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Essential Fatty Acids in the Fetal and Newborn Lamb

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Abstract

The concentrations of linoleic and linolenic acids and their metabolites in the liver, kidney, brain, erythrocytes and plasma of fetal lambs at various stages of gestation, and of newborn and 2-week-old suckled lambs was determined. Throughout gestation the fetal tissues, erythrocytes and plasma all contained low levels of linoleic and linolenic acids together with consistently high levels of their long-chain polyunsaturated metabolites. The triene : tetraene (eicosa-5,8,11-trienoic acid/arachidonic acid) ratio was always 0·4 or less except at birth when it reached 0·6 in liver and 0·9 in plasma. Milk intake significantly increased the linoleic and linolenic acid levels in the lamb by 2 weeks after birth. These results show that the developing fetal lamb should not be regarded as being deficient in essential fatty acids, as suggested by previous investigators. It is proposed that the total metabolites of linoleic and linolenic acids are the most appropriate measure of the essential fatty acid status of the fetal lamb.

Introduction

It has been suggested that late-term and newborn lambs are deficient in essential fatty acids (EFA) (Shorland *et al.* 1966; Noble 1973). This assessment is based on the presence of low levels of linoleic [18 : 2(n – 6)] and linolenic [18 : 3(n – 3)] acids in various tissues and high ratios of eicosa-5,8,11-trienoic [20 : 3(n – 9)] to arachidonic [20 : 4(n – 6)] acids in liver and plasma. However, fetal lambs at birth showed no clinical signs of EFA-deficiency (Payne and Rattray 1982).

The direct placental transfer of unesterified linoleic and linolenic acids from the ewe to the late-term fetus appears to be negligible (Elphick *et al.* 1979; Leat and Harrison 1980). In primates and rabbits the placental transfer and subsequent metabolism of EFA has been shown to vary with gestational age (Robertson and Sprecher 1968; Portman *et al.* 1969; Biezenski 1970), while little is known about the placental transfer and subsequent metabolism in the fetal lamb at different stages of gestation.

This paper reports the concentrations of linoleic and linolenic acids and their derived long-chain polyunsaturated fatty acid (LCP) metabolites in tissue, erythrocyte and plasma lipids of fetal lambs at different stages of gestation and compares them with the levels in 2-week-old suckled lambs. The data obtained was used to reassess the EFA status of the fetal lamb using criteria more appropriate to this species.

Materials and Methods

Tissue and Blood Samples

Liver, kidney and brain were removed from 27 fetal lambs of mean age 57 (range 53–60), 91 (range 79–107) and 135 (range 126–144) days and from seven unsuckled newborn lambs. Fetal gestational ages were estimated by crown–rump length measurements (Barcroft 1946; Stephenson and Lambourne 1960). Jugular blood samples

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were taken from 13 chronically cannulated fetal lambs of mean age 125 (range 120–128), 134 (range 132–137) and 142 (range 140–144) days and from four unsuckled newborn lambs. Liver, kidney, brain and blood samples were obtained from three or four 2-week-old suckled lambs.

Extraction of Lipids and Analysis of Fatty Acid Methyl Esters

Tissue and plasma lipids were extracted with chloroform–methanol (2 : 1 v/v, containing 10 mg/l butylated hydroxytoluene) using the method of Folch *et al.* (1957). Erythrocyte lipids were extracted by the method of Rose and Oklander (1965). An internal standard of heptadecanoic acid (17 : 0) was added to lipid extracts before trans-methylation to allow a quantitative assessment of fatty acids based on the wet weight of tissue. The concentrations of fatty acids were expressed as per millilitre of plasma or per millilitre of packed erythrocytes. Portions of the plasma lipid extracts were fractionated into cholesteryl esters, triglycerides, unesterified fatty acids and phospholipids by thin-layer chromatography and quantified by the method of Christie *et al.* (1970). Fatty acid methyl esters were prepared by saponification followed by trans-esterification with boron trifluoride–methanol (Metcalfe *et al.* 1966), and separated by gas–liquid chromatography (Shimadzu GC-6AM, Japan) using a 75 m support-coated open tubular (SCOT) capillary column coated with either SP-2310 or SP-2340 (Chromalytic, Melbourne, Australia). Separations were achieved by temperature programming from 150 to 195°C at 2°C min^{-1} with a nitrogen carrier gas flow of 150 mm s^{-1} and fatty acid compositions were calculated from peak areas using an integrator (Shimadzu Chromatopac-E1A, Japan). Commercial standards (Nu-Chek-Prep, Minnesota, U.S.A.) and standards of eicosatrienoic acid isolated from EFA-deficient rat brain were used to identify the individual fatty acids.

