these changes are shown in Tables I and II. The percentage of oleic acid in the TG fraction increased at peak lactation (14) days and was at a maximum during involution (Table I). The percentage of linoleic acid increased during late pregnancy, but then declined markedly during peak lactation and involution. Although the concentration of lipid phosphorus did not change during pregnancy and lactation, there was an increase in the percentage of palmitic acid in the PL fraction and a decrease in the percentages of stearic and arachidonic acids (Table II).

In the period immediately before parturition, there were considerable changes in lipid metabolism in the rat. These included an abrupt fall in lipoprotein lipase activity in adipose tissue and an increase in plasma TG levels (9). Furthermore, food intake was greater and there was an increase in the synthesis of fatty acids in the liver (10). The accumulation of TG in the liver at this time may have been related to these changes. The concentration of TG in the liver remained high in early lactation, but plasma TG fell rapidly soon after parturition, probably due to a rapid increase in lipoprotein lipase activity in mammary tissue which allowed it to take up TG for milk production (9). Although food intake and the synthesis of fatty acids in the liver were even higher during lactation (10), the values for the concentration of TG in both plasma and liver were low at peak lactation (9, Table I), and probably reflected a rapid turnover of plasma TG and their uptake by the mammary gland. At weaning there was a rapid fall in mammary lipoprotein lipase activity (9) which gave rise to hypertriglyceridemia and the accumulation of TG in the liver.

The changes in fatty acid composition may be a further consequence of these changes in lipid metabolism. McKay and Kaunitz (3) attributed the changes in pregnant rats to a mobilization of fat depots and an increase in the extra mitochondrial synthesis of fatty acids relative to mitochondrial transformations to stearic and arachidonic acids. These factors may have been important in the lactating rat also. It seems relevant also to draw attention to the similarity between the changes in fatty acid composition that occurred during lactation and those observed after feeding fat deficient diets (11). In both cases, the changes coincided with an increase in hepatic lipogenesis. As the animals used in the present work received a nutritionally adequate diet ad libitum, the importance of this similarity remains to be established.

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receiving protected soybean oil supplement was virtually indistinguishable from that from nonruminants.

**INTRODUCTION**

Phosphatidyl choline constitutes the principal glycerolipid in bile of all mammalian species examined to date. In ruminants (1), fatty acid composition of bile phosphatidyl choline is characterized by low levels of linoleic acid and its longer chain metabolites. It also is characterized by appreciable amounts of polyunsaturated fatty acids not normally found in phospholipids in quantity, such as linolenic acid and certain conjugated fatty acids that are intermediates in the ruminal biohydrogenation of linoleic and linolenic acids. Bile phosphatidyl choline from nonruminant species, e.g., the pig (1), rat, dog, and man (2), however, contain linoleic acid as the major unsaturated fatty acid component. In this study, the composition of bile phosphatidyl choline has been examined in sheep fed control diets or diets containing unprotected maize oil, or tallow or soybean oil protected by formaldehyde or treated protein against biohydrogenation by rumen microorganisms (3).

**MATERIALS AND METHODS**

Four groups of 5 6-month-old Cheviot lambs were fed either a control diet of hay and concentrates or diets in which a proportion of the concentrates, i.e., high energy cereals such as rolled oats or flaked maize, was replaced either by protected soybean oil (3), protected tallow (3), or by unprotected maize oil (30 g/day of supplementary fat or 12% or total calories). The total fat intake of lambs on the control diet was 12 g/day, and on the fat supplemented diets, it was 40 g/day. The fats protected with formaldehyde-treated protein were donated by Alta Lipids UK Ltd. (London, England). Animals were maintained on the diets for 6 weeks when they were killed and the gall bladders removed. The gall bladder contents were acidified to pH 4 with concentrated HCl before being extracted with chloroform:methanol (2:1, v/v). Phosphatidyl choline was isolated by silicic acid column chromatography followed by preparative thin layer chromatography (TLC) as described earlier (1). The methyl ester derivatives, prepared by sodium methoxide catalyzed methanolation, were separated by gas liquid chromatography (GLC) on packed columns of EGSS-X, and were identified by their retention times relative to authentic standards and by silver nitrate TLC (1). trans-Monoenoic components were estimated by GLC after separation along with saturated components by TLC on silica gel layers impregnated with silver nitrate (10% by wt); hexane:diethyl ether (9:1, v/v) was the developing solvent. Phosphatidyl choline was converted to diglycerides by phospholipase C hydrolysis and acetylated prior to separation into molecular species by silver nitrate TLC by methods described in detail elsewhere (1).

