EFFECT OF WHOLE-MILK AND REPLACEMENT-MILK FEEDING ON THE FATTY ACID COMPOSITION OF LYMPH LIPIDS IN YOUNG CALVES

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Abstract

Lipids in thoracic duct and intestinal lymph from young calves fed whole milk or replacement milk were analysed. Lymph triglyceride contained C_8 and longer fatty acids with an average molecular weight of 260. The major components were 14 : 0, 16 : 0, 18 : 0, and 18 : 1, with small amounts of branched-chain and odd carbon-number fatty acids. C_{10} fatty acid from milk lipid was apparently absorbed entirely into lymph. The composition of lymph triglyceride closely reflected that of the diet and showed no diurnal variation when the calves were fed once daily. Triglyceride from blood plasma resembled that from lymph except for higher proportions of 18 : 2 and 18 : 3.

Feeding of replacement milk depressed lymph flow to 72% of that during whole-milk feeding and this was apparently due to a decrease in blood flow through the intestinal capillaries. There was also a reduction in the recovery of dietary long-chain fatty acid as neutral lipid in lymph during replacement-milk feeding (50% compared with 83%) which could not be accounted for by malabsorption of saturated fatty acids in the replacement milk. These effects were attributed to the presence of a factor in the replacement milk which may have arisen from the oxidation of unsaturated fatty acids.

I. INTRODUCTION

The young milk-fed calf, unlike the adult bovine, derives most of its energy from dietary lipid. Previous studies have shown that most of this lipid is absorbed by way of the lymph (Shannon and Lascelles 1967). The opportunity was taken to use samples from these studies to compare the fatty acid composition of lymph lipid with that of the dietary lipid.

In addition, the previous studies in young calves were extended to examine the efficiency of absorption of a mixture of animal and vegetable lipids included in a commercial replacement milk with that of lipid in fresh whole milk. Particular attention was paid to the relative efficiency of absorption of the different fatty acids in the diet.

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II. Materials and Methods

(a) *Animals*

Samples were obtained from calves used in previous studies (Shannon and Lascelles 1967, 1968) in which recirculating lymphatico-venous shunts were established between either the thoracic or intestinal lymph ducts and the jugular vein. The calves were Friesians of either sex and surgery was carried out in the first week of life. Fresh whole milk (4·54 litres, obtained each day from the Dairy Research Unit's milk vat) was fed either in one or two equal feeds daily. In addition, for studies using replacement milk, thoracic duct–venous shunts were established in four bull calves of mixed breeds (Friesian, Guernsey, and Jersey cross breeds). These calves weighed an average of 32·0 kg at the time of operation and their care and management was as previously described (Shannon and Lascelles 1967).

The replacement milk (Denkavit, Permewan Wright Ltd., Abbotsford, Vic.) was prepared from skim-milk powder, butter-milk powder, and a homogenized fat mixture which was stated to contain coconut oil, lecithin, mutton tallow, beef dripping, and lard, together with vitamin and mineral supplements. The composition of the replacement milk as determined in this laboratory did not differ from the manufacturers specification, which was: minimum crude protein 30%, crude fat 17–19%, maximum total salt (NaCl) 0·3%.

(b) *Analytical Techniques*

Determinations were made of total esterified fatty acid (T.E.F.A.), phospholipid, free fatty acid (F.F.A.), total protein, and albumin in lymph and blood plasma as previously described (Shannon and Lascelles 1967, 1968). Total cholesterol in lymph and blood plasma was estimated by the method of Abell *et al.* (1952). Total lipid in milk was determined by the Babcock method as described by Davis and MacDonald (1953). Lipids were extracted with chloroform–methanol (2 : 1 v/v) and separated by thin-layer chromatography as reported by Hartmann and Lascelles (1965). Some lipid samples were separated into neutral lipid and phospholipid fractions on small columns of silicic acid (Kernohan, Lascelles, and Wadsworth 1971). Methyl esters of fatty acids were prepared by dimethyl carbonate-induced transesterification and analysed by gas–liquid chromatography as reported by Wadsworth (1968*a*). Methyl esters prepared in this way from the neutral lipid of lymph and blood plasma represent triglyceride fatty acids since lymph and plasma contain little partial glyceride and dimethyl carbonate-induced transesterification does not esterify cholesterol ester or F.F.A. (Wadsworth 1968*a*). Butyl esters were prepared from the lipid in whole milk and replacement milk using dibutyl carbonate and analysed by temperature-programmed gas–liquid chromatography.

