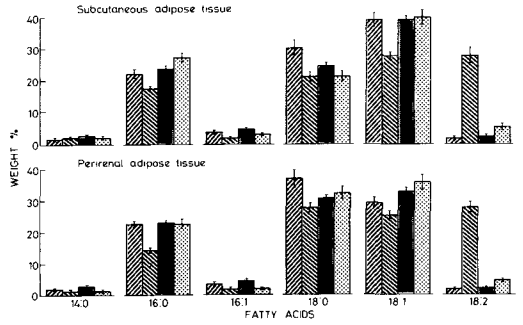


FIG. 2. Effect of feeding protected fats on the fatty acid composition of triglycerides from adipose tissues of lambs. ▨ Control, ▧ safflower oil, ▩ tallow, ⬚ palm oil. The lambs (8-10 weeks of age) were fed either 800 g per day chopped lucerne and crushed oats (1:1) (control diet) or other diets containing lucerne (350 g), oats (140 g), and formaldehyde treated oil: casein (2:1, w/w) supplements (210 g) (7). Lambs were slaughtered after 8 weeks and tissues were immediately transferred to chloroform: methanol (2:1, v/v). Means and standard errors for four lambs.

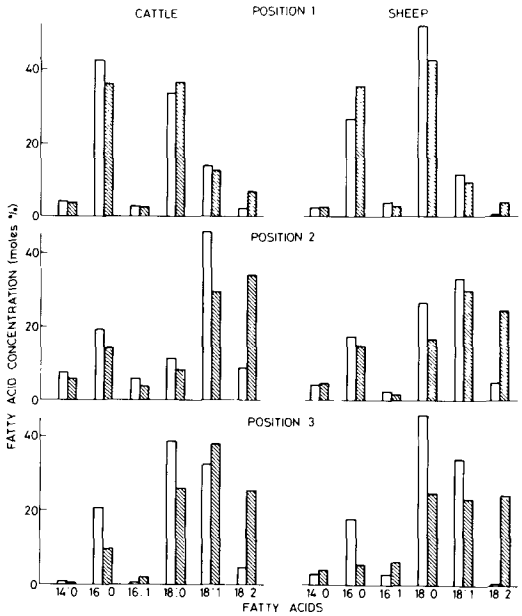


FIG. 3. Intrapositional distribution of major fatty acids in the triglycerides from perirenal adipose tissue from cattle and sheep which had received unsupplemented ( □ ) and 18:2 supplemented ( ▨ ) diets. The protected sunflower seed supplements (8) were fed to mature sheep and cattle for 8-10 weeks prior to slaughter. The basal diets contained lucerne and oats (1:1, w/w), and supplemented diets contained 30% by wt of lipid supplement. Each value represents a single animal.

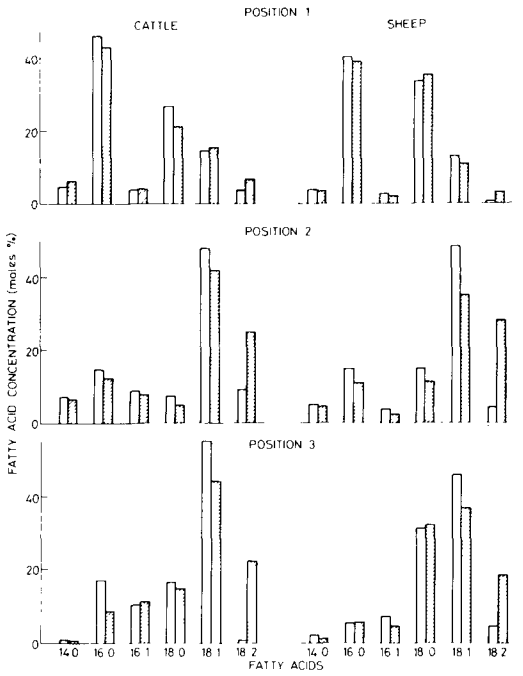


FIG. 4. Intrapositional distribution of major fatty acids in the triglycerides from subcutaneous adipose tissue from cattle and sheep which had received unsupplemented ( □ ) and 18:2 supplemented ( ▨ ) diets. The protected sunflower seed supplements (8) were fed to mature sheep and cattle for 8-10 weeks prior to slaughter. The basal diets contained lucerne and oats (1:1, w/w), and supplemented diets contained 30% by wt of lipid supplement. Each value represents a single animal.

lambs of protected safflower oil containing large proportions of 18:2 led to a clear increase in the proportion of 18:2 in the subcutaneous and perirenal adipose tissue TGs (Fig. 2). This was associated with a decline in the proportions of 16:0, 18:0, and 18:1. The feeding of protected tallow or palm oil caused lesser alterations in the fatty acid composition of adipose tissue TG (Fig. 2). The proportion of 18:2 was increased on feeding the protected palm oil, and there were also increases in the proportion of 16:0 (subcutaneous) and 18:1 (perirenal) on this diet. In addition, the feeding of protected palm oil and tallow caused reductions in the proportion of 18:0 in the fat from both tissue sites (Fig. 2).