**RESULTS AND DISCUSSION**

The yield of phosphatidyl choline from bile of sheep on the 4 diets did not vary significantly, and averaged 0.342 g/100 ml bile, an amount only one-third of the level in more mature animals (1). Fatty acid compositions of bile phosphatidyl cholines are listed in Table I. In that of the control animals, 16:0, 18:0, and 18:1 (cis) were the major components, along with an appreciable amount of 18:2 (n-6) and considerably more 20:4 (n-6) than was detected in the same lipid from more mature sheep (1). Also, 18:3 (n-3) and fatty acids containing conjugated double bond systems, which were major components in the bile of the mature animals, were only present in trace amounts in the younger animals used in this study. The reason for this effect was probably because the mature sheep in the earlier work were pasture fed, whereas the younger ones in this work received hay concentrate diets; the latter have much higher 18:2 contents relative to 18:3 than fresh grass (4).

Not unexpectedly, the composition of bile phosphatidyl choline from sheep receiving the protected tallow supplement did not differ markedly from that of the control animals, and, indeed, the only significant difference was a drop in the concentration of 16:1. This fatty acid is often present in higher concentrations in the tissues of animals on low fat diets than in those receiving high fat diets (5). With sheep receiving the protected soybean oil supplement, on the other hand, there was a greater than 3-fold increase in the concentration of linoleic acid in the bile phosphatidyl choline entirely at the expense of the cis-monoenoic acids. Indeed, the fatty acid composition in this instance was very similar to that of bile phosphatidyl choline from nonruminant animals. Bile phosphatidyl choline from sheep fed unprotected maize oil supplement contained twice as much 18:2 (n-6) as that of controls, again at the expense of the cis-monoenoic fatty acids. Therefore, an appreciable portion of the linoleic acid in the maize oil supplement must have escaped biohydrogenation. With none of the fat supplements were the levels of the longer chain metabolites of
<table>
<thead>
<tr>
<th>Dietary fat supplement</th>
<th>16:0</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1 cis</th>
<th>18:1 trans</th>
<th>18:2 (n-6)</th>
<th>18:2 conja</th>
<th>20:4</th>
<th>otherb</th>
<th>Amount of phosphatidyl cholinec (g/100 g bile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>25.9</td>
<td>3.3</td>
<td>15.9</td>
<td>29.8</td>
<td>1.6</td>
<td>8.9</td>
<td>1.2</td>
<td>6.9</td>
<td>6.5</td>
<td>0.451</td>
</tr>
<tr>
<td>±4.6</td>
<td>±0.7</td>
<td>±3.8</td>
<td>±5.6</td>
<td>±0.8</td>
<td>±3.2</td>
<td>±0.5</td>
<td>±1.7</td>
<td></td>
<td></td>
<td>±0.221</td>
</tr>
<tr>
<td>Protected tallow</td>
<td>26.1</td>
<td>1.5e</td>
<td>17.1</td>
<td>24.6</td>
<td>1.1</td>
<td>10.3</td>
<td>0.9</td>
<td>10.2</td>
<td>8.2</td>
<td>0.185</td>
</tr>
<tr>
<td>±3.7</td>
<td>±0.2</td>
<td>±1.4</td>
<td>±3.3</td>
<td>±0.5</td>
<td>±0.9</td>
<td>±0.5</td>
<td>±4.0</td>
<td></td>
<td></td>
<td>±0.073</td>
</tr>
<tr>
<td>Protected soybean oil</td>
<td>22.7</td>
<td>1.4e</td>
<td>17.7</td>
<td>14.3d</td>
<td>1.4</td>
<td>28.1e</td>
<td>0.6</td>
<td>9.4</td>
<td>4.4</td>
<td>0.344</td>
</tr>
<tr>
<td>±2.1</td>
<td>±0.3</td>
<td>±1.4</td>
<td>±4.8</td>
<td>±0.6</td>
<td>±6.0</td>
<td>±0.2</td>
<td>±1.8</td>
<td></td>
<td></td>
<td>±0.123</td>
</tr>
<tr>
<td>Unprotected maize oil</td>
<td>24.6</td>
<td>1.7e</td>
<td>17.7</td>
<td>18.6</td>
<td>2.5</td>
<td>19.5</td>
<td>1.6</td>
<td>8.3</td>
<td>5.5</td>
<td>0.388</td>
</tr>
<tr>
<td>±4.1</td>
<td>±0.7</td>
<td>±3.9</td>
<td>±5.6</td>
<td>±0.7</td>
<td>±4.7</td>
<td>±0.6</td>
<td>±2.4</td>
<td></td>
<td></td>
<td>±0.172</td>
</tr>
</tbody>
</table>

