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Fatty acid composition of liver lipids of young lambs

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1. The changes in the lipid composition of livers from lambs receiving either ewe's milk or a reconstituted low-fat milk powder have been studied during the first 8 d after birth.

2. At birth the phospholipid and unesterified fatty acid fractions constituted the major proportion of the liver lipids. In the lambs on the artificial diet the relative concentration of the phospholipid fraction increased and there were corresponding decreases in the relative concentrations of all the other fractions, whereas in the naturally fed lambs the relative concentration of the phospholipids remained similar to that found in the livers at birth.

3. In the liver lipids at birth there were only low concentrations of the C_{18} polyunsaturated fatty acids, although there were appreciable concentrations of the higher C_{20} and C_{22} polyunsaturated fatty acids.

4. After birth there was a large increase in the concentration of the C_{18} polyunsaturated fatty acids in the livers of the lambs on the natural diet and a decrease in the 20:3 ω9 : 20:4 ω6 (triene : tetraene) ratio. In the lambs receiving the artificial diet the concentration of the C_{18} polyunsaturated fatty acids and the triene : tetraene ratio remained similar to that observed at birth.

5. These changes are discussed in relationship to the metabolism of the essential fatty acids in monogastric animals.

In a previous experiment (Noble, Steele & Moore, 1971) the compositions of the plasma lipids of lambs suckled naturally for 8 d after birth were compared with those of the plasma lipids of lambs given an artificial low-fat diet for a similar period. Although the linoleic acid content of the colostrum and milk of the ewes during early lactation never exceeded 1 % of the total fatty acids present, and the content of linolenic acid was even lower (Noble, Steele & Moore, 1970), it was found that there were large increases in the concentrations of linoleic acid and linolenic acid in the plasma lipids of the lambs during the first few days after birth. Investigations with two further groups of lambs receiving either ewe's milk or the artificial low-fat diet has yielded information on the lipid changes that occur in some of the other major tissues of the lamb during the first 8 d after birth, and details are now given of the lipid changes that occurred in the livers of these lambs.

EXPERIMENTAL

Eleven pairs of twin lambs were obtained from a flock of pure-bred Cheviot ewes. The diet and handling of the ewes were as detailed previously (Noble *et al.* 1971). The lambs were divided at birth into two groups. One member of each twin pair was suckled naturally and remained with its mother; the other member was removed from its mother and was given *ad lib.* a diet of a reconstituted low-fat dried milk powder (Unigate Dairies Ltd, London). The total fat content and fatty acid composition of the diets received by the two groups of lambs were similar to those given in detail

previously (Noble *et al.* 1971). Although the concentration of 18:2 in the milk fats was of the same order in both the natural and artificial diets, the concentration of total fat in the artificial diet was negligible (i.e. 0·03 % of the dry matter) when compared with that of the ewe's milk. Four lambs were slaughtered immediately after birth; the eighteen remaining lambs were slaughtered at intervals of 2, 4 and 8 d after birth. The tissues were removed as quickly as possible after death and the lipids extracted with 2:1 (v/v) chloroform : methanol (Folch, Lees & Stanley, 1957). The solvent was removed from a portion of the lipid extract and the total fat content of the livers was determined gravimetrically. After fractionation by thin-layer chromatography, the fatty acid compositions of the major lipid fractions were determined by the methods described previously in detail by Moore & Williams (1964), Noble & Moore (1964) and Moore, Noble & Steele (1969). The absolute concentrations of the various lipid fractions in the tissues were determined by the addition to each fraction of a known amount of n-heptadecanoic acid as an internal standard (Christie, Noble & Moore, 1970). No attempt was made to determine the concentration of free cholesterol. After fractionation on 3 g columns of silicic acid (Moore & Doran, 1962), the C_{18} monoenoic fatty acids in the total phospholipids and the neutral lipids from the lipid extracts of the livers were separated into *cis*- and *trans*-isomers by thin-layer chromatography on silica gel impregnated with silver nitrate (Morris, 1966). The proportion of the positional isomers in each of these fractions was determined by analysis on a gas–liquid chromatograph fitted with a single-flame ionization detector using a support-coated open capillary column (1525 cm × 0·05 cm) with a stationary phase of diethylene glycol succinate (Perkin–Elmer Ltd, Beaconsfield, England). The retention volume of each of these C_{18} monoenoic isomers was identified by the use of authentic acids obtained from the Hormel Institute (Austin, Minnesota, USA). The positional distribution of the double bond in each isomer was further checked by oxidation of the methyl ester fractions (Chang & Sweeley, 1962) and analysis of the resultant monocarboxylic and dicarboxylic acids by gas chromatography, as described by Moore & Williams (1966).