*Positional distribution of fatty acids:* Figure 3 shows the positional distribution of fatty acids in the perirenal adipose tissue TG from mature sheep and cattle fed conventional diets or similar diets supplemented with protected 18:2. Figure 4 shows corresponding results for the subcutaneous adipose tissue TG. The 18:2 which was protected against ruminal hydrogenation was incorporated predominantly at positions 2 and 3 of the TGs from both adipose tissue sites. Furthermore, this incorporation of 18:2 did not consistently alter the interpositional specificity of the other major fatty acids, with the possible exception of 16:0, which tended to be replaced more effectively by 18:2 on position 3 than on position 2, particularly in perirenal tissue (Fig. 3). Examination of the positional distribution of fatty acids in perirenal adipose tissue from sheep fed protected palm oil revealed that the interpositional specificity of the major fatty acids was not altered, with the possible exception of position 1, where 16:0 was slightly enhanced and there was a decrease in the proportion of 18:0 (S.C. Mills, unpublished study, 1974).

### Milk Triglyceride

*Fatty acid composition:* Table II shows the effect of feeding protected 18:2 on the fatty acid composition of the three TG fractions separated from cows' milk by Sephadex LH-20 chromatography. The protected 18:2 was found in all three fractions, but the greatest amounts were present in the highest mol wt

TABLE II

Fatty Acid Composition (in Moles %) of Milk Triglyceride Fractions from Cows Fed Control Diets or Similar Diets Supplemented with Protected 18:2

| Fatty acid | Control[a] | | | | 18:2 supplemented[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | Original triglyceride | Fractions[b] I | II | III | Original triglyceride | Fractions[b] I | II | III |
| 4:0 | 8.1 | 7.2 | 8.0 | 8.4 | 5.3 | 8.4 | 10.3 | 13.4 |
| 6:0 | 4.2 | 2.0 | 5.1 | 4.5 | 5.1 | 3.1 | 3.1 | 5.0 |
| 8:0 | 1.7 | 0.7 | 1.9 | 2.8 | 2.4 | 1.2 | 3.5 | 4.1 |
| 10:0 | 2.9 | 1.5 | 3.0 | 3.8 | 4.3 | 2.8 | 5.1 | 5.5 |
| 12:0 | 2.7 | 1.8 | 2.6 | 3.4 | 3.7 | 2.7 | 4.0 | 4.1 |
| 14:0 | 7.9 | 6.0 | 7.9 | 8.9 | 8.3 | 6.8 | 8.7 | 8.3 |
| 16:0 | 20.3 | 18.3 | 20.3 | 20.4 | 15.9 | 14.7 | 15.8 | 14.8 |
| 16:1 | 3.0 | 3.4 | 2.7 | 2.8 | 1.5 | 1.6 | 1.0 | 1.3 |
| 18:0 | 13.9 | 15.8 | 13.4 | 11.6 | 12.3 | 14.2 | 11.3 | 10.0 |
| 18:1 | 26.1 | 29.9 | 25.2 | 21.4 | 19.1 | 21.8 | 17.6 | 15.6 |
| 18:2 | 5.0 | 5.1 | 5.9 | 2.9 | 18.1 | 18.6 | 16.6 | 13.4 |
| 18:3 | 1.2 | 2.2 | 0.9 | 0.5 | 1.5 | 1.6 | 0.8 | 0:4 |

[a]The control and basal diets contained lucerne and oats (1:1, w/w). The formaldehyde treated supplement, safflower oil:casein (2:1, w/w) (7), was fed at 800 g per day.

[b]Fractions I, II, and III were isolated by gel filtration of milk triglyceride on Sephadex LH-20 using procedures described in the text.

fraction (fraction I). This fraction also contained the greatest concentrations of the other long chain fatty acids, e.g., 18:0 and 18:1.

*Positional distribution of fatty acids:* Figure 5 shows the positional distribution of fatty acids in the high mol wt fraction (fraction I) of milk TG from cows fed conventional diets or diets supplemented with protected 18:2. The characteristic features of these milk TGs are (a) a preferential esterification of short and medium chain length fatty acids ($C_4$-$C_{12}$) at position 3: (b) a preferential esterification of 16:0 and 14:0 at positions 1 and 2; (c) ca. random esterification of 18:0 and 18:1, i.e., these acids are located in ca. equal proportions on all three positions; and (d) the protected 18:2 incorporated at all three positions, with the highest proportion at position 3.