III. Experimental Protocol and Results

(a) *Whole-milk Feeding*

In an earlier study (Shannon and Lascelles 1967) calves with thoracic duct–venous shunts were used in an investigation of once-daily feeding. It was found that there was a definite pattern of absorption on the once-daily regime, lipid concentration and output in lymph falling after feeding to reach a minimum level at 2–6 hr, rising to attain a maximum value at 10 hr and falling off thereafter. Four samples were taken from each of the three calves for fatty acid analysis. Samples were obtained immediately before feeding, during the periods of minimum and maximum neutral lipid output, and at the end of the 24-hr collection period. Triglyceride was separated from lymph lipid by thin-layer chromatography and submitted to gas–liquid chromatography.

Analysis by gas–liquid chromatography, using Apiezon L and the polyesters of ethylene glycol adipate and diethylene glycol succinate as stationary phases, allowed the following homologous series of fatty acids to be tentatively identified (cf.

Wadsworth 1968*a*, 1968*b*) in lymph triglyceride: n-saturated C_8–C_{18} (including odd carbon numbers); iso-methyl-branched, saturated C_{13}–C_{18}; anteiso-methyl-branched, saturated C_{15} and C_{17}; and mono-unsaturated C_{10} and C_{12}–C_{18}. A number of long-chain, unsaturated components, including 18 : 2 and 20 : 4 and probably including 18 : 1 isomers with the double bond near the methyl terminus, were also identified, together with 18 : 2 conjugated isomers. The proportions of the major fatty acids in lymph triglyceride are reported in Table 1. A similar range of fatty acids was tenta-

TABLE 1

COMPARISON OF THE FATTY ACID COMPOSITION OF LYMPH TRIGLYCERIDE AND LIPID FROM WHOLE MILK FED TO WEEK-OLD CALVES

Values (weight percentage of total fatty acids) are means ± standard errors for one sample from each of three calves. Only major components (> 1% of total) of chain length > C_9 are reported

Conditions for Gas–Liquid Chromatography	Fatty Acid	Lymph Triglyceride	Milk Lipid
1·52 m by 0·64 cm (O.D.) column packed with 12% diethylene glycol succinate polyester on Gas Chrom P and operated isothermally at 180°C for lymph triglyceride and temperature-programmed from 75 to 200°C at 4°C/min for milk lipid	10 : 0	2·0±0·3	2·1±0·3
	12 : 0	3·3±0·5	2·6±0·3
	14 : 0	11·1±1·2	9·4±1·3
	16 : 0	28·8±0·3	26·8±0·9
	18 : 0	10·6±0·3**	13·2±0·4
	18 : 1	31·9±2·3	31·6±3·3
	18 : 2	1·9±0·2	3·0±0·4
	18 : 3	2·4±0·1*	1·8±0·2
	Remainder	8·2±0·2*	9·7±0·4

* $P < 0·05$, ** $P < 0·01$ (significance of differences using Student's t-test).

tively identified in triglyceride from blood plasma, cholesterol ester from blood plasma and lymph, and in lymph phospholipid. However, phospholipid from blood was totally deficient in fatty acids of chain length less than C_{12}, contained only small proportions of C_{15} and C_{17} odd carbon-number and C_{15}–C_{18} branched-chain fatty acids, and only one minor, long-chain component (18 : 1 with the double bond near the methyl terminus).

The results of the fatty acid composition of lymph triglyceride were submitted to an analysis of variance. There were significant between-calf differences for all fatty acids except 18 : 0. However, the absence of significant between-times differences indicated that the fatty acid composition of lymph triglyceride did not change throughout the day on the once-daily feeding regime.

