FATTY ACID COMPONENTS OF OVINE EXTRAHEPATIC TISSUES AFTER RUMEN DEVELOPMENT

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Summary

The lipids of ovine heart muscle, kidney, skeletal muscle, and depot fat have been separated into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions by chromatography on silicic acid. Fatty acid components of these fractions have been identified and estimated by gas chromatography, and include two unusual major components from muscle and kidney phospholipid. The interrelationships between these results and comparative values for other species are discussed.

I. Introduction

Detailed information on the fatty acids in the lipids of organs other than the liver is meagre (Shorland 1962), and particularly so with ruminant lipids. Klenk and Ditt (1934) have reported on the fatty acid composition of ox heart muscle, while Ault and Brown (1934) investigated the phospholipids of ox adrenals, and the lipids of sheep muscle were investigated by Hartman and Shorland (1957). As part of an investigation of the lipid metabolism of ruminants (Masters 1963*a*, 1963*b*), however, information of a more exact nature than was available in the literature was required.

Further, although ruminant depot fats have been reported more fully than the organ lipids (Hilditch and Longnecker 1937; Hilditch and Pedelty 1941; Hartman and Shorland 1961), it was necessary to have additional information on the fatty acid composition of the different lipid fractions of this source.

For these reasons the lipids of ovine heart, kidney, skeletal muscle, and depot fat have been fractionated into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions by chromatography on silicic acid. Fatty acid components of these fractions have been determined by gas chromatography.

II. Methods

The tissues in these experiments were obtained from freshly slaughtered young rams. Heart and kidneys were excised as well as samples of lean skeletal muscle and depot fat. Perinephric and myocardial depot fat were removed and the tissues were stored in closed vessels at −10°C in the dark until required.

The lipids were the extracted from these samples, fractionated on silicic acid columns, and the fatty acid components determined by gas chromatography. These techniques have been fully described previously (Horgan and Masters 1963).

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III. Results

Total lipid concentrations in the extrahepatic tissues were: heart, 3·9% of fresh tissue weight; kidney, 3·9%; skeletal muscle, 2·7%.

The data, presented in Table 1, illustrate the relative percentage composition of the main lipid fractions in ovine heart, kidney, skeletal muscle, and depot fat. Phospholipid is the dominant fraction in the first three of these tissues, with triglyceride the major component of depot fat. On a comparative basis, phospholipid decreases in the sequence heart, kidney, muscle, depot fat, while triglyceride increases in the same sequence. All these tissues, including depot fat, possess appreciable quantities of each of the four lipid fractions.

In Tables 2, 3, 4, and 5, are listed the fatty acid composition of ovine heart, kidney, skeletal muscle, and depot fat, respectively. The fatty acids listed (C_{12}–C_{22}) represent, in general, more than 95% of all the fatty acids present, but it was noticeable that the phospholipid of cardiac and skeletal muscle contained nearly 5% of short-chain ($<C_{12}$) fatty acids.

Table 1

SILICIC ACID CHROMATOGRAPHY OF OVINE TISSUE LIPIDS

Values for each component refer to the percentage by weight of the total tissue lipids

Fraction	Heart	Kidney	Muscle	Depot Fat
Cholesterol ester	8	11	8	5
Triglyceride	7	12	36	79
Free fatty acid	11	21	10	12
Phospholipid	74	56	46	4

In the phospholipid of heart, kidney, and muscle, two unidentified fatty acids were present as major components (A?, B?). The chromatographic data of these unsaturated acids [carbon numbers: polyethyleneglycol adipate (175°C), 15·6, 17·6; Apiezon L (198°C), 16·6, 18·6] do not coincide with previously reported tissue fatty acid components, and their identity is being investigated by further analytical procedures.

From relative retention data and the behaviour following bromination, the acid designated 18 : 2? is thought to be of the *trans* variety.