Table III shows the positional distribution of fatty acids in the high mol wt fraction (fraction I) of milk TG from goats fed conventional diets or diets supplemented with protected 18:2. The positional specificity of the shorter chain acids (8:0 and 10:0), 16:0, 18:0, and 18:1 was similar to that in cows' milk TGs (Fig. 5), but the 18:2 was distributed more evenly in the goat TGs.

The lower mol wt TG fractions from cows' milk (fractions II and III) were reacted with pancreatic lipase. The reaction products were isolated and their fatty acid compositions determined (Table IV). Although in this case it is not possible to distinguish between the fatty acids on position 1 and those on position 3 of the glyceride molecule, it is possible, by comparing the concentrations of particular fatty acids at the respective 2 and 1(3) positions, to conclude that these lower mol wt TGs probably have a positional distribution of fatty acids which is similar to that of the high mol wt fraction (Fig. 5). This is indicated by (a) the greater concentrations of the shorter chain length acids (4:0 - 8:0) at the 1(3) position, (b) the greater concentration of 16:0 at the 2 position, and (c) the ca. equal proportions of 18:2 at the 2 and the 1(3) positions.

### Lymph Triglyceride

Figure 6 shows the positional distribution of fatty acids in TG from the thoracic lymph duct of sheep fed control diets or similar diets supplemented with protected 18:2. A feature of these results is the pronounced interpositional specificity of 16:0; this fatty acid was located predominantly on position 2. Similar results were obtained by Garton and Duncan (19) using pancreatic lipase to isolate the 2-monoglycerides. Of the other principal fatty acids, 18:0 was preferentially esterified to positions 1 and 3, 18:1 was located on all three positions in ca. equal proportions, and 18:2 was preferred at position 3.

The incorporation of protected 18:2 into the lymph TG caused slight alterations to the interpositional locations of the other major fatty acids, i.e., 18:2 substituted for 18:0 on position 3, 16:0 on positions 1 and 2, and 18:1 on positions 1 and 2 (Fig. 6).

### Lymph and Biliary Phospholipids

Figure 7 shows the positional distribution of

TABLE III

Intrapositional Distribution (in Moles %) of Fatty Acids in the High Mol Wt Triglycerides from Goats' Milk[a]

| Fatty acids | Control | | | Supplemented | | |
|---|---|---|---|---|---|---|
| | Position | | | Position | | |
| | 1 | 2 | 3 | 1 | 2 | 3 |
| 8:0 | 1.1 | 0.2 | 8.0 | 2.0 | 0.9 | 7.3 |
| 10:0 | 3.5 | 11.8 | 14.3 | 10.4 | 3.4 | 14.7 |
| 12:0 | 3.1 | 6.1 | 4.3 | 7.3 | 3.5 | 3.9 |
| 14:0 | 5.7 | 12.8 | 6.1 | 16.3 | 6.8 | 4.2 |
| 16:0 | 31.0 | 26.5 | 5.7 | 17.4 | 24.4 | 10.1 |
| 18:0 | 9.7 | 6.5 | 9.2 | 6.5 | 13.2 | 6.1 |
| 18:1 | 18.8 | 18.0 | 27.8 | 15.9 | 23.0 | 21.7 |
| 18:2 | 0.7 | 2.1 | 4.9 | 18.9 | 15.9 | 13.5 |

[a]Goats were fed diets of lucerne and oats or similar diets supplemented with formaldehyde-treated safflower oil/casein (7). High mol wt triglyceride fraction I was prepared by gel filtration as described in the text.

TABLE IV

Composition (in Moles %) of Fatty Acids at Position 2 and at Position 1(3) of Triglycerides from Control and 18:2-Enriched Bovine Milk[a]

| Fatty acid | Fraction II[b] | | | | Fraction III[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | | 18:2-enriched | | Control | | 18:2-enriched | |
| | Position[c] | | | | | | | |
| | 2 | 1(3) | 2 | 1(3) | 2 | 1(3) | 2 | 1(3) |
| 4:0 | 3.2 | 10.4 | 6.1 | 13.7 | 0.9 | 12.5 | 2.9 | 17.7 |
| 6:0 | 2.4 | 6.4 | 5.7 | 1.8 | 3.1 | 5.5 | 2.0 | 7.5 |
| 8:0 | 1.4 | 2.2 | 2.4 | 3.9 | 1.4 | 3.7 | 2.8 | 4.6 |
| 10:0 | 2.8 | 3.1 | 5.3 | 4.9 | 4.9 | 3.6 | 5.5 | 5.6 |
| 12:0 | 3.0 | 2.4 | 5.3 | 3.4 | 4.6 | 3.1 | 6.0 | 3.3 |
| 14:0 | 8.5 | 7.6 | 9.4 | 8.1 | 11.3 | 8.4 | 11.4 | 6.9 |
| 16:0 | 24.4 | 18.3 | 16.7 | 15.0 | 26.8 | 19.0 | 19.1 | 12.9 |
| 16:1 | 4.6 | 1.8 | 2.2 | - | 4.1 | 2.3 | 2.4 | 0.9 |
| 18:0 | 12.7 | 13.8 | 9.7 | 11.7 | 11.4 | 12.5 | 10.5 | 9.8 |
| 18:1 | 24.5 | 25.6 | 15.6 | 18.1 | 23.9 | 21.9 | 16.7 | 15.0 |
| 18:2 | 4.6 | 6.6 | 15.4 | 16.7 | 3.7 | 2.8 | 14.8 | 12.8 |

[a]The control and basal diets contained lucerne and oats (1:1; w/w). The formaldehyde-treated supplement, safflower oil:casein (2:1, w/w) (7), was fed at 800 g per day.

