COMPARISON OF LINOLEIC ACID AND STEARIC ACID ABSORPTION BY THE GALLBLADDER

DEWEY H. NEIDERHISER and CLIFFORD K. HARMON

Medical Research Service, Veterans Administration Hospital and The Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106 (U.S.A.)

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Summary

The absorption of [14C]linoleic acid and [14C]stearic acid in guinea pig bile by the in situ guinea pig gallbladder was compared. Linoleic acid was adsorbed at a faster rate than was stearic acid. Differences were also observed in the incorporation of these two fatty acids into complex lipids of the gallbladder mucosa. A greater portion of adsorbed linoleic acid was incorporated into triacylglycerol whereas a greater portion of stearic acid was incorporated into sphingomyelin. The significance of these findings in relation to the fatty acid composition of bile is discussed.

Introduction

The role of the gallbladder in gallstone formation has been the subject of several studies [1-4]. Swell et al. [2] suggested that the absence of the gallbladder results in a decreased bile salt pool which may lead to supersaturation of the bile with cholesterol. We have studied lipid absorption by the gallbladder [3,4] for its possible implications in cholesterol gallstone formation. Bile salts and phosphatidylcholine, the major phospholipid found in bile, solubilize cholesterol by a micellar mechanism [5]. Selective absorption of these lipids from a bile that is saturated with cholesterol could lead to a supersaturated state. Studies on the absorption of bile salts show that they are not absorbed [6] and that phosphatidylcholine is adsorbed only slightly [4]. However, we found that oleic acid (18 : 1) and lysophosphatidylcholine, the hydrolytic products released from bile phosphatidylcholine through the action of phospholipase A₂, are readily absorbed by the gallbladder [3,4]. In acute cholecystitis, lysophosphatidylcholine has been demonstrated in human bile [7]. The major portion of the oleic acid absorbed by the gallbladder is transported from the gallbladder and metabolized to CO₂ [3]; whereas, the major portion of ab-
sorbed lysophosphatidylcholine remains in the gallbladder wall and is converted back to phosphatidylcholine. One enzyme that we have found in gallbladder mucosa that catalyzes this reaction is lysophosphatidylcholine acyltransferase [4,8].

In man, 20–30% of the total fatty acids in bile phosphatidylcholine are present as linoleic acid (18:2) and 4 to 5% as stearic acid (18:0) [9]. Linoleic acid would be released from bile phosphatidylcholine through the action of phospholipase A₂ while stearic acid would be released through phospholipase A₁. Further, increased concentrations of stearic acid have been reported from gallbladder bile of patients with biliary tract obstruction [10]. Therefore, we compared the absorption of these two fatty acids by the gallbladder. Differences in the degree of unsaturation (carbon-carbon double bond) have previously been shown to affect the overall absorption of fatty acids by the intestine [11]. In the present study, we observed marked differences in both the overall absorption and metabolism of the saturated fatty acid, stearic acid, and the di-unsaturated fatty acid, linoleic acid, by the situ guinea pig gallbladder.

Materials and Methods

[1-14C]linoleic acid (50 Ci/mol) and [1-14C]stearic acid (10.9 Ci/mol) were purchased from New England Nuclear, Boston, Mass. They were purified prior to use by thin layer chromatography on silica gel G plates (Analtech, Wilmington, Del.) with the solvent system containing light petroleum/diethyl ether/acetic acid (80 : 20 : 1, by vol.) [12]. Carrier stearic acid and carrier linoleic acid (Applied Science Labs., State College, Pa.) were used without further purification. After methylation [13], gas liquid chromatography showed single peaks corresponding to standard stearic or linoleic acid and no detectable contaminating fatty acids were found. Quantification using heptadecanoic acid as the internal standard showed that each fatty acid contained more than 95% stearic or linoleic acid. Solutions of carrier and radioactive fatty acid were stored separately in chloroform at −10°C.

Radioactivity was measured in a scintillation solution which contained Cab-O-Sil/toluene/ethanol and BBOT as previously described [3]. Corrections for quenching were made by channel ratio determinations and data are expressed in disintegrations per minute (dpm). Phospholipid phosphorus and triacylglycerol in the gallbladder extracts after separation by thin layer chromatography were assayed as previously described [3,4]. The total bile acid [14], phosphatidylcholine [4] and cholesterol [4] content of bile were measured as indicated. The solvent system used to separate phospholipids contained chloroform/methanol/water (65 : 25 : 4, by vol.) [15].