In addition to those acids shown in the tables, five further components were recognizable as trace quantities. The relative retention data and saturation of these methyl esters are consistent with a tentative identification of chain lengths as 13 (sat.), 15 (unsat.), 19 (sat.), 20 (br.), and 22 (br.). The identification of the acids C_{20} (1 double bond) and C_{20} (2 double bonds) must also be considered tentative, in view of the lack of suitable reference standards.

A comparison of the results in Tables 2–5 shows that the percentage of palmitic acid varies quite considerably between different lipid fractions in the same tissue. Depot fat displays the lowest percentage of palmitic acid of all the cholesterol ester fractions, but the highest in triglyceride and phospholipid.

TABLE 2

FATTY ACID COMPOSITION OF OVINE HEART LIPIDS

Values for each component refer to the percentage by weight of the total fatty acid methyl esters in each fraction. 'Trace' means that the amount of fatty acid present is less than 0·5%

Fatty Acid*	Cholesterol Esters	Triglycerides	Free Fatty Acids	Phospholipids
12 : 0	Trace	Trace	Trace	Trace
14 (br.)†	Trace	Trace	Trace	„
14 : 0	3·5	0·7	0·6	„
14 : 1	Trace	Trace	Trace	„
15 (br.)	Trace	Trace	„	„
15 : 0	0·8	0·5	„	„
15 : 1	Trace	Trace	„	„
A?	„	„	„	12·7
16 (br.)	„	„	„	Trace
16 : 0	32·8	21·2	21·3	19·0
16 : 1	1·9	1·2	2·0	1·7
17 (br.)	0·9	0·7	0·5	Trace
17 : 0	0·4	1·7	0·8	0·8
16 : 2	Trace	Trace	Trace	Trace
B?	„	„	„	2·9
18 (br.)	„	„	„	Trace
18 : 0	20·6	29·7	25·0	9·6
18 : 1	31·7	36·3	39·6	26·4
18 : 2	3·8	4·3	5·4	17·0
18 : 2?	Trace	Trace	Trace	Trace
18 : 3	1·0	1·5	1·7	1·7
20 : 0	Trace	Trace	Trace	Trace
20 : 1	Trace	Trace	Trace	Trace
20 : 2	Trace	„	Trace	Trace
21 : 0	0·6	„	0·6	1·3
20 : 4	0·9	0·6	1·2	3·3
22 : 0	Trace	Trace	Trace	0·9

* Numerals indicate chain length and number of double bonds present, respectively.

† br. = singly methyl branched chain.

There is a broad similarity in the distribution of the C_{18} acids of the cholesterol ester fraction of depot fat, heart tissue, and skeletal muscle. However, kidney possesses a higher level of linoleic acid which is in contrast to the higher degree of saturation of the fatty acids of the triglyceride fraction of this tissue. The distribution

of the C_{18} acids of the free fatty acid fraction of depot fat, heart, and skeletal muscle is also similar, but there is a higher content of stearic acid and linoleic acid and a lower oleic acid content in kidney tissue. Also in the phospholipid fraction of these tissues linoleic acid is present to a greater extent than in the other fractions, especially in heart tissue, where it is present as the major component. In general, the phospholipid

TABLE 3

FATTY ACID COMPOSITION OF OVINE KIDNEY LIPIDS

Values for each component refer to the percentage by weight of the total fatty acid methyl esters in each fraction. 'Trace' means that the amount of fatty acid present is less than 0·5%

Fatty Acid	Cholesterol Esters	Triglycerides	Free Fatty Acids	Phospholipids
12 : 0	0·6	Trace	Trace	Trace
14 (br.)*	Trace	"	"	"
14 : 0	0·6	"	"	"
14 : 1	Trace	"	"	"
15 (br.)	Trace	"	"	"
15 : 0	0·9	"	"	"
15 : 1	Trace	"	"	"
A?	"	"	0·7	3·0
16 (br.)	"	"	Trace	Trace
16 : 0	24·1	15·0	20·4	15·1
16 : 1	1·6	0·8	1·6	0·9
17 (br.)	Trace	Trace	Trace	Trace
17 : 0	1·5	0·9	0·7	1·1
16 : 2	Trace	Trace	Trace	Trace
B?	"	"	"	0·7
18 (br.)	"	"	"	Trace
18 : 0	24·1	40·0	35·6	24·5
18 : 1	29·7	34·1	13·5	24·5
18 : 2	8·6	3·2	14·4	12·2
18 : 2?	Trace	Trace	Trace	Trace
18 : 3	1·5	0·8	3·3	2·0
20 : 0	1·0	Trace	Trace	Trace
20 : 1	Trace	Trace	Trace	Trace
20 : 2	"	"	"	Trace
21 : 0	"	"	"	2·6
20 : 4	3·8	2·1	3·8	10·2
22 : 0	1·1	0·9	1·7	2·1