RESULTS

The percentage compositions of the liver lipids, expressed as 'weight percentages of the total fatty acids', for the naturally fed and artificially fed lambs are given in Table 1. At birth the phospholipid and unesterified fatty acid fractions constituted the major proportion of the lipids in the liver, accounting for some 67 and 18% respectively of the total fatty acids present. By the 2nd day after birth the relative concentration of the phospholipid fatty acids in the livers of the artificially fed lambs had increased to 80% of the total fatty acids present and there were corresponding decreases in the relative concentrations of all the other fractions; the relative concentration of the phospholipid fraction on the 4th and 8th days after birth was similar to that observed on the 2nd day after birth. In the livers of the naturally fed lambs the relative concentration of the phospholipid fraction remained similar to that observed at birth, but the relative concentrations of the cholesteryl ester and triglyceride fractions tended to increase. In the livers of both groups of lambs the relative concentra-

Table 1. *Concentrations (weight percentages of the total fatty acids) of the fatty acids in each of the major lipid fractions of the livers from the lambs receiving either their natural diet or an artificial low-fat diet*

		Day 2		Day 4		Day 8		
	Day 0	Low-fat	Natural	Low-fat	Natural	Low-fat	Natural	SE
Total lipid content (g/100 g)	10·7	9·4	14·0	13·9	14·9	14·6	17·4	1·321
Percentage distribution of fatty acids:								
Cholesteryl ester fatty acids	5·0	5·35	5·28	2·31***	11·07	4·05***	9·45	0·701
Triglyceride fatty acids	5·19	3·54***	6·86	1·76***	6·94	3·47***	10·22	0·776
Unesterified fatty acids	18·4	8·95	13·2	15·2*.	17·7	14·8	14·8	1·511
Monoglyceride fatty acids	4·55	2·47*	1·29	2·71	1·95	0·49	1·19	0·512
Phospholipid fatty acids	66·8	79·7***	73·4	78·0***	62·3	77·2***	64·4	1·482

Significance of differences between dietary treatments: * $P < 0.05$; *** $P < 0.001$.

Table 2. *Fatty acid compositions (major components, weight percentages of total) of the liver cholesteryl esters from the lambs receiving either their natural diet or an artificial low-fat diet*

		Day 2		Day 4		Day 8		
Fatty acid	Day 0	Low-fat	Natural	Low-fat	Natural	Low-fat	Natural	SE
16 : 0	30·5	21·3***	16·9	22·1***	14·5	28·1***	15·5	0·902
16 : 1	12·3	7·2*	4·2	7·3*	4·0	12·3***	2·7	1·175
18 : 0	7·8	9·5	6·5	11·6**	5·4	10·7	7·9	1·726
18 : 1	36·8	44·4***	60·9	39·9***	59·2	36·5***	49·1	2·615
18 : 2 ω6	1·7	2·0**	4·8	4·0	4·5	1·5***	11·1	0·676
18 : 3 ω3	0·9	0·7	1·0	0·7*	1·7	0·8***	3·4	0·475
20 : 3 ω9	2·4	2·8**	0·9	3·7***	0·6	1·3	0·4	0·562
20 : 4 ω6	1·5	4·3***	1·0	3·4*	1·3	0·5	0·9	0·743

Significance of differences between dietary treatments: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 3. *Fatty acid composition (major components, weight percentages of total) of the liver phospholipids from the lambs receiving either their natural diet or an artificial low-fat diet*

