Fatty acid composition of tissue phospholipids of the foetal calf and neonatal lamb, deer calf and piglet as compared with the cow, sheep, deer and pig

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1. The fatty acid compositions of muscle and brain phospholipids of foetal calves, neonatal lambs, deer calves and piglets, and mature cattle, sheep, deer and pigs were determined. The cattle, sheep and deer had previously grazed ryegrass–clover pastures, and the pigs had been given rations based on barley. Two steers and four sheep had been given protected polyunsaturated lipid-protein supplements.

2. In muscle phospholipids the values for triene:tetraene were 1·5 for neonatal lambs and 0·3 for foetal calves. Levels of linoleic acid were low compared with those in older animals but levels of the fatty acids 22:5ω3 and 22:6ω3 were comparatively high. For arachidonic acid there was little difference between young and mature animals.

3. In muscle phospholipids of neonatal piglets and deer calves values for triene:tetraene were low. The piglet also had a low value for 22:5ω3:22:6ω3 compared with those in deer, calves or lambs. This ratio showed a proportionately greater increase with maturity in the pig than in cattle and sheep. Whilst the neonatal deer had higher linoleic acid levels than the other young ruminants, the fatty acid composition of muscle phospholipids of mature deer was rather similar to that in other ruminants.

4. Phospholipids of brain showed little difference in fatty acid composition between foetuses or neonates and the mature animals. There was higher 22:4ω6 content in the adult ruminant with even higher levels in sheep given protected polyunsaturated fat. Linoleic acid was barely evident in any animal. The 22:6ω3 content was as high in the foetal or neonatal ruminant brain as in the adult, and higher than in the piglet. The fatty acid composition of brain phospholipids of young deer was similar to that in other ruminants.

5. In other tissue phospholipids in foetal or neonatal ruminants and piglets there were high levels of 22:6ω3 in liver and low levels in lung. The neonatal animals, in particular, had high palmitic acid levels in lung. Hearts of young ruminants contained high levels of 20:5ω3 and C_{18}-aldehyde derived from plasmalogens. Piglet heart contained higher linoleic acid and arachidonic acid, possibly due to increased entry of linoleic acid across the placenta from the sow.

Young foetal and neonatal ruminants such as bovine calves and lambs contain low levels of linoleic and linolenic acids in the tissue lipids (Leat, 1966; Scott, Setchell & Bassett, 1967; Noble, Steele & Moore, 1971). As a result it has been suggested that these animals could well be verging on a state of essential fatty acid deficiency, particularly if due recognisance is made of the value for the ratio, triene:tetraene (Noble, 1973).

Since the report of Van Duyne, Parker, Havel & Holm (1960) that unesterified palmitic acid was not freely permeable across the placenta of the sheep and the fact that tissues of newborn ruminants were low in linoleic and linolenic acids it has often been accepted that the placenta of the sheep is almost impermeable to acids such as linoleic and linolenic acids. In most analyses for fatty acids of newborn ruminants results for acids up to C_{20} only have usually been reported. Shorland, Body & Gass (1966), however, published analyses of minced foetal lambs which included significant amounts of C_{22} polyunsaturated fatty acids (PUFA) derived from linolenic acid. In addition a few reports have shown that phospholipids of various tissues of 1 week old and newborn sheep contained C_{22} acids derived from linolenic acid (Payne & Masters, 1971; Noble, Steele & Moore, 1970, 1971). This indicated that there was some amount of placental transfer of maternal fatty acids since these acids could not have been synthesized endogenously (Holman, 1968).

In any assessment of whether ruminants are likely to be deficient in linoleic acid, all acids which can be derived from this acid must be taken into account. In addition, though Holman (1968) has suggested that linolenic acid should be considered in a different context from linoleic acid and essential fatty acid deficiency other workers have produced some evidence for linolenic acid to be considered as an essential fatty acid (Rivers & Davidson, 1974; Sinclair, Fiennes, Hay, Watson, Crawford & Hart, 1974). The fact that linolenic acid can cause promotion of growth and affect the level of 20:3ω9 as does linoleic acid (Mohrhauer & Holman, 1963; Pudelkewicz, Seuffert & Holman, 1968) suggests that linolenic derivatives should also be considered.

It was postulated that placental transfer of essential fatty acids may well be much greater than has been previously thought, and that the total quantity of PUFA is sufficient to make up for low quantities of linoleic and arachidonic acids.

As relevant fatty acid analyses were not available to test this hypothesis, this paper describes the fatty acid composition of tissue phospholipids of foetal and neonatal ruminants and non-ruminants, together with results for muscle and brain in adult animals.