* br. = singly methyl branched chain.

fractions of all these tissues contain fatty acids with a higher degree of unsaturation than in other tissues; the cholesterol ester fractions of kidney and heart tissue are more unsaturated than the corresponding triglyceride fraction; whilst skeletal muscle and depot fat show a constant amount of saturation in all fractions except the phospholipid fraction.

There is little arachidonic acid in depot fat or in the triglyceride fraction of the other tissues; but it is a considerable component of the phospolipid and free fatty acid fractions of heart, kidney, and skeletal muscle, and in the cholesterol ester fraction of kidney.

TABLE 4

FATTY ACID COMPOSITION OF OVINE MUSCLE LIPIDS

Values for each component refer to the percentage by weight of the total fatty acid methyl esters in each fraction. 'Trace' means that the amount of fatty acid present is less than 0·5%

Fatty Acid	Cholesterol Esters	Triglycerides	Free Fatty Acids	Phospholipids
12 : 0	Trace	Trace	Trace	Trace
14 (br.)*	Trace	Trace	„	Trace
14 : 0	2·4	1·6	„	0·8
14 : 1	Trace	Trace	„	Trace
15 (br.)	Trace	Trace	„	„
15 : 0	0·7	0·5	„	„
15 : 1	Trace	Trace	„	„
A?	„	„	„	3·8
16 (br.)	„	„	„	Trace
16 : 0	24·4	29·7	20·3	13·3
16 : 1	2·0	2·2	3·3	1·4
17 (br.)	Trace	Trace	Trace	Trace
17 : 0	1·1	1·1	2·0	0·7
16 : 2	Trace	Trace	Trace	Trace
B?	„	„	„	4·3
18 (br.)	„	„	„	Trace
18 : 0	21·2	20·8	21·0	16·3
18 : 1	40·5	38·2	39·3	31·2
18 : 2	3·6	3·7	3·5	11·3
18 : 2?	Trace	Trace	Trace	Trace
18 : 3	1·0	0·8	0·7	4·4
20 : 0	0·6	Trace	Trace	Trace
20 : 1	Trace	Trace	Trace	Trace
20 : 2	„	„	Trace	Trace
21 : 0	„	„	1·0	0·9
20 : 4	0·8	„	3·9	6·2
22 : 0	Trace	„	2·0	3·5

* br. = singly methyl branched chain.

Of the two unidentified fatty acid components (A?; B?), the first (A?) is usually present in the greater quantity and reaches its maximum concentration in cardiac phospholipid. The second of these acids (B?) has its highest concentration in skeletal muscle. Trace quantities of these fatty acids appear in other fractions and other tissues.

Myristic acid only appears as a major component in depot fat (particularly triglyceride) and the other minor and trace components have a fairly general distribution. Depot fat differs from the other tissues, though, in possessing little fatty acid of chain length longer than C_{18}.