Results

Tissue Fatty Acids

Low concentrations of linoleic and linolenic acids were present in the liver, kidney and brain of the fetal and newborn lambs (Table 1). However, there were high concentrations of their LCP metabolites which accounted for 17–22% of the total fatty acids present in liver, 13–16% in kidney and 15–19% in brain. These LCP fatty acids tended to increase towards late term, particularly in the brain. All the fetal tissues contained high concentrations of the non-essential oleic acid, which together with its metabolite eicosatrienoic acid increased towards term. The triene : tetraene ratio was 0·4 or less in all the fetal tissues throughout gestation except at birth when it reached 0·6 in the liver.

There were marked increases in the tissue linoleic and linolenic acid concentrations following the ingestion of milk after birth. The metabolites of both linoleic and linolenic acids also increased in the liver and kidney, whereas only the metabolites of linoleic acid increased in the brain. All the tissues of the suckled lambs exhibited triene : tetraene ratios of 0·1.

Erythrocyte and Plasma Fatty Acids

Both the erythrocyte and plasma total lipids of the fetal lambs contained low concentrations of linoleic and linolenic acids together with relatively higher concentrations of their LCP metabolites which remained fairly constant throughout the last third of the gestation period (Table 2). The concentrations of oleic acid were high relative to the levels of other fatty acids in the fetal erythrocytes and plasma, and together with eicosatrienoic acid increased towards term. The triene : tetraene ratio was always 0·4 or less throughout the last third of gestation, but reached 0·9 in the plasma at birth.

There were large increases in the concentrations of linoleic and linolenic acids in the erythrocytes and plasma of the 2-week-old suckled lambs, accompanied by increases in their metabolites in the plasma only. The erythrocytes of these lambs showed decreased

Table 1. Fatty acid concentrations (mg/100 g wet tissue) in tissue total lipids of fetal, newborn and 2-week-old lambs

No. of animals given in parentheses

Fatty acid	Mean fetal age (days): 57 (9)	91 (9)	135 (9)	Newborn (7)	Two weeks after birth (3)
		Liver			
18 : 1	265·1	244·1	236·1	410·9	394·3
18 : 2(n – 6)	26·0	8·6	8·0	9·1	183·0
18 : 3(n – 3)	0·9	0·5	0·7	0·7	20·3
20 : 3(n – 9)	18·7	19·9	28·2	35·3	18·2
20 : 4(n – 6)	96·4	51·4	72·8	62·7	206·4
20 : 5(n – 3)	24·1	19·5	9·1	10·8	27·0
22 : 5(n – 3)	31·0	33·6	37·1	36·1	51·6
22 : 6(n – 3)	72·5	50·0	57·2	64·9	101·3
LCP (n – 6)[A]	105·0	58·2	79·3	72·8	225·1
	±8·4	±3·3	±3·8	±5·5	±5·3
LCP (n – 3)[B]	127·5	103·1	103·4	111·8	180·0
	±7·0	±6·8	±11·0	±7·4	±15·9
20 : 3(n – 9)/20 : 4(n – 6)	0·2	0·4	0·4	0·6	0·1
Total fatty acids	1078·4	828·4	913·5	1145·8	1810·2
	±29·0	±32·0	±37·2	±122·4	±62·0
		Kidney			
18 : 1	244·5	285·8	337·0	365·6	326·5
18 : 2(n – 6)	9·1	8·6	9·1	7·8	97·8
18 : 3(n – 3)	0·9	0·9	1·6	0·7	5·5
20 : 3(n – 9)	18·0	18·0	25·9	24·2	10·3
20 : 4(n – 6)	54·0	52·4	65·9	66·0	110·3
20 : 5(n – 3)	8·9	19·0	20·1	12·2	24·9
22 : 5(n – 3)	9·7	14·0	13·5	7·3	17·2
22 : 6(n – 3)	18·8	21·7	20·8	16·6	29·9
LCP (n – 6)	58·8	56·0	71·7	70·6	117·1
	±5·6	±4·6	±4·0	±3·4	±8·9
LCP (n – 3)	37·4	54·1	54·4	36·1	72·0
	±2·3	±4·7	±3·9	±3·0	±9·4
20 : 3(n – 9)/20 : 4(n – 6)	0·3	0·3	0·4	0·4	0·1
Total fatty acids	655·7	720·0	837·6	845·6	992·3
	±27·7	±34·8	±57·5	±52·7	±59·1
		Brain			
18 : 1	163·9	183·3	367·7	658·4	640·8
18 : 2(n – 6)	1·5	1·9	1·4	1·5	7·2
18 : 3(n – 3)	0·5	0·3	0·6	0·8	1·4
20 : 3(n – 9)	6·9	7·2	9·4	15·0	16·4
20 : 4(n – 6)	33·4	35·7	83·3	94·9	116·5
20 : 5(n – 3)	1·9	4·0	1·3	3·8	2·9
22 : 5(n – 3)	8·6	9·9	10·2	18·9	12·3
22 : 6(n – 3)	59·8	99·2	144·7	210·5	217·7
LCP (n – 6)	41·7	43·4	114·5	131·7	159·5
	±4·0	±4·8	±7·4	±9·8	±6·2
LCP (n – 3)	70·3	113·0	156·0	233·2	232·9
	±9·4	±8·0	±9·8	±16·2	±34·8
20 : 3(n – 9)/20 : 4(n – 6)	0·2	0·2	0·1	0·2	0·1
Total fatty acids	780·7	999·0	1467·4	2296·3	2218·5
	±11·2	±57·2	±87·9	±178·2	±134·3