		Day 2		Day 4		Day 8		
Fatty acid	Day 0	Low-fat	Natural	Low-fat	Natural	Low-fat	Natural	SE
16 : 0	23·5	17·3	17·4	14·9	15·3	16·3***	12·7	0·801
16 : 1	4·5	3·5	1·5	3·1	1·1	5·0**	0·7	0·423
18 : 0	14·7	16·4***	20·9	18·3**	21·7	17·0***	25·9	0·944
18 : 1	27·6	33·9***	25·1	40·7***	27·2	34·1***	19·5	1·57
18 : 2 ω6	1·8	1·4***	4·2	1·5***	5·6	2·0***	9·4	0·341
18 : 3 ω3	0·1	0·1	0·5	0·2*	0·7	0·1***	1·3	0·252
20 : 3 ω9	4·8	4·6***	1·8	4·7***	1·4	7·3***	0·8	0·147
20 : 4 ω6	5·7	5·2***	8·1	4·1***	7·2	5·0***	10·5	0·669
20 : 5 ω3	—	1·3***	2·6	0·7***	2·3	0·9***	1·9	0·167
22 : 5 ω3	4·1	3·8**	5·3	2·8***	5·2	3·2***	7·0	0·524
22 : 6 ω3	9·0	8·1	9·2	5·6	7·3	3·7***	7·5	0·873

Significance of differences between dietary treatments: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 4. *Fatty acid compositions (major components, weight percentages of total) of the liver unesterified fatty acids from the lambs receiving either their natural diet or an artificial low-fat diet*

		Day 2		Day 4		Day 8		
Fatty acid	Day 0	Low-fat	Natural	Low-fat	Natural	Low-fat	Natural	SE
16:0	22·5	14·7	16·3	12·4	14·7	14·1	15·8	1·248
16:1	7·6	5·1***	1·9	5·8***	2·2	6·5***	1·1	0·665
18:0	10·2	11·9***	15·9	10·4***	15·2	11·8***	19·7	0·750
18:1	36·6	38·1**	33·5	46·8***	39·1	38·7***	31·0	1·301
18:2ω6	1·0	1·6***	5·3	0·9***	5·5	2·3***	9·1	0·348
18:3ω3	0·1	0·1**	1·0	0·2***	1·3	0·1***	1·4	0·266
20:3ω9	5·9	5·2***	1·5	5·4***	1·3	7·5***	0·5	0·312
20:4ω6	6·0	6·8*	7·1	5·4	5·7	5·5**	7·2	0·459
20:5ω3	1·1	1·7**	2·6	1·2**	2·2	1·1	1·5	0·294
22:5ω3	3·7	4·5	4·9	3·4	4·0	2·8	4·5	1·055
22:6ω3	4·3	7·8	6·3	6·0	4·1	3·5	4·0	1·174

Significance of differences between dietary treatments: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 5. *Fatty acid compositions (major components, weight percentages of total) of the liver triglycerides from the lambs receiving either their natural diet or an artificial low-fat diet*

		Day 2		Day 4		Day 8		
Fatty acid	Day 0	Low-fat	Natural	Low-fat	Natural	Low-fat	Natural	SE
16:0	31·1	25·4	25·8	22·0*	26·2	26·2	26·2	1·59
16:1	6·7	7·2***	3·1	5·7**	2·5	9·9***	1·8	0·876
18:0	9·7	13·0	12·1	14·7	13·4	11·0	15·6	0·924
18:1	33·6	36·1	35·7	43·2*	39·4	36·4	38·1	1·57
18:2ω6	1·9	1·9***	6·6	2·2***	5·2	1·5***	9·0	0·467
18:3ω3	0·1	0·3***	1·5	0·5***	1·5	0·1***	2·0	0·217
20:3ω9	3·0	3·9	1·4	2·6	0·7	4·3	0·5	0·538
20:4ω6	4·0	5·6	6·9	2·9	2·8	3·5	4·2	0·388
22:5ω3	2·1	—	—	—	—	—	—	—
22:6ω3	2·2	—	—	—	—	—	—	—

Significance of differences between dietary treatments: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 6. *Fatty acid compositions (major components, weight percentages of total) of the liver monoglycerides from the lambs receiving either their natural diet or an artificial low-fat diet*