A comparison of the fatty acid composition of lymph triglyceride from three calves with that of the dietary milk lipid was made and the results are presented in Table 1. It may be seen that the composition of the lymph triglyceride closely reflected that of the diet, significant differences being small in magnitude.

(*b*) *Replacement Milk*

The experiment followed the same design as that described previously (Shannon and Lascelles 1969), consisting of an experimental period during which replacement milk was fed (230 g dry powder plus warm water to 2·27 litres given twice daily), preceded and followed by control periods during which fresh, whole milk (2·27 litres)

was fed twice daily. An adjustment period of 3–4 days was allowed at each change of diet before commencing collection of samples at hourly intervals for a period of 12 hr. Although the calves were fed from two batches of the replacement milk there were no appreciable differences in the results obtained. The average amount of lipid ingested over a 12-hr period by the calves was 83·4, 51·0, and 109·2 g for whole-milk, replacement-milk, and whole-milk feeding, respectively.

Comparisons of the average lymph flow, concentration of total protein, and output of neutral lipid (derived by subtracting values for phospholipid fatty acid from those for T.E.F.A.), phospholipid, and F.F.A. in thoracic duct lymph when the calves were alternately fed whole milk, replacement milk, and whole milk are presented in Table 2. It may be seen that, whereas total protein concentration

TABLE 2

LYMPH FLOW, TOTAL PROTEIN CONCENTRATION, AND OUTPUT OF NEUTRAL LIPID, PHOSPHOLIPID, AND F.F.A. IN THORACIC DUCT LYMPH FROM FOUR CALVES FED REPLACEMENT MILK

Values are means ± standard errors for a total of 52 samples collected in each of three 12-hr periods when the calves were being fed alternately whole milk, replacement milk, and whole milk

Parameter Measured	Whole-milk Feeding	Replacement-milk Feeding	Whole-milk Feeding
Lymph flow (ml/hr)	387·3 ±12·1	274·6 ±6·8	373·8 ±7·4
Total protein concn. (g/100 ml)	3·83 ± 0·06	3·75 ±0·05	3·64 ±0·07
Neutral lipid output (g/hr)	4·855± 0·276	1·999±0·113	6·395±0·391
Phospholipid output (g/hr)	0·738± 0·030	0·431±0·017	0·886±0·035
F.F.A. output (g/hr)	0·077± 0·008	0·038±0·002	0·089±0·006

changed only slightly during replacement-milk feeding, lymph flow and lipid output dropped markedly. These changes were reversed upon returning to the whole-milk diet. Analysis of variance (see Shannon and Lascelles 1969) revealed significant quadratic components of the between-treatments terms for lymph flow, neutral lipid concentration, and the outputs of neutral lipid, phospholipid, and F.F.A. indicating that replacement-milk feeding was responsible for the depression of these variates. There were no significant treatment effects for total protein concentration or albumin : globulin ratio in lymph. Since the plasma total protein concentration also remained constant in these calves, it follows that the lymph : plasma ratio for total protein concentration was unaltered by replacement-milk feeding.

Replacement-milk feeding, compared with whole-milk feeding, did not significantly alter the relative proportions of the lipid fractions in plasma. On the other hand, the concentrations of total lipid, phospholipid, and F.F.A. in the lymph were notably lower when the calves were being fed replacement milk (cf. Table 2). These changes were reversed when the calves were returned to whole-milk feeding. Total cholesterol values in the lymph, however, were unaltered by the changing dietary regimes. The percentage contributions made by phospholipid and total

cholesterol to the total lipid concentration in lymph during whole-milk feeding (10·5 and 2·5% respectively) were increased following replacement-milk feeding (16·1 and 4·6% respectively).