[b]Fractions II and III were prepared by gel fractionation of milk triglycerides on Sephadex LH-20 as described in the text.

[c]The 2-monoglycerides and free fatty acids were isolated by thin layer chromatography after pancreatic lipase hydrolysis of respective fractions (17). This procedure does not allow the separate isolation of fatty acids on positions 1 and 3, i.e., 1(3).

fatty acids in the biliary and lymph phospholipids from sheep fed conventional diets or similar diets supplemented with protected 18:2. The biliary phospholipids from lambs fed conventional diets showed a positional distribution of fatty acids which is characteristic for most phospholipids (1), i.e., the proportion of saturated fatty acids (16:0 and 18:0) was highest on position 1 and that of the unsaturated fatty acids (18:1 and 18:2) was highest on position 2 (Fig. 7). This marked degree of positional specificity was also evident for the biliary phospholipids obtained from lambs fed protected 18:2 (Fig. 7). The proportion of 18:2 in the biliary phospholipids was considerably enhanced by feeding the protected lipid supplement, and this fatty acid was predominantly located at position 2, mainly at the expense of 18:1.

The positional specificity of fatty acids in the lymph phospholipids was different from that in the bile. 16:0 and 18:1 were distributed equally between positions 1 and 2. 18:0 showed a slight preference for position 1 in the control situation, but this was reversed by feeding the protected 18:2. 18:2 itself showed a

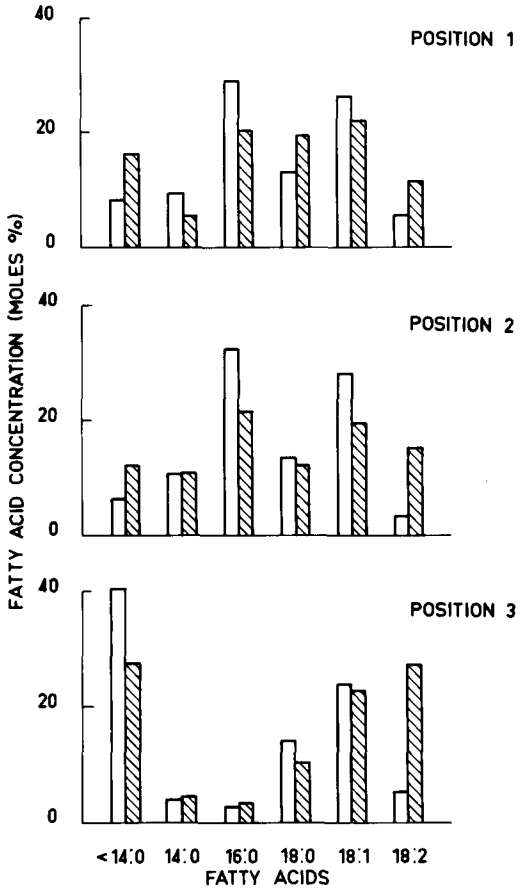


FIG. 5. Intrapositional distribution of fatty acids in high mol wt triglycerides from cows' milk. Cows were receiving unsupplemented ( □ ) or 18:1 supplemented ( ▨ ) diets. The 18:2 supplement was formaldehyde treated safflower oil: casein (2:1, w/w) (7) and was fed at 800 g per day; the balance of the ration was lucerne and oats (1:1, w/w). The unsupplemented diet consisted solely of lucerne and oats (1:1, w/w).