Male guinea pigs (Beaumanor Farms, Aurora, Ohio) weighing 250–300 g were used in all experiments. All animals were fasted for 24 h prior to surgery. Guinea pig hepatic bile and gallbladder bile were collected as previously described [3,16] and stored separately at −10°C. The bile used for in situ experiments was a mixture of hepatic and gallbladder bile which contained 7.2 ± 0.2 μmol/ml total bile acid, 27 ± 3 nmol/ml phosphatidylcholine and 8.2 ± 1.1 nmol/ml cholesterol. The bile was rendered aseptic by passage through a 0.45 μm Millipore filter. The appropriate amount of radioactive fatty acid (usually
400 000–500 000 dpm) was mixed with carrier fatty acid, usually 1 μmol. After removal of the chloroform, bile was added to make a final concentration of 1 μmol of fatty acid per ml of bile. To these solutions, 100 μg/ml of bromosulfophthalein, a dye that is not absorbed by the gallbladder [3,4,16] was added.

The in situ guinea pig gallbladders were prepared as previously described [3,4]. The cystic duct was separated from the blood vessels and cannulated. After removal of the bile from the gallbladder, 1 ml of the appropriate bile solution was instilled through a cannula, the cannula was then removed and the cystic duct tied off. The guinea pig was placed in a metabolic chamber and expired CO₂ was collected in 2 M NaOH. The 0–12 h animals were fasted during the experimental period, whereas for the 24-h animals, food and water were placed in the metabolic chamber. The animals remained anesthetized for 6–8 h after surgery. At the scheduled time (0–24 h after surgery) the guinea pig was killed and the gallbladder and liver were excised. The lumen of the gallbladder was exhaustively washed four times with 0.5 ml of 1% albumin in Krebs-Ringer phosphate buffer, pH 7.2, and the combined washes were made to 5 ml. In some experiments, the gallbladder was washed with Krebs-Ringer phosphate buffer alone. The washed gallbladder was extracted with boiling chloroform/methanol (2:1) and the combined extracts were made to 4 ml. Aliquots of the gallbladder wash, gallbladder extract, expired CO₂ in 2 M NaOH and urine were measured for radioactivity. The liver was homogenized and made to 50 ml with water and the carcass was homogenized in 500 ml of water and aliquots were radioassayed as previously described [3,4]. The recovery of the non-absorbable marker, bromosulfophthalein, in the wash was measured spectrophotometrically [17].

In certain experiments, blood was collected from gallbladder vein after instillation of 1.2 μCi of [14C]linoleic acid (0.1 μmol in 1 ml of bile) and serum was processed as previously described [3]. For these experiments, the bile plus [14C]linoleic acid was instilled into the gallbladder for 15 min. A vein leading from the gallbladder to the liver was then severed and blood was collected from this vein in microcapillary tubes for 30 min. Radioactivity was measured in 10 μl aliquot of the serum. The serum proteins were separated by electrophoresis on cellulose acetate strips and the radioactivity in the protein bands (stained with bromphenol blue) was measured. The serum proteins were also separated by electrophoresis on 7% polyacrylamide gels. Protein bands were indicated by either Amido-schwarz stain with subsequent destaining or with commassie blue, without destaining (destaining removed radioactivity from the gels). Protein bands were sliced into 1-mm sections and the distribution of radioactivity within the bands was measured.

Aliquots of the gallbladder extract, usually 0.1 ml, were analyzed by thin layer chromatography with the solvent systems described above and the areas corresponding to standard triacylglycerol, phosphatidylcholine, phosphatidylethanolamine, sphingomyelin and fatty acid were assayed for radioactivity. In certain experiments, areas were also analyzed for phospholipid phosphorus or triacylglycerol.

The following criteria were used to determine whether an experiment was satisfactory. First, over 90% of the non-absorbable marker, bromosulfophthal-
ein, was recovered for all animals (0–24 h). Second, the instilled bile solution was concentrated by the gallbladder from 1 ml to less than 0.4 ml for the 6, 12, and 24-h animals. For the 1–4 h animals, some concentration occurred; however, a specific change in volume was not used as a criterium. Third, when examined histologically, the gallbladder mucosa and lamina propria were intact and the mucosa was assessed as normal when examined independently by a pathologist using criteria previously established [3,4,16,18].

Results

The portion of the diunsaturated fatty acid, linoleic acid, absorbed from bile instilled into the guinea pig gallbladder for 6 h was markedly greater than that observed for the saturated fatty acid, stearic acid (Tables I and II). After instillation of 1 μmol of [14C]linoleic acid in 1 ml of bile, 5.0 ± 3.2% of the radioactivity was recovered in the gallbladder lumen 6 h later (Table 1). After instillation of the same quantity of [14C]stearic acid for 6 h, 65.9 ± 5.9% of the radioactivity was recovered in the gallbladder lumen. The radioactivity which disappeared from the gallbladder lumen was recovered in the gallbladder wall, expired CO₂, liver, urine and carcass for both the linoleic acid and stearic acid animals. Our findings on linoleic acid uptake by the gallbladder are similar to those observed previously for oleic acid uptake [3].