Lipids from lymph and plasma were separated on silicic acid columns and submitted to gas–liquid chromatography. The compositions of lymph triglyceride and phospholipid obtained during feeding of whole milk or replacement milk are compared in Table 3. It may be seen that the feeding of replacement milk produced

TABLE 3

EFFECT OF FEEDING REPLACEMENT MILK ON THE FATTY ACID COMPOSITION OF TRIGLYCERIDE AND PHOSPHOLIPID IN THE LYMPH AND TRIGLYCERIDE IN THE BLOOD PLASMA OF WEEK-OLD CALVES

In the case of lymph triglyceride, values (weight percentage of total fatty acids) are means ± standard errors for one sample from each of four calves; for lymph phospholipid, values represent one calf; for blood plasma, values represent one sample from each of three calves. Only major components (> 1% of total) of chain length > C_9 are reported. Gas–liquid chromatography conditions: 1·52 m by 0·64 cm (O.D.) columns packed with 10% EGSP-Z on Gas Chrom Q and operated isothermally at 170°C for lymph and plasma lipids and temperature-programmed from 70 to 200°C at 4°C/min for replacement-milk lipid

Fatty Acid	Lymph Triglyceride		Replacement-milk Lipid	Lymph Phospholipid		Plasma Triglyceride	
	Before	After		Before	After	Before	After
10 : 0	1·7±0·2*	0·7±0·2	2·4	—	—	1·6±0·8	0·7±0·6
12 : 0	3·6±0·2**	9·6±1·4	9·7	Trace	Trace	2·2±0·4	3·0±0·7
14 : 0	11·8±0·5***	6·6±0·5	5·3	2·3	1·0	9·2±1·0*	4·7±0·4
16 : 0	33·1±2·6	24·5±1·8	17·5	17·7	14·4	33·5±2·6	28·1±1·0
18 : 0	10·6±0·9	14·5±2·2	18·1	24·7	28·4	7·2±1·7	12·8±6·9
18 : 1	26·4±2·8*	34·3±0·9	34·1	26·1	25·7	21·4±1·3	25·4±1·8
18 : 2	2·5±0·8	2·7±0·7	3·0	16·6	19·9	10·6±1·5	15·1±4·5
18 : 3	0·6±0·5	0·9±0·6	1·0	3·4	3·4	2·1±1·1	2·6±1·4
Remainder	9·7±2·2	6·3±1·1	9·0	9·2	7·3	12·1±0·4**	7·6±0·8

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ (significance of differences using Student's t-test).

significant changes in the composition of lymph triglyceride toward that of the diet. The composition of lymph phospholipid changed little by comparison. The changes induced in lymph triglyceride by replacement-milk feeding were reflected in blood plasma triglyceride (Table 3) although most of these changes were not statistically significant.

IV. DISCUSSION

(*a*) *Fatty Acid Composition of Lymph Lipid*

The range of fatty acids found in the lipids of thoracic duct lymph from calves fed whole milk was similar to that in lymph from cows (Wadsworth 1968*a*) and to that of dietary milk fat (Tables 1 and 3). This finding agrees with those of Heath, Adams, and Morris (1964) for milk-fed lambs and Toullec (1968) for 3–6-week-old calves. In the latter studies, the lymph contained smaller proportions of C_{10} and C_{12} fatty acids than the diets whereas in the present study there were no significant differences between the two during whole-milk feeding (Table 1). It has been assumed, following the work of Bloom, Chaikoff, and Reinhardt (1951), that fatty acids of chain length C_{10} and smaller are absorbed predominantly via the portal vein. The present results confirm this finding for C_4–C_8 fatty acids, but suggest that during whole-milk feeding

the lymphatics may be the more important pathway of absorption for C_{10} fatty acid in the pre-ruminant calf.

Triglyceride from blood plasma showed a similar composition to that of lymph triglyceride on whole-milk feeding and changed to some extent towards that of the diet when calves were fed replacement milk (Table 3). These observations are probably related to the finding that chylomicrons and very low density lipoproteins ($D < 1 \cdot 005$) can be readily detected in blood serum of milk-fed calves (Wadsworth, unpublished data).

It was previously assumed that the average molecular weight of fatty acids in calf lipids was 280 (Shannon and Lascelles 1967) but the present data indicate a value closer to 260 for fatty acid in lymph triglyceride (Tables 1 and 3), whereas that for phospholipid fatty acid is closer to 280 (Table 3).