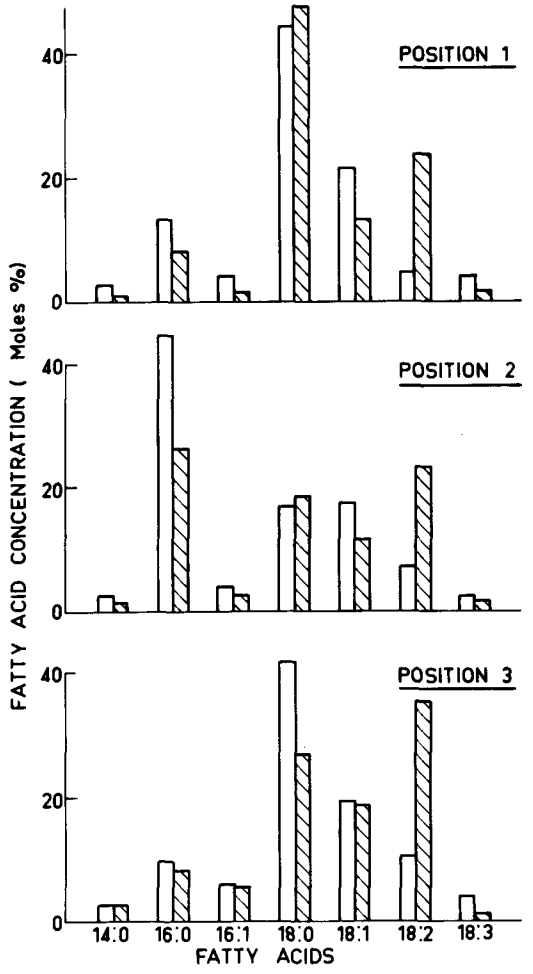


FIG. 6. Intrapositional distribution of fatty acids in thoracic lymph triglycerides from a 1 year old sheep which had received an unsupplemented ( □ ) diet and a similar sheep fed an 18:2 supplemented ( ▨ ) diet. The control and basal diets were lucerne and oats (1:1, w/w). The lipid supplement of 200 g per day formaldehyde-treated safflower oil:casein (2:1, w/w), was fed for 3 weeks prior to thoracic duct cannulation. Cannulation and collection of lymph was carried out under general anaesthesia at ca. 12 hr post feeding.

preference for the 2 position in the lymph phospholipids from sheep fed control diets, but this also tended to be reversed by feeding the protected 18:2 supplements (Fig. 7).

#### Liver Triglyceride

The positional distribution of fatty acids in TG from the livers of sheep fed conventional diets or similar diets supplemented with protected 18:2 is shown in Figure 8. Octadecenoic acid (18:1) showed a preference for positions 2 and 3 in the control sheep, but no marked positional specificity at all for the supplemented animals. Myristic and palmitic acids were located preferentially on positions 1 and 2, while 18:0 was preferentially on positions 1 and 3. The location of 18:2 was largely restricted to positions 2 and 3 for the lipid supplemented animal, and the proportions of all other fatty acids, except 14:0 and 18:3, were reduced at these sites.

### *PIGS*

#### Pig Adipose Tissue Triglyceride

*Fatty acid composition:* Pigs were fed 18:2 in the form of (a) crushed safflower seeds, (b) crushed sunflower seeds, (c) formaldehyde treated homogenized sunflower seed based sup-

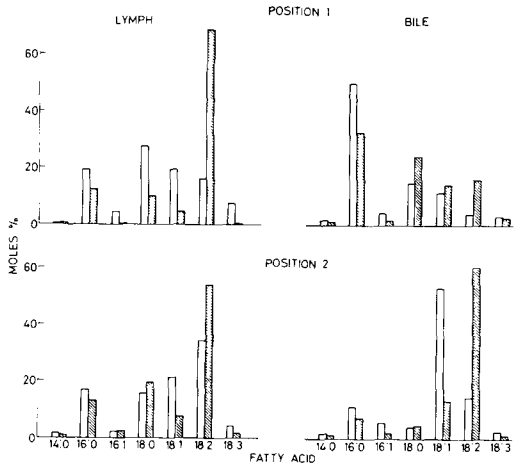


FIG. 7. Intrapositional distribution of fatty acids in biliary and lymph phospholipids from sheep which had received unsupplemented ( ☐ ) or 18:2 supplemented ( ▨ ) diets. The biliary phospholipids were obtained from young lambs, as in Figure 2, whereas the lymph phospholipids were obtained from older sheep as in Figure 6.