[A] LCP(n – 6), total metabolites of linoleic acid (mean ± s.e.m.).
[B] LCP(n – 3), total metabolites of linolenic acid (mean ± s.e.m.).

concentrations of the metabolites of linoleic and linolenic acids. Both the erythrocytes and plasma of the suckled lambs exhibited triene : tetraene ratios of 0·1.

Plasma Lipid Classes

The concentrations and proportions of the various plasma lipid classes of the fetal lamb remained fairly constant throughout the last third of gestation until birth when

Table 2. Fatty acid concentrations (μg/ml) in erythrocyte and plasma total lipids of late-term, newborn and 2-week-old lambs

No. of animals given in parentheses

Fatty acid	Mean fetal age (days): 125 (5)	134 (5)	142 (3)	Newborn (4)	Two weeks after birth (4)
		Erythrocyte			
18 : 1	450·6	488·3	495·6	564·5	502·4
18 : 2(n – 6)	17·4	16·3	14·4	17·1	49·7
18 : 3(n – 3)	1·1	0·5	0·5	1·4	2·5
20 : 3(n – 9)	4·6	6·8	4·9	7·3	2·1
20 : 4(n – 6)	45·0	47·9	36·3	35·5	17·6
20 : 5(n – 3)	6·9	7·5	6·0	7·8	4·7
22 : 5(n – 3)	14·5	19·0	12·7	14·4	7·9
22 : 6(n – 3)	13·7	16·9	7·4	9·1	4·3
LCP (n – 6)[A]	49·0	50·8	37·8	36·8	18·3
	±4·9	±5·1	±8·4	±1·8	±2·4
LCP (n – 3)[B]	35·1	43·5	26·0	31·3	16·8
	±4·4	±4·3	±4·3	±2·1	±2·0
20 : 3(n – 9)/20 : 4(n – 6)	0·1	0·1	0·1	0·2	0·1
Total fatty acids	737·1	806·2	769·6	848·8	734·1
	±39·1	±42·2	±20·6	±57·3	±46·7
		Plasma			
18 : 1	74·9	84·0	99·4	330·4	866·7
18 : 2(n – 6)	3·4	2·7	2·8	8·0	362·2
18 : 3(n – 3)	0·5	0·2	0·2	0·5	54·7
20 : 3(n – 9)	2·5	3·3	3·4	7·8	5·8
20 : 4(n – 6)	11·5	7·5	11·5	8·7	52·6
20 : 5(n – 3)	3·4	2·5	2·3	3·3	14·0
22 : 5(n – 3)	5·1	3·7	4·8	3·9	12·8
22 : 6(n – 3)	6·7	4·7	5·5	7·1	12·0
LCP (n – 6)	12·0	7·9	12·3	9·3	57·2
	±1·4	±0·9	±0·9	±1·6	±14·0
LCP (n – 3)	15·2	11·0	12·7	14·4	38·7
	±1·0	±1·4	±0·5	±1·8	±7·5
20 : 3(n – 9)/20 : 4(n – 6)	0·2	0·4	0·3	0·9	0·1
Total fatty acids	212·1	210·1	251·6	596·3	2329·0
	±14·1	±13·5	±11·6	±40·8	±180·2