		Day 2		Day 4		Day 8		
Fatty acid	Day 0	Low-fat	Natural	Low-fat	Natural	Low-fat	Natural	SE
16:0	23·8	24·6	23·2	20·6	23·0	24·3	18·5	3·249
16:1	5·7	5·1	3·3	2·9	4·7	5·2*	2·4	1·257
18:0	10·9	16·2*	20·3	20·9*	15·5	17·1**	22·2	1·738
18:1	32·0	36·8	34·5	43·7	42·1	38·6*	32·9	2·624
18:2ω6	1·2	0·9**	2·7	0·9***	3·2	0·9***	6·2	0·492
18:3ω3	0·1	0·3	0·5	0·1***	0·7	0·4***	1·0	0·145
20:3ω9	4·3	5·2***	2·1	4·3***	1·4	6·3***	1·0	0·348
20:4ω6	4·5	2·2	3·3	1·6	1·7	1·7***	5·5	0·777
22:5ω3	3·8	—	—	—	—	—	—	—
22:6ω3	5·4	—	—	—	—	—	—	—

Significance of differences between dietary treatments: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tions of the unesterified fatty acid and monoglyceride fractions were similar during the period between the 2nd and the 8th day after birth.

The fatty acid compositions of the cholesteryl ester, phospholipid, unesterified fatty acid, triglyceride and monoglyceride fractions from the livers of the naturally fed and artificially fed lambs after birth are given in Tables 2–6 respectively.

The major fatty acids in all the liver lipid fractions at birth were 16:0, 18:0 and 18:1; the cholesteryl ester and triglyceride fractions contained relatively higher concentrations of 16:0 than did the other lipid fractions. All the lipid fractions of the liver in the newborn lamb contained an appreciable concentration of 16:1. At birth, the concentrations of 18:2 did not exceed 2% of the total fatty acids present in any of the liver lipid fractions, and the concentrations of 18:3 were even less. However, there were appreciable concentrations of the higher polyunsaturated fatty acids in the liver lipids; at birth the C_{20} and C_{22} polyunsaturated fatty acids together accounted for 20–24% of the total fatty acids present in the liver phospholipids and unesterified fatty acids. During the first 8 d after birth there were marked increases in the concentrations of 18:2 and 18:3 in all the lipid fractions in the livers of the lambs receiving the natural diet. In all the liver lipids the increases in the concentrations of these C_{18} polyunsaturated fatty acids were accompanied by decreases in the concentrations of 20:3ω9. In the phospholipid fraction the 20:3ω9 to 20:4ω6 ratio (triene:tetraene ratio) at birth was of the order of 0·85, but after 8 d on the natural diet the ratio had decreased to 0·07. In the liver phospholipids of the naturally fed lambs there was a marked increase in the concentration of 18:0 and decreases in the concentrations of 16:0, 16:1 and 18:1 during the 8 d after birth. In the naturally fed lambs during the same period, there was a marked increase in the concentration of 18:1 and decreases in the concentrations of 16:0 and 16:1 in the liver cholesteryl esters; in the triglyceride, unesterified fatty acid and monoglyceride fractions there was an increase in the concentration of 18:0 and decreases in the concentrations of 16:0 and 16:1.

In contrast to the results for the suckled animals, the concentrations of 18:2 and 18:3 in all the liver lipid fractions of the lambs receiving the artificial diet during the 8 d after birth remained similar to the values found immediately after birth. The concentration of 20:3ω9 also remained similar to that observed in the liver lipids at birth and resulted in the maintenance of a high triene:tetraene ratio throughout the whole 8 d period. In the main, the changes in the fatty acid concentrations in the lipid fractions of the livers from the artificially fed lambs were small in comparison with the changes that occurred in the suckled lambs and were confined generally to increases in the concentration of 18:1 and decreases in the concentration of 16:0. The concentration of 16:1 in all the lipid fractions of the livers of the lambs on the low-fat diet remained similar to the relatively high concentration observed immediately after birth.