\(a\) 9-cis, 11-trans-Octadecadienoic acid.

\(b\) Including 18:3 (n-6), 18:3 (n-3), cis-9, trans-11, cis-15-octadecatrienoic acid, 20:3 (n-6), 20:4 (n-3), 22:3 (n-6), 22:4 (n-6), 22:5 (n-3) and 22:6 (n-3).

\(c\) Mean ± SD of 5 animals.

\(d\) Significantly (P<0.01) different from control group.

\(e\) Significantly (P<0.001) different from control group.
TABLE II
Variation in Proportions (% of the Principal Molecular Species in Bile Phosphatidyl Cholines with Dietary Treatment

<table>
<thead>
<tr>
<th>Dietary fat supplement</th>
<th>Saturated-saturated</th>
<th>Saturated-monoenoic</th>
<th>Monoenoic-monoenoic</th>
<th>Saturated-dienoic</th>
<th>Monoenoic-dienoic</th>
<th>Polyenoic$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protected tallow</td>
<td>2.4</td>
<td>44.0</td>
<td>5.1</td>
<td>19.7</td>
<td>4.1</td>
<td>24.7</td>
</tr>
<tr>
<td>Protected soybean oil</td>
<td>1.6</td>
<td>23.6</td>
<td>3.3</td>
<td>43.2</td>
<td>8.1</td>
<td>20.2</td>
</tr>
<tr>
<td>None (control)</td>
<td>3.4</td>
<td>52.6</td>
<td>7.6</td>
<td>12.2</td>
<td>3.1</td>
<td>21.1</td>
</tr>
<tr>
<td>Unprotected maize oil</td>
<td>1.5</td>
<td>34.0</td>
<td>4.9</td>
<td>32.0</td>
<td>4.4</td>
<td>23.2</td>
</tr>
</tbody>
</table>

$^a$Three or more double bonds.

linoleic acid, in bile, such as 20:4 (n-6), altered significantly.

Phosphatidyl cholines from the bile of sheep in each group were pooled and separated into molecular species, in the form of diglyceride acetate derivatives by means of silver nitrate chromatography. The results are listed in Table II. More than half of the control phosphatidyl choline consisted of the molecular species containing one saturated and one monoenoic fatty acid (SM). In bile phosphatidyl choline from sheep on fat supplemented diets, this species tended to decrease and was replaced largely by the molecular species containing one saturated and one dienoic fatty acid (SD). In that from the sheep receiving the protected soybean oil supplement, for example, the proportion of the SM fraction was only half that of the control, while the proportion of the SD fraction was more than 3 times that of the control. Although the amounts of the molecular species from the 4 groups varied markedly, fatty acid compositions of corresponding fractions were very similar.

These results provided confirmation of the suggestion (1) that the principal structural requirement of phosphatidyl choline for its detergent function in bile is that it be liquid, for which purpose it contains in one position, probably position 2, those polyunsaturated fatty acids most readily available or those not required for more essential purposes. In the normal pasture fed ruminant, linoleic acid and related metabolites are in short supply because of biohydrogenation. The animal then utilizes any available linolenic acid, the essential status of which has been questioned (6), and conjugated unsaturated fatty acids for the synthesis of bile phosphatidyl choline. In nonruminants on an adequate diet, tissue concentration of linoleic acid would not be limiting, and this is the polyunsaturated fatty acid most readily available for the purpose. However, it was clear that when the supply of linoleic acid to ruminant tissues was not limited, as with the sheep given the protected soybean oil supplement, the biliary phosphatidyl choline was almost indistinguishable in composition from that of nonruminants.

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