[A,B] As for Table 1.

the plasma unesterified fatty acid fraction increased considerably (Table 3), although the cholesteryl esters and phospholipids together still accounted for 53% of the plasma lipids. The cholesteryl esters and phospholipids were the principal lipid classes in the

plasma of the late-term fetal and 2-week-old lambs and accounted for 79–87% of the plasma lipids.

Table 3. Amounts[A] (μg/ml) and proportions[B] of plasma lipid classes of late-term, newborn and 2-week-old lambs

No. of animals	Mean fetal age (days)	Amounts and proportion in plasma of: Cholesteryl ester	Triglyceride	Unesterified fatty acid	Phospholipid
5	125	194 ± 19(50)	42 ± 4(11)	29 ± 3 (8)	118 ± 11(31)
5	134	181 ± 13(52)	30 ± 2 (9)	18 ± 2 (5)	116 ± 10(34)
3	142	166 ± 24(49)	30 ± 2 (9)	14 ± 0 (4)	127 ± 10(38)
4	Newborn	170 ± 14(28)	39 ± 2 (6)	244 ± 20(41)	148 ± 11(25)
4	Two weeks after birth	676 ± 55(38)	302 ± 21(17)	60 ± 3 (3)	718 ± 6(41)

[A] Mean ± s.e.m. [B] Percentages in parentheses.

Discussion

An important feature of these results was the consistently high concentrations of the LCP metabolites of linoleic and linolenic acids in the tissue and plasma lipids of the fetal and newborn lambs, despite the presence of low concentrations of their precursors. It is these metabolites which are the biologically active fatty acids in EFA metabolism, rather than their precursors linoleic and linolenic acids (Holman 1970). The LCP constitute a substantial proportion of the cellular phospholipids and also serve as precursors of the prostaglandins and related compounds (Kunau and Holman 1977) which have physiologic roles in the developing fetus (Challis and Patrick 1980). Although linoleic acid has been shown to regulate transepidermal water loss in the rat (Prottey 1977), this is unlikely to be important to the fetus in an aqueous intra-uterine environment. The presence of high concentrations of the LCP(n – 6) and LCP(n – 3) throughout the gestation period does not support the contention that the fetal lamb is EFA-deficient because one of the characteristic features of deficiency is significantly reduced levels of LCP (Mohrhauer and Holman 1963).

The arachidonic and docosahexaenoic [22 : 6(n – 3)] acids which make up a substantial portion of the LCP in the fetal tissues and plasma are unlikely to be derived directly from the maternal unesterified fatty acids because this maternal lipid fraction contains only trace levels of these fatty acids (Leat and Harrison 1980). They are more likely to be synthesized from linoleic or linolenic acid either in the placental or fetal tissues, or both, as suggested by the results of Shand and Noble (1979, 1981). Despite reports that the sheep placenta is impermeable to fatty acids including the EFA (Elphick *et al.* 1979; Leat and Harrison 1980), the presence of small amounts of linoleic and linolenic acids in all the fetal tissues and plasma suggests some placental transfer of these fatty acids from the maternal circulation since they could not have been synthesized in the fetus (Holman 1968). The possibility that other maternal plasma lipids such as the cholesteryl esters and phospholipids, which are rich sources of linoleic and linolenic acids (Christie 1978), are hydrolysed by the placenta to release linoleic and linolenic acids which are then transferred to the fetus, must also be considered. The phospholipase A enzyme identified in the sheep placenta by Grieves and Liggins (1976) may be related to such placental transport mechanisms.