Table 7 shows the proportions of the major positional and geometrical isomers in the C_{18} monoenoic acid fraction in the neutral lipids (i.e cholesteryl esters, unesterified fatty acids, triglycerides and monoglycerides) and phospholipids in the livers of the lambs at birth and at 8 d. Although at birth the major C_{18} monoenoic acid in both

fractions was the *cis*-9-isomer, there was a relatively high proportion of the *cis*-11-isomer; the proportions of the *trans*-isomers were small. However, by the 8th day after birth the concentration of both the *trans*-9- and *trans*-11-monoenes, in particular the *trans*-11-isomer, had increased in the livers of the suckled lambs. Only trace amounts of the *cis*-11 isomer were found in the liver lipids of the 8-d-old lambs that had received the natural diet. However, in the livers of the 8-d-old lambs given the artificial diet, the proportion of the *cis*-11 isomer was similar to that found in the livers of the lambs at birth.

Table 7. *Compositions of the* 18:1 *(percentage of total* 18:1*) in the neutral lipids and phospholipids of the livers from the lambs receiving either their natural diet or an artificial low-fat diet*

	Neutral lipids			Phospholipids		
		Day 8			Day 8	
Isomer	Day 0	Low-fat	Natural	Day 0	Low-fat	Natural
Δ9-*trans*	1·2	0·5	2·1	Trace	2·1	1·6
Δ11-*trans*	1·2	1·3	10·0	Trace	1·7	5·6
Δ9-*cis*	73·3	77·9	87·9	78·4	79·8	92·9
Δ11-*cis*	24·3	20·3	Trace	21·6	16·3	Trace

DISCUSSION

Investigations with adult sheep (Peters & Smith, 1964) indicate that, in common with other animals, the two main components in the lipids of the liver are the phospholipids and triglycerides, the former constituting some 70% of the total lipids present. Although the proportion of the phospholipid fraction in the liver lipids of the newborn lamb (Table 1) is similar to that found in the liver lipids of adult sheep (Peters & Smith, 1964), the concentration of the triglyceride fraction is very much less and that of the unesterified fatty acid very much higher than in the adult. It is tempting to correlate the increased pool size of unesterified fatty acids in the liver at birth with the selective uptake, by the developing foetus of unesterified fatty acids from the maternal circulation (McBride & Korn, 1964; Van Duyne, Parker, Havel & Holm, 1960) and the elevated pool size of unesterified fatty acids in the plasma at birth (Noble *et al.* 1971; Van Duyne & Havel, 1959). The proportion of unesterified fatty acids in the liver lipids remained high during the 8 d after birth in both groups of lambs (Table 1). Although no similar information appears to be available for the newborn lamb, the investigations of Body, Shorland & Gass (1966) into the lipid composition of foetal lambs by analyses of complete homogenates also indicated high concentrations of unesterified fatty acids in the total lipid extracts. The possibility that the high concentrations of unesterified fatty acids observed in the livers of the lambs in the present work merely reflects the hydrolysis of lipids during storage and sample preparation must also be considered. However, when the lipids of adult sheep liver were extracted, stored and analysed by the procedures used in the investigation now reported, the unesterified fatty acid fraction was found to comprise only about

1% of the total liver lipids (R. C. Noble, W. Steele & J. H. Moore, unpublished observations). Furthermore, the accumulation of partial glycerides in the lipid extracts might be taken as indicative of hydrolytic cleavage, but in the present work the proportion of monoglycerides was in all samples less than 5% of the total lipid present, whilst only trace concentrations of diglycerides and lysophosphatides could be detected. The dissimilarity between the fatty acid composition of the unesterified fatty acid fraction and that of the triglyceride or phospholipid fractions is not consistent with the view that the high concentrations of the unesterified fatty acids in the livers of the lambs were due to hydrolysis *post mortem*.