MATERIALS AND METHODS

Animal tissues

Tissues were obtained from seven foetal bovine calves (mean body-weight 23 kg) available at slaughter of the cows, from six Romney lambs slaughtered immediately after birth and from six piglets and two deer calves which died probably as a result of asphyxiation before they suckled. Muscle samples were from the hind leg, being predominantly *vastus lateralis*. Samples of muscle and brain were also taken from the cows and adult sheep, pigs and deer. The cows, sheep and deer had been grazing ryegrass–clover pasture typical of that in New Zealand while the adult pigs had been given rations based largely on barley and maize with a small amount of meat meal.

In addition, a few samples were obtained from two steers, 18 months old, and two wethers, 9 months old, which had been given a protected lipid supplement that resulted in high linoleic acid levels in triglycerides and other lipids. This supplement, supplied by Alta Lipids (NZ), Upper Hutt, New Zealand, contained 270 g linoleic acid/kg. These animals are designated 'poly' sheep and cattle.

All samples of tissue were deep frozen at $-20°$ until analysed.

Extraction methods

The tissues (usually 5 g minced finely with scissors and sampled internally from a slab of tissue) were extracted with chloroform–methanol (2:1, v/v) (Folch, Lees & Sloane-Stanley, 1957). For most tissues, after concentration of the chloroform extract by rotary evaporation under vacuum, the residue was taken up in 2 ml chloroform–methanol, and for separation of phospholipids by thin-layer chromatography 60 μl was applied to plates coated with silica gel G (Merck, Darmstadt). After developing the chromatogram in hexane–diethyl ether–acetic acid (80:20:1, by vol.), the areas of silica gel corresponding to phospholipids were removed and the samples were transmethylated (Metcalfe, Schmitz & Pelka, 1966). The methyl esters were then separated using a gas–liquid chromatograph (Model FM402; Hewlett Packard, Avondale, Pennsylvania, USA) with a 45 mm ID $\times$ 1·20 m glass column containing 170 g diethylene glycol succinate/kg Gas Chrom Q. at 170° (Applied Science Laboratories, State College, Pennsylvania, USA). Relative peak areas and hence composition (% total fatty acids) were calculated as described by Bartlett & Iverson (1966). Inclusion of an internal standard, methyl margarate, to the extent of 10–20% of total fatty acids during the methylation enabled the PUFA acid content to be calculated on a wet

weight basis (Payne, 1978). Relevant acids were identified by comparison with certain standards and by comparison of equivalent chain lengths, calculated according to the method of Woodford & van Gent (1960), with published values (Hoffstetter, Sen & Holman, 1965). Final confirmation of the identity of relevant acids in relation to the number of double bonds was established by gas–liquid chromatography and mass spectrometry.

RESULTS

Fatty acid composition of muscle phospholipids

These results are shown in Table 1. There was a significant amount of the eicosatrienoic acid (20:3ω9) in the lambs but much less in the calf. Thus the value for triene:tetraene of 1·5 for the lambs is significantly higher than that (0·3) for the calves ($P < 0{\cdot}01$).

Linoleic acid levels were much higher in the mature animal than in the foetal or newborn animal but for the higher derivative, arachidonic acid (20:4ω6), the difference was much less. In the bovine calf linolenic acid and eicosapentaenoic acid, 20:5ω3, increased to a greater extent with maturity than did the C_{22} acids which remained relatively static.

In the piglets there was a massive increase in linoleic acid with maturity but a sharp decrease in arachidonic acid. The value for 22:5ω3:22:6ω3 increased with maturity also. As might be expected with higher amounts of linoleic acid present the 20:3ω9 acid content was low. As for the pig, the major difference in fatty acid composition between the deer calves and other young ruminants was the higher level of linoleic acid. The fatty acid composition in the mature deer was similar to that in cattle and sheep in most respects, except for a high level of linolenic acid with correspondingly lower levels of oleic acid.

Fatty acid composition of brain lipids

The composition of brain phospholipids changed little with maturity (Table 2). There was an increase in the docosatetraenoic acid, 22:4ω6, with particularly high levels in the 'poly' sheep, presumably as a result of the higher linoleic acid intake. There was virtually no change with maturity in linoleic acid content, which was very low in brains of all animals, or in arachidonic acid. In the brains of foetal or neonatal animals there were high levels of 22:6ω3. Though in muscle of piglets there were comparable levels of 22:6ω3 to that in lambs and calves, in the piglet brain the level, while still high, was much lower than that in the ruminants. In contrast to muscle, the fatty acid composition of brain phospholipids of deer calves is similar to that of calves.

Fatty acid composition of phospholipids of liver, heart, lung and intestine

Marked differences between tissues (see Table 3) included: (*a*) high levels of the 22ω3 PUFA in liver and low levels in lungs; (*b*) high concentrations of palmitic acid in lung, particularly in the neonatal animals; (*c*) relatively high levels of the acid, 20:5ω3, and the C_{16}-aldehyde derived from plasmalogen in the hearts of calves and lambs compared to the levels in other tissues.