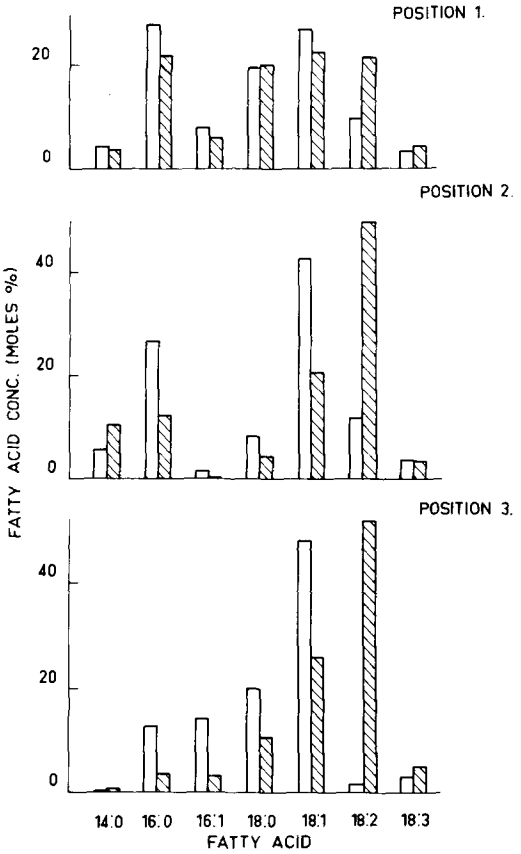


FIG. 8. Intrapositional distribution of fatty acids on liver triglycerides isolated from sheep receiving unsupplemented ( ☐ ) and 18:2 supplemented ( ▨ ) diets. The protected sunflower seed supplements (8) were fed to mature sheep for 8-10 weeks prior to slaughter. The basal diets contained lucerne and oats (1:1, w/w), and supplemented diets contained 30% by wt of lipid supplement. Each value represents a single animal.

plements prepared for ruminant feeding (8), or (d) 18:2-enriched cows' milk.

Figure 9 shows the fatty acid composition of subcutaneous adipose tissue TG from these pigs and from other pigs fed control diets. Feeding the oilseeds, oilseed supplements or 18:2-enriched cows' milk caused substantial increases in the proportion of 18:2 and reductions in the proportions of 16:0 and 18:1. The proportion of 18:2 in the tissues from pigs fed 18:2-enriched cows' milk was similar to that in the milk diet (Table I).

*Positional distribution of fatty acids:* Stereospecific analyses of perirenal adipose tissue TGs from pigs fed 18:2-enriched cows' milk showed that the 18:2 was incorporated specifically onto positions 1 and 3, and there were no marked effects of this incorporation on the interpositional specificity of the other major fatty acids, with the possible exception of 18:1 in position 3 (Fig. 10). The positional specificity, however, was markedly different from that in ruminant adipose tissues (Figs. 3 and 4), particularly because position 2 of pig TG was occupied predominatly by 16:0. Stearic acid (18:0) was mainly on position 1, and the other major fatty acid (18:1) was distributed with a preference similar to 18:2, i.e., on positions 1 and 3. Myristic acid (14:0), like 16:0, was placed mainly on position 2.

## DISCUSSION

The tissue fatty acid composition of ruminants can be altered by feeding fats which are protected from ruminal hydrogenation (7,8,20). The feeding of such fats containing polyenoic fatty acids, e.g., 18:2, will result in elevated concentrations of the respective fatty acids in the lipids of various organs and tissues (7,8,20) (Fig. 2, Table II). This incorporation of protected polyenoic fatty acids into ruminant tissue lipids is equivalent to that observed when polyenoic fats are fed to monogastric species (Fig. 9).

The feeding of protected nonpolyenoic fats, containing saturated or monoenoic fatty acids, to ruminants also increases the concentration of the major component fatty acids in the respective tissue lipids (Fig. 2). The extent of this alteration is, however, less than that observed when protected polyenoic fats are fed, and this

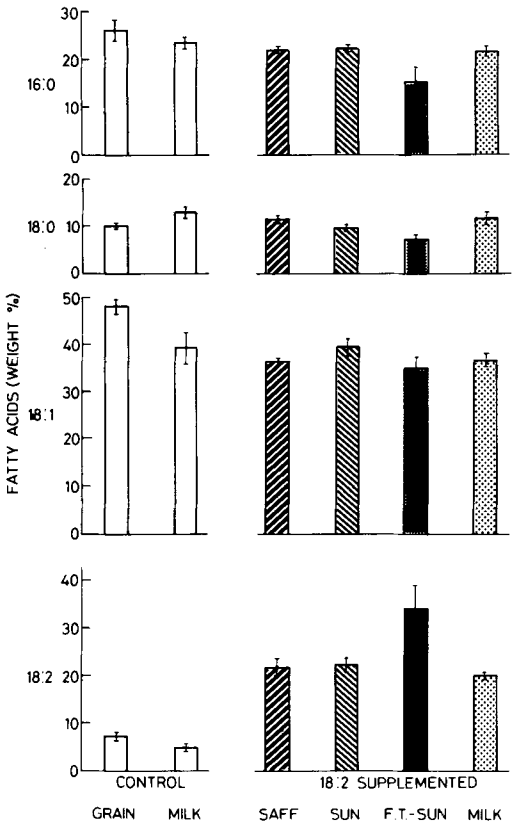


FIG. 9. Effect of feeding different dietary fats on the fatty acid composition of pig subcutaneous adipose tissue. Values are the mean ± standard error for three animals. Saff = safflower seeds, Sun = sunflower seeds, F-T Sun = formaldehyde-treated sunflower seed supplements (8). 18:2 – enriched cows' milk was obtained by feeding F-T Sun to lactating cows. The milk diets were fed as the sole diet for periods of 3-12 months prior to slaughter. The basal grain diet contained sorghum, barley and meat meal. This diet was supplemented with oilseeds or oilseed supplements (ca. 30% by wt of diet) and fed ad libitum for 12 weeks prior to slaughter.