The triene : tetraene ratio was originally proposed by Holman (1960) as a measure of EFA status in non-ruminant species, and a ratio exceeding 0·4 was considered to be abnormal. Based on the triene : tetraene ratios encountered in the present study, which were 0·4 or less in all tissues during gestation, except in liver and plasma at birth, it is not possible to regard the fetal lamb as EFA-deficient. By comparison, the triene : tetraene ratios reached 4·7 in EFA-deficient rats (Sinclair and Collins 1970) and 6·0 in humans (Holman 1977). Previous workers who had reported high ratios in fetal and newborn lambs all based their calculations on results obtained using conventional packed gas–liquid chromatography columns (Shorland *et al.* 1966; Noble *et al.* 1971*a*, 1971*b*; Leat *et al.* 1978) on which eicosatrienoic acid could elute near or with eicosadienoic acid [20 : 2(n – 6)], dihomogamma linolenic acid [20 : 3(n – 6)] and docosanoic acid (22 : 0) while arachidonic acid [20 : 4(n – 6)] can overlap with eicosa-11,14,17-trienoic acid [20 : 3(n – 3)] and docosaenoic acid (22 : 1) (Ackman 1980; Sinclair 1980). The results reported in this paper were obtained using capillary gas–liquid chromatography and were not subject to errors caused by the co-chromatographing of the various fatty acids.

The accumulation of eicosatrienoic acid in non-ruminant tissues has been accepted as being a characteristic of EFA-deficiency (Holman 1968). This fatty acid is produced from oleic acid by the same enzymic sequence that converts linoleic and linolenic acids to their LCP metabolites, with the rate of production depending on the relative levels of the three precursor fatty acids. Under conditions of EFA-deficiency, eicosatrienoic acid is produced because of the markedly reduced availability of linoleic and linolenic acids leaving oleic acid as the major substrate for the enzymic sequence (Holman 1970). The high concentrations of oleic acid found in the fetal lamb are largely derived from maternal acetate and glucose (Vernon *et al.* 1981) and this is characteristic of ruminant lipids (de Gier and van Deenen 1964; Payne and Masters 1971). The synthesis of oleate in the fetus is also further enhanced by the presence of unusually low levels of linoleic acid which normally inhibit the Δ^9-desaturase, an enzyme required for oleate synthesis (Jeffcoat and James 1977). Therefore the presence of small quantities of eicosatrienoic acid in the sheep fetus (ranging from 0·6 to 3·0% of the total fatty acids present) is best explained as a normal consequence of an abundant supply of oleic acid relative to the low levels of linoleic and linolenic acids, rather than an indication of a deficient EFA status. By comparison, levels of eicosatrienoic exceeding 10% in EFA-deficient rats (Hassam *et al.* 1977) and humans (Friedman *et al.* 1976) were commonly reported. In fact, the presence of eicosatrienoic acid in the fetus is in itself evidence of functional enzymes in the feto-placental unit which convert linoleic and linolenic acids to their LCP metabolites thus accounting for the high concentrations of the latter present in the fetus throughout gestation and at birth.

In spite of the large increases in the concentrations of linoleic and linolenic acids in the erythrocytes of the 2-week-old suckled lambs, the observed decrease in their LCP metabolites probably resulted from the change in the erythrocyte populations at birth from the fetal to the mature type (Perk *et al.* 1964) where the aging process is normally characterized by increases in linoleic and linolenic acids compensated by decreases in their LCP metabolites (van Gastel *et al.* 1965; Phillips *et al.* 1969).

An increased sympathetic nervous activity at birth causes a rapid mobilization of fatty acids from the adipose tissue (van Duyne *et al.* 1960), and this accounts for the marked increases in the plasma unesterified fatty acid fraction and plasma oleic acid. While the oleic acid content of the liver and brain also increased at birth, it was mostly

located in the phospholipid fraction which at all times accounted for more than 80% of the tissue total lipids (Christie 1978; M. A. Rajion and J. G. McLean, unpublished observations).

The pattern of increased tissue EFA and LCP levels and increased concentrations of the plasma cholesteryl esters, triglycerides and phospholipids in the 2-week-old suckled lambs all reflected the high fat intake provided by the milk (Noble *et al.* 1971*a*, 1971*b*; Payne 1978; Leat and Harrison 1980).

These results show that the traditional parameters used for assessing EFA status are not appropriate for the fetal lamb because of the consequences of limited placental transfer of unesterified linoleic and linolenic acids and the high levels of oleic acid present in the fetus. In addition, tissues of the fetal lamb were shown to contain substantial concentrations of total LCP. It is proposed that the total metabolites of linoleic and linolenic acids are the most appropriate measure of EFA status of the fetal lamb, which should not be regarded as being EFA-deficient. Further experiments are required to determine precisely how the fetal lamb obtains adequate amounts of EFA and LCP.

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