In the piglet organs the amount of the acid 22:6ω3 was greater than that of 22:5ω3, as found in muscle. The piglet heart also contained higher linoleic and arachidonic acid than calf and lamb hearts, presumably as a result of increased linoleic acid transfer across the placenta of the sow. An observation of interest in this respect is that there is a relatively high content of an acid corresponding to linolenic acid or possibly 20:1ω9 whereas the content of higher PUFA derivatives was low.

Table 1. *The mean fatty acid composition (g/kg fatty acids) of leg muscle phospholipids in foetal calves, neonatal lambs, neonatal piglets, neonatal deer, adult cows, sheep, pigs, deer and cattle and sheep given protected polyunsaturated supplements* ('poly' animals)*

(No. of animals given in parentheses)

		Fatty acid														
Animal		16:0 ald	16:0	16:1ω9	18:0	18:1ω9	18:2ω6	18:3ω3	20:3ω9	20:3ω6	20:4ω6	20:5ω3	22:4ω6	22:5ω6	22:5ω3	22:6ω3
Calves	(7)	42	120	29	118	361	42	7	24	17	70	14	14	6	70	24
Cows	(4)	37	108	7	94	235	199	48	tr	18	86	50	16	tr	68	12
'Poly' cows	(2)	34	138	7	179	100	368	5	11	17	75	14	9	5	20	2
Lambs	(5)	38	104	21	98	441	7	11	72	tr	48	26	7	5	60	41
Sheep	(5)	41	105	11	122	232	141	66	15	8	83	68	6	tr	51	21
'Poly' sheep	(4)	35	85	tr	127	94	425	33	10	4	112	19	7	8	29	14
Piglets	(6)	34	124	20	119	267	77	3	11	13	184	4	16	23	10	34
Pigs	(5)	30	126	ND	111	131	318	11	5	14	109	17	8	8	23	24
Deer calves	(2)	54	88	18	132	201	93	30	15	32	77	50	9	ND	80	45
Deer	(4)	81	69	16	106	98	211	135	3	11	87	77	3	ND	45	20
Pooled SD		14	17	6	18	46	29	19	5	5	19	12	6	4	21	14

ald, aldehyde; tr, trace; ND, not detected.

* For details, see p. 46.

Table 2. *The fatty acid composition (g/kg fatty acids) of brain phospholipids in foetal calves, neonatal lambs, neonatal piglets, neonatal deer, adult cows, sheep, pigs, deer and cattle and sheep given protected polyunsaturated supplements* ('poly' animals)*

(No. of animals given in parentheses)

		Fatty acid														
Animal		16:0 ald	16:0	16:1ω9	18:0	18:1ω9	18:2ω6	18:3ω3	20:3ω9	20:3ω6	20:4ω6	20:5ω3	22:4ω6	22:5ω6	22:5ω3	22:6ω3
Calves	(7)	25	171	14	147	249	5	15	10	7	71	5	30	3	30	202
Cows	(4)	23	141	7	164	277	6	32	6	6	81	10	41	19	19	161
'Poly' cows	(2)	13	115	7	137	189	4	20	20	11	164	14	66	17	8	199
Lambs	(4)	8	126	5	152	205	2	15	30	ND	74	24	3	ND	42	288
Sheep	(6)	10	161	6	154	275	tr	30	12	5	73	14	38	4	29	194
'Poly' sheep	(2)	20	160	4	146	233	3	9	2	2	90	10	93	10	12	183
Piglets	(2)	14	223	20	206	181	5	7	10	2	120	8	45	ND	38	115
Pigs	(3)	23	151	8	182	254	9	21	9	10	112	14	53	14	5	110
Deer calves	(2)	27	166	13	166	223	tr	17	9	8	79	7	29	ND	35	174
Deer	(2)	41	147	8	169	274	13	31	5	7	101	10	67	ND	16	132
Pooled SD		7	20	3	19	29	3	7	5	3	12	5	16	3	10	42

ald, aldehyde; tr, trace; ND, not detected.

* For details, see p. 46.