Previous work with adult and foetal sheep has shown that there are large differences between the fatty acid compositions of foetal and of adult tissues. A comparison of the fatty acid composition of whole foetal and adult homogenates (Shorland, Body & Gass, 1966) indicated that the tissues of the adult contained higher concentrations of 18:0, 18:2 and 18:3, and of the C_{15} and C_{17} normal and branched-chain acids, but lower concentrations of 18:1 than the tissues of the foetus. Shorland *et al.* (1966) also noted that the foetus contained appreciable concentrations of the C_{20} and C_{22} polyunsaturated fatty acids in the phospholipids. Similar differences have been observed in lipids extracted from individual foetal and maternal tissues (Leat, 1966; Scott, Setchell & Bassett, 1967). In agreement with these results for foetal tissues, the lipids from the livers of the newborn lambs in the experiment now described, in particular the phospholipid fraction, had very low concentrations of 18:2 and 18:3 but appreciable concentrations of the C_{20} and C_{22} polyunsaturated fatty acids. The total changes that occurred in the fatty acid composition of the livers from the lambs receiving the low-fat artificial diet in this investigation were similar to those observed previously in the lipids of the plasma (Noble *et al.* 1971). In the lambs receiving their natural diet, the concentrations of 18:2 and 18:3 increased considerably over the 8 d after birth but, whereas in the plasma these changes were mainly confined to the phospholipid and cholesteryl ester fractions, the concentrations of 18:2 and 18:3 increased in all the major lipid fractions of the liver. These changes were accompanied by a general decrease in the concentration of 20:3ω9 and a concomitant decrease in the triene:tetraene ratio in the phospholipid fraction. These results are in general accord with the previous observations on the plasma lipid changes that occurred in lambs receiving ewe's milk (Noble *et al.* 1971). They also show the widespread nature of the changes in the essential fatty acid status of the lamb during the 1st week after birth when given a diet which, according to criteria based on results for monogastric animals (Holman, 1968), provides less than the minimum requirement of essential fatty acids (Noble *et al.* 1971).

Investigations into the fatty acid composition of ovine foetal lipids (Scott *et al.* 1967) showed that, although substantial concentrations of the C_{20} and C_{22} polyunsaturated fatty acids could be found in the phospholipids of the liver, there were only low concentrations of 18:2 and 18:3. From these results, it was suggested that there may be a rapid conversion of the C_{18} polyunsaturated fatty acids into the C_{20} and higher polyunsaturated fatty acids by the foetal tissues. Although there is no evidence to the contrary, investigations into the distribution of the fatty acids in the

phospholipids from lamb erythrocytes has led De Gier & Van Deenen (1964) to suggest that, in sheep, there is a marked inability to convert 18:2 into 20:4ω6. In this respect, it is interesting to note that, although in the lambs receiving their natural diet the concentration of 18:2 in the liver lipids increased markedly, the concentration of 20:4ω6 in all the lipid fractions except the phospholipids remained similar to that in the livers of the lambs at birth (Tables 2–6).

In the investigations reported here all the major liver lipid fractions at birth contained high concentrations of 16:1. These concentrations were maintained throughout the 8 d period in the lambs receiving the artificial diet but were quickly reduced in the lambs receiving the natural diet. These results are similar to those of the previous investigation into the plasma lipid changes during the first 8 d after birth (Noble *et al.* 1971). High concentrations of 16:1 have also been observed in the various lipid fractions from the livers of foetal lambs (Scott *et al.* 1967). It was suggested previously (Noble *et al.* 1971) that the high concentration of 16:1 found in the plasma of lambs at birth was a reflection of the dependence of the foetus during development upon synthesis *de novo* of fatty acids and the subsequent desaturation of the 16:0 thus produced. Under conditions of fat depletion, in which synthesis *de novo* would play a more important part in fatty acid metabolism, an accumulation of 16:1 has also been noted (Mead, 1957; Sand, Sen & Schlenk, 1965). In the work now described, the higher concentrations of 16:1 in the livers of the newborn lambs and of those lambs receiving the low-fat artificial diet was also accompanied by notable concentrations of the *cis*-11-octadecenoic acid (Table 7), indicating subsequent chain elongation of the accumulating 16:1. In the lambs receiving their natural diet there were negligible concentrations of the *cis*-11-isomer, but the concentration of the *trans*-9- and *trans*-11-octadecenoic acids had been increased, reflecting the greater dependence of the naturally fed lambs upon fatty acids from lipids of exogenous origin.

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