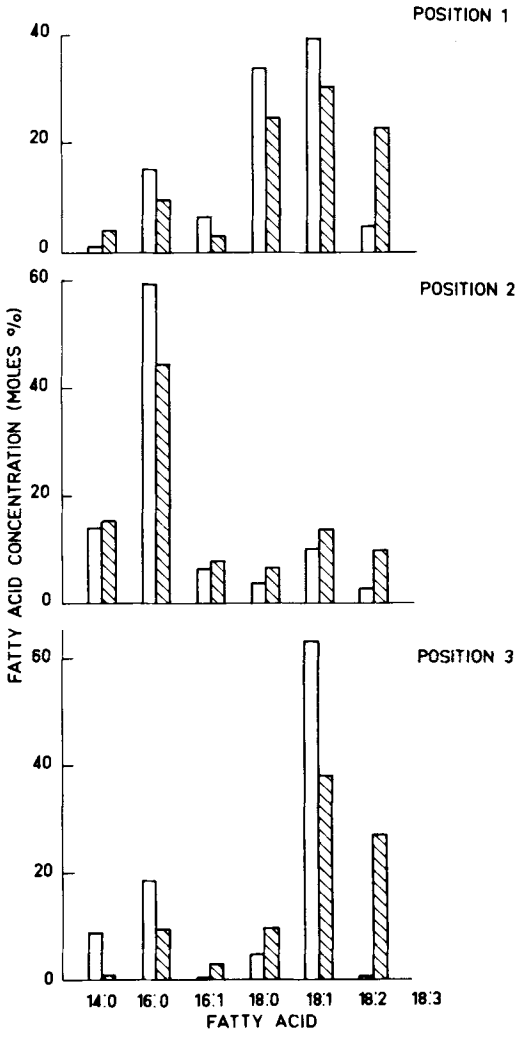


FIG. 10. Intra positional distribution of fatty acids in the perirenal adipose tissue of pigs which had received conventional cows' milk ( □ ) or 18:2-enriched cows' milk ( ▨ ). The milk diets, as in Figure 9 were fed for 3 months prior to slaughter. The pigs were introduced to the milk diets at 2-4 weeks of age.

is probably due to the metabolic transformation of the component acids, e.g., 16:0 and 18:0, prior to their acylation to form TG (21,22). It is also possible that these non-polyenoic fatty acids do not suppress the normal endogenous synthesis of tissue fatty acids to the same extent as do the polyenoic fatty acids (23). This latter effect may also partially explain the reduction in the proportions of 16:0, 18:0, and 18:1 as a consequence of 18:2 incorporation into adipose tissue TGs of ruminants (Fig. 2) and pigs (Fig. 9).

The incorporated 18:2 was specifically located on positions 2 and 3 of ruminant adipose tissue TG (Figs. 3 and 4) and on positions 1 and 3 of porcine adipose tissue TG (Fig. 10). This distribution of 18:2 confirms the established observations pertaining to the disposition of unsaturated fatty acids in the respective TGs and highlights the important differences that exist between pigs and other species (1).

The feeding of protected linoleic acid not only enhances the proportion of 18:2 in ruminant adipose tissue TGs, but also increases the proportion of this acid in milk TGs from these species (7,8,20) (Table II). Stereospecific analyses of a high mol wt milk TG fraction showed that this 18:2 was present in ca. equal

proportions for all three positions of the goats' milk TGs (Table III), but tended to be preferentially located on position 3 of the cows' milk TGs (Fig. 5).

There were also positional specificities for the other component fatty acids of the high mol wt milk TGs, and these specificities are in general agreement with previously published analyses carried out on milk TGs from cows, sheep, goats, and humans (15,24-26). The shorter chain length acids ($< C_{14}$) were predominantly on position 3; 16:0 was predominantly on positions 1 and 2; and 18:1 was rather evenly distributed. The lack of any marked preference for 18:0 on position 1 is in contrast to the previous observations (15,24-26). We also did not show any marked preference of 14:0 for the 2 position of milk TGs (26). These different observations may be due to the different methods of isolating high mol wt TG fractions, i.e., Sephadex LH-20 versus silicic acid chromatography (26).

Ruminant lymphatic TGs show a marked specificity for 16:0 at position 2 (19) (Fig. 6); and this possibly results from the competition between 18:0 and 16:0 for acylation at positions 1 and/or 3 (27). Considerable amounts of 18:0 are produced by ruminal hydrogenation of the conventional dietary lipids, and this fatty acid is presented to the small intestine in the nonesterified form (28). In addition, 16:0 from basal dietary components would also exist as nonesterified fatty acid in the intestinal lumen (28). On the other hand, protected dietary TGs are not hydrolyzed during passage through the rumen (20). Thus, the feeding of protected lipid supplement would result in an intestinal mixture of nonesterified fatty acids, derived from the basal diet, and intact TGs, derived from the supplement.