Table 3. *The fatty acid composition (g/kg fatty acids) of phospholipids of other tissues of foetal calves, neonatal lambs, neonatal piglets and neonatal deer*

(No. of animals given in parentheses)

Animal	Tissue		Fatty acid														
			16:0 ald	16:0	16:1ω9	18:0	18:1ω9	18:2ω6	18:3ω3	20:3ω9	20:3ω6	20:4ω6	20:5ω3	22:4ω6	22:5ω6	22:5ω3	22:6ω3
Calves	Liver	(2)	3	142	25	169	144	46	2	6	38	128	26	4	ND	125	100
	Heart	(3)	46	152	36	107	270	75	12	5	30	103	35	15	ND	55	25
	Lung	(3)	37	24	45	100	271	45	15	13	27	77	6	5	ND	36	17
	Intestine	(2)	1	143	26	178	297	95	8	7	25	111	4	6	ND	50	31
Lambs	Liver	(3)	2	118	15	87	225	21	11	53	ND	76	38	5	ND	133	191
	Heart	(2)	68	110	7	93	282	20	11	82	ND	87	86	ND	ND	77	60
	Lung	(3)	24	403	45	53	274	5	4	32	ND	41	16	3	ND	41	32
	Intestine	(3)	3	109	5	102	341	5	8	64	ND	84	56	ND	ND	83	122
Piglets	Liver	(6)	5	108	35	174	181	59	3	16	14	219	ND	11	25	11	86
	Heart	(6)	39	101	19	105	206	102	74	10	8	230	11	18	29	11	23
	Lung	(3)	19	326	69	90	201	35	3	6	8	119	3	13	13	6	21
	Intestine	(6)	10	126	12	142	225	47	31	9	16	215	8	21	23	15	62
Pooled SD			9	30	8	17	24	16	7	9	11	36	7	6	7	13	26

ald, aldehyde; ND, not detected.

DISCUSSION

As in adults the fatty acid composition of phospholipids of tissues of neonatal and foetal animals differed both within and between species. Thus, hearts of all animals studied contained higher levels of linoleic and arachidonic acids than other tissues but pig heart had much higher levels.

The tissues which were analysed were mostly those expected to contribute significantly to the total content of PUFA (see Payne, 1978).

Fatty acid compositions of phospholipids of liver, heart, lung and intestine were analysed in only a few young ruminants, principally to confirm that these tissues would not contribute to any great extent (> 10%) to the total content of PUFA. Though the results presented for these tissues can not be analysed statistically there are clearly certain differences but in many instances their biological significance is not known.

From the 22ω3 PUFA values for muscle and brain of the 'poly' ruminants it is apparent that the feeding of a diet high in PUFA has reduced the level of these acids. Mohrhauer & Holman (1963) have reported that linoleic acid inhibits conversion of linolenic acid to higher acids in the rat. The intake of linoleic acid in the 'poly' animals not only decreased the total 22ω3 acid content but also increased 22:5ω6 content as has been reported in the rat by Galli, Agradi & Paoletti (1974). However, there is no real basis to the suggestion that the fatty acid ratio, 22:5ω6:22:6ω3, is an index of linolenic acid deficiency. In the pig the intake of linoleic acid contained in grain (a moderate amount compared with the amount fed to the 'poly' animals) caused little change in total 22:ω3 acids but some decrease in 22:6ω3. This indicates that the relationship between linoleic and the higher derivatives is not clear. The differences in values for 22:5ω3:22:6ω3 between muscle and brain in calves, lambs and piglets suggest that linoleic acid content is not a determinant of the ratio. It seems that other factors, perhaps related to the membrane structure required in tissues of particular species, dictate the fatty acid composition.

The higher palmitic acid level in the lung of neonatal animals as compared with the foetal calf is possibly a result of surfactant production just before parturition (Fujiwara, Adams, Sipos & El-Salawy, 1968).

The presence of high levels of 22:6ω3 in the brains of all species, particularly ruminants, suggests there is an efficient conversion of linolenic acid to 22:6ω3. A selective uptake of linolenic acid or linoleic acid by rat brain has been demonstrated by Dhopeshwarkar & Mead (1973). The lack of change in the fatty acid composition of brain in 'poly' animals as great as in muscle suggests that uptake of linoleic acid into adult ruminant brain is not great despite the report in the review of Dhopeshwarkar & Mead (1973) that incorporation of radioactive linoleic acid from the carotid artery is high. It is apparent that there is only a small turnover of phospholipids in brain and feeding on high linoleic acid intakes would require a very long period of feeding to have much effect. Though linolenic acid intake in the adult ruminant is low compared to linoleic acid intake, preferential uptake by the brain ensures the maintenance of high 22:6ω3 levels.

The relative constancy of the level of arachidonic acid in tissues of ruminants of all ages and conditions suggests that this is the really essential acid and linoleic acid is only a store to be converted to arachidonic acid as needed. In the piglet there appears to be a different point of equilibrium as the level of arachidonic acid is much higher than that in the adult pig or ruminants. This maintenance of constant levels of arachidonic acid may well be related to its role as the source of prostaglandins.

From these analyses for fatty acids it would be thought that lambs and calves had very low linoleic acid levels. However, in terms of the C20:3ω9:C20:4ω6 ratio only lambs would be classified as deficient (Holman, 1960). Further explanation on this deficiency,

which is more apparent than real, is to be found in a subsequent report (Payne, 1978).

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