TABLE 5

FATTY ACID COMPOSITION OF OVINE DEPOT FAT LIPIDS

Values for each component refer to the percentage by weight of the total fatty acid methyl esters in each fraction. 'Trace' means that the amount of fatty acid present is less than 0·5%

Fatty Acid	Cholesterol Esters	Triglycerides	Free Fatty Acids	Phospholipids
12 : 0	Trace	Trace	Trace	Trace
14 (br.)*	Trace	Trace	Trace	Trace
14 : 0	2·9	17·5	3·5	2·7
14 : 1	Trace	Trace	Trace	Trace
15 (br.)	Trace	1·2	Trace	„
15 : 0	0·7	6·0	0·8	„
15 : 1	Trace	Trace	Trace	„
A?	—	—	—	„
16 (br.)	Trace	Trace	Trace	„
16 : 0	18·2	33·8	22·1	29·6
16 : 1	Trace	3·4	3·6	3·0
17 (br.)	Trace	0·6	Trace	Trace
17 : 0	0·6	1·0	1·3	„
16 : 2	Trace	Trace	Trace	„
B?	—	—	—	„
18 (br.)	Trace	Trace	Trace	„
18 : 0	26·2	12·1	23·1	19·0
18 : 1	42·5	19·0	34·5	35·3
18 : 2	4·4	1·4	4·3	6·3
18 : 2?	Trace	Trace	Trace	Trace
18 : 3	1·4	0·5	3·0	2·8
20 : 0	Trace	Trace	0·7	0·6
20 : 1	Trace	Trace	Trace	Trace
20 : 2	„	„	„	„
21 : 0	„	„	„	„
20 : 4	0·6	„	0·5	0·6
22 : 0	Trace	„	Trace	Trace

* br. = singly methyl branched chain.

IV. DISCUSSION

Although a number of workers have reported partial fractionations of the lipids from extrahepatic tissues (Bloor 1943; Williams *et al.* 1945; Futter and Shorland 1957; Kochen, Marinetti, and Stotz 1960), the separation of these lipids into cholesterol ester, triglyceride, free fatty acid, and phospholipid fractions does

not appear in the available literature. It is suggested that this more complete fractionation, when combined with an analysis of the constituent fatty acids (Tables 1–5), constitutes a more precise and useful basis for metabolic studies than the usual treatment of lipid as an entity, or fractionation on the basis of acetone solubility (Lis, Tinoco, and Okey 1961; Masters 1963*a*, 1963*b*). The appreciable quantities present in all the lipid fractions (Table 1), the different metabolic significance of each of these lipid groups (Deuel 1955), and the difference in fatty acid composition of the individual fractions (Tables 2–5) all support this proposition.

The high percentages of phospholipid in ovine extrahepatic tissues, and relative values between these different sources (Table 1), are in agreement with previous investigations of other species (Deuel 1955), but comparative values are not available for the other lipid fractions.

With regard to fatty acid composition, depot fat is the most extensively investigated of these tissues, and a number of interspecies comparisons have been made in this respect (Garton 1961). Results in this paper (Table 5) agree with previous work in that they exemplify the lack of long-chain fatty acids ($>C_{18}$), the high saturation, and the unusual fatty acids characteristic of ruminant depot fats (Hilditch and Pedelty 1941; Garton 1961). In the triglyceride fraction of ovine depot fat, however, higher values have been obtained for myristic and palmitic acids and lower values for stearic and oleic acids. These differences may be caused partly by the different fractionation procedures, by the different tissue sites, or by other variables such as age, sex, breed, and nutritional history.

In respect of other tissues investigated, there is little basis for interspecies comparisons. Whereas Futter and Shorland (1957) reported that the fatty acid compositions of rabbit kidney and muscle broadly resembled one another and that of rabbit liver, there is quite extensive dissimilarity between these tissues in the sheep (Tables 1–4; Horgan and Masters 1963). As a whole, rumen hydrogenation is reflected in the low percentages of long-chain polyunsaturated fatty acid components, and the comparatively high saturation of ovine tissue cholesterol esters (Getz *et al.* 1962; Duncan and Garton 1962). Phospholipid is the most highly saturated fraction of ovine liver lipids, but the least saturated fraction in ovine heart, kidney, and muscle.

A further point of interest here is the occurrence of two unusual major fatty acid components (A and B) in the phospholipid of extrahepatic tissues (Tables 2–4). The appreciable concentration and uncommon localization of these acids may be significant and the distribution and interrelationships of these compounds are being further investigated.

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