The lack of any specific location of 18:2 at position 2 of lymphatic TGs from sheep fed protected safflower oil (Fig. 6) is in contrast to the specific location of 18:2 at this position in safflower oil and other linoleic enriched seed oils (1,29). This nonretention of the dietary 2-monoglyceride structure is not consistent with results obtained in nonruminants (30,31) and may suggest that, in ruminants, the protected dietary TGs are more extensively hydrolyzed prior to absorption, or that intestinal monoglyceride hydrolases (32,33) are more active than in nonruminants. Any such conclusion, however, must be considered cautiously in view of the presence in the ruminant intestine of large amounts of nonesterified fatty acids derived from ruminal hydrolysis and hydrogenation of basal dietary lipids.

An analogy exists between the stereospecific arrangement of fatty acids on TG from ruminant milk (Fig. 5, Table III) and lymph (Fig. 6) (15,19,25,26). TGs from both secretory products contain relatively large proportions of the 16:0 located at position 2. This analogy may reflect the importance of lymph TGs as a source of milk TGs (34-36). The lymph TG enters the circulatory system as chylomicrons or very low density lipoproteins (VLDL), and the TG may be partially hydrolyzed by mammary gland lipoprotein lipase (37,38). The resultant 2-monoglycerides, containing relatively large proportions of 16:0, could then serve as precursors for mammary gland TG biosynthesis, a pathway similar to that present in the intestine (39,40). Such a pathway in the mammary gland has been previously considered (36,41), but conclusive evidence is not yet available to fully support this idea (35,42).

Adipose tissue lipoprotein lipase (43), like mammary gland lipoprotein lipase (37,38), also has been shown to specifically hydrolyze the 1 and 3 positions of TGs. Accordingly, since chylomicrons and/or VLDL provide a source of fatty acids for adipose tissue TG biosynthesis (44), one might also expect to find a specific location of 16:0 at the 2 position of these TGs. That this is not the case for ruminants could suggest a rearrangement of fatty acids on the TG molecule (45), possibly involving adipose tissue monoglyceride hydrolase (32,33).

That the stereospecificity of ruminant liver TGs (Fig. 8) does bear some relationship to that of ruminant lymph TGs (Fig. 6) may be due to hepatic uptake of intact chylomicron and/or VLDL triglycerides (44,46).

TGs from tissues and secretory products other than ruminant liver, milk, and lymph also show a preferential location of 16:0 at position 2, e.g., TGs from human milk (24), pig milk (47,48), and pig adipose tissue (Fig. 10) (1,4,5). Whether these observations can also be related to a specific location of 16:0 at position 2 of the respective chylomicron of VLDL TGs awaits further investigation. These investigations must be carried out with due regard to the diet and nutritional status of the animal (24,47,49,50). In addition, the question of the stereospecificity of chylomicron TGs is only satisfactorily resolved by analyzing lymphatic chylomicrons and not serum chylomicrons, owing to the rapid stereospecific rearrangements that take place during lipoprotein transport and metabolism (see accompanying paper by Nestel et al.).

With most tissue phospholipids, as with most tissue TGs, there is a characteristic positional distribution of fatty acids. This distribution is

such that the unsaturated fatty acids are generally located at position 2 and the saturated fatty acids at position 1 (1).

The lymph phospholipids from ruminants fed control diets tend to show this specific positional location for 18:0 and 18:2 (Fig. 7), but this specificity was not apparent for 16:0 and 18:1. The feeding of protected 18:2 resulted in a substantial elevation in the 18:2 content of lymph phospholipids, but this 18:2 tended to be evenly distributed between positions 1 and 2 of the phospholipids (Fig. 7). (The concentration of 18:2 at position 1 was actually greater than at position 2.)

Biliary phospholipids from sheep fed protected 18:2 also showed an enhanced concentration of this acid, but the 18:2 was specifically located at position 2 (Fig. 7). This would indicate that biliary phospholipids are not the direct precursor of the linoleate-enriched lymphatic phospholipids. As a corollary, these linoleate-enriched lymphatic phospholipids are probably synthesized de novo by the intestinal cells in response to the enhanced absorption of TG.

Feeding linoleate-enriched oils to rats results in the appearance of a dilinoleyl phosphatidylcholine in lymph chylomicrons (51). Furthermore, it has been shown that this phospholipid is synthesized de novo and is not derived from biliary phospholipid (51).

We did not attempt to separate the individual lymph phosphoglycerides from sheep fed protected 18:2, but the high concentration of 18:2 at position 1 (69%) and at position 2 (54%) would suggest the presence of considerable amounts of dilinoleyl phosphoglyceride, presumably synthesized de novo by the intestinal mucosa as in rats fed safflower oil (51).

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