(*b*) *Replacement-milk Feeding*

It can be calculated from the data in Table 2 that the neutral lipid transported in the thoracic duct lymph represented, on average, approximately 83% of the long-chain ($> C_8$) fatty acid supplied in the whole-milk diet. On the other hand, the estimated recovery in lymph of fatty acid supplied by the replacement-milk diet was approximately 50%. It would appear that the relative efficiency of absorption was reduced when the replacement milk was fed. Indeed, Hartmann and White (1970) reported that the apparent digestibility of lipid was significantly lower for calves fed replacement milk (89·3%) compared with that for calves fed whole milk (97·4%) and similar experiments to those reported here confirmed the reduced recovery in lymph of long-chain fatty acid supplied by the replacement-milk diet (Hartmann and Waterson, personal communication).

A recent study by Radostits and Bell (1968) found low apparent digestibility of replacement lipid with negative digestibility coefficients for palmitic and stearic acids. This suggested selective malabsorption of long-chain, saturated fatty acids and, indeed, there was significantly less stearic acid in lymph triglyceride than in dietary-milk lipid in the present study, although the proportions of most of the other fatty acids did not differ between lymph triglyceride and the diet (Tables 1 and 3). However, Blomstrand, Dahlback, and Linder (1959) reported that, of the radioactively labelled stearic acid absorbed into the lymph of human patients, 20% was recovered in phospholipids, compared with only 3–5% for palmitic, oleic, and linoleic acids. This suggests that the decreased proportion of 18 : 0 in lymph triglyceride compared with dietary lipid (Tables 1 and 3) may have been due to incorporation into lymph phospholipid of relatively more of the dietary stearic acid than of the other fatty acids. The high proportion of 18 : 0 in lymph phospholipid (Table 3) supports this suggestion and it may be concluded that there was little, if any, selective malabsorption of individual fatty acids during replacement-milk feeding in the present experiments.

On the other hand, lymph flow was considerably reduced during replacement-milk feeding compared with whole-milk feeding (Table 2), probably reflecting changes in blood flow and thus in lymph formation in the intestinal region, similar to that previously found in calves fed skim milk (Shannon and Lascelles 1969). The replacement-milk diet contained some 15 times the amount of lipid in the skim milk diet whereas the flow of lymph from the thoracic duct was comparable when calves were

fed the two different diets. It is suggested, therefore, that a factor (or factors) in the artificial diet (other than the level of lipid ingested) depressed lymph formation.

The factors responsible for the reduction in lymph formation observed in the present study may have been products from oxidative deterioration of the replacement-milk lipid during storage (Adams *et al.* 1959; Bhalareo, Inoue, and Kummerow 1963). However, the feeding of air-oxidized replacement milk to a calf failed to produce any reduction in lymph flow (Hartmann and Waterson, personal communication). In the latter study, the feeding of fresh replacement milk to calves did not reduce lymph flow from the thoracic duct. Thus, the cause of the reduced lymph flow observed in the present calves (Table 2) remains uncertain.

It is evident that the poor absorption of lipid in the replacement milk cannot be explained on the basis of the present results. It is suggested that further studies on such factors as disturbances of curd formation in the abomasum associated with heat treatment of the replacement milk during manufacture (Roy 1970) may prove fruitful in this regard.

It has been found that the output of endogenous lipid in lymph is independent of the amount or composition of dietary lipid (Boucrot and Clément 1968). Since the output of neutral lipid in lymph was reduced during replacement-milk feeding (Table 2) it would be expected that endogenous lipid contributed a greater proportion of the total lipid to lymph during feeding of replacement milk than of whole milk. This is consistent with the increased proportions of phospholipid and total cholesterol in lymph lipid and with the lesser resemblance between lymph triglyceride and dietary lipid during replacement-milk feeding (Table 3) than during feeding of whole milk (Table 1). In this connection an experiment in which a young calf was prepared with a re-entrant bile cannula (Wadsworth and Lascelles, unpublished data) indicated that 0·5–0·7 g/hr of bile phospholipid entered the gut irrespective of the level of lipid in the diet. This would represent about 10–15% of the daily transport of neutral lipid in thoracic duct lymph (Shannon and Lascelles 1967), and is probably the major source of endogenous lipid in thoracic duct lymph of calves.

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