The relative recoveries of [14C]linoleic acid and [14C]stearic acid in the guinea pig gallbladder contents and respective [14C]CO₂ formation at various intervals over a 24-h period are presented in Table II. For all the animals studied, the recovery of the non-absorbable marker was greater than 90%. The data clearly show that the overall absorption and metabolism to CO₂ of stearic acid is less rapid than that of linoleic acid. 3-h after instillation, 90% of the [14C]linoleic acid had been absorbed by the gallbladder whereas only 12% of the [14C]stearic acid had been absorbed. At the end of the 24-h interval, the

| TABLE I |
| DISTRIBUION OF RADIOACTIVITY 6 h AFTER INSTILLATION OF [1-14C]LINOLEIC ACID OR [1-14C]STEARIC ACID INTO THE IN SITU GUINEA PIG GALLBLADDER |

1 μmol of [14C]linoleic or [14C]stearic acid (400000–500000 dpm) in 1 ml of guinea pig bile containing bromosulfophthalein (100 μg/ml) was instilled into the gallbladder for 6 h. Values represent mean percent recovery of radioactivity ± S.D.; there were 6 animals in each group.

<table>
<thead>
<tr>
<th></th>
<th>[1-14C]linoleic acid a (% of instilled)</th>
<th>[1-14C]stearic acid b (% of instilled)</th>
<th>[1-14C]oleic acid c (% of instilled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallbladder contents</td>
<td>5.0 ± 3.2</td>
<td>65.9 ± 5.9</td>
<td>5.5 ± 3.4</td>
</tr>
<tr>
<td>Gallbladder wall</td>
<td>16.6 ± 4.0</td>
<td>7.2 ± 1.2</td>
<td>23.2 ± 6.2</td>
</tr>
<tr>
<td>Expired CO₂</td>
<td>32.9 ± 3.6</td>
<td>14.9 ± 3.2</td>
<td>35.2 ± 6.6</td>
</tr>
<tr>
<td>Carcass</td>
<td>25.7 ± 6.3</td>
<td>8.5 ± 3.6</td>
<td>19.1 ± 2.5</td>
</tr>
<tr>
<td>Liver</td>
<td>18.3 ± 7.8</td>
<td>4.3 ± 2.0</td>
<td>9.8 ± 3.3</td>
</tr>
<tr>
<td>Urine</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total Recovery</td>
<td>99.7 ± 5.8</td>
<td>100.9 ± 4.8</td>
<td>93.2 ± 4.0</td>
</tr>
</tbody>
</table>

a Bromosulfophthalein recovery was 95 ± 3% (91–99%).

b Bromosulfophthalein recovery was 96 ± 4% (92–100%).

c Data taken from previous study (see ref. 3).
TABLE II

DISTRIBUTION OF RADIOACTIVITY BETWEEN GALLBLADDER CONTENTS AND EXPIRED CO₂
AFTER INSTILLATION OF [1-¹⁴C]LINOLEIC OR [1-¹⁴C]STEARIC ACID INTO THE IN SITU
GUINEA PIG GALLBLADDER

1 µmol of [¹⁴C]linoleic acid or [¹⁴C]stearic acid (400000–500000 dpm) in 1 ml of guinea pig bile containing bromosulfophthalein (100 µg/ml) was instilled into the gallbladder at 0 time. Values represent mean ± S.D., there were 3 animals in each group.

<table>
<thead>
<tr>
<th>Hours after instillation</th>
<th>% of instilled</th>
<th>Expired CO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gallbladder contents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Linoleic</td>
<td>Stearic</td>
</tr>
<tr>
<td>1</td>
<td>48.9 ± 3.7</td>
<td>94.7 ± 1.6</td>
</tr>
<tr>
<td>2</td>
<td>37.4 ± 7.7</td>
<td>93.2 ± 5.0</td>
</tr>
<tr>
<td>3</td>
<td>8.6 ± 4.5</td>
<td>88.5 ± 4.8</td>
</tr>
<tr>
<td>4</td>
<td>5.8 ± 3.8</td>
<td>81.1 ± 4.0</td>
</tr>
<tr>
<td>6</td>
<td>5.0 ± 3.2</td>
<td>65.9 ± 5.9</td>
</tr>
<tr>
<td>12</td>
<td>1.4 ± 0.4</td>
<td>15.9 ± 2.5</td>
</tr>
<tr>
<td>24</td>
<td>1.8 ± 0.6</td>
<td>9.8 ± 4.7</td>
</tr>
</tbody>
</table>

percent of radioactivity remaining in the gallbladder lumen for the stearic acid group (9.8 ± 4.7%) approached that of the linoleic acid group (1.8 ± 0.6%). Similarly, the amount of [¹⁴C]CO₂ recovered at each interval was lower for the stearic acid group than for the linoleic group. At the end of the 24-h interval, the total amount of instilled radioactivity recovered as [¹⁴C]CO₂ for the stearic acid group (57.5 ± 6.3%) approached that for the linoleic acid group (63.5 ± 4.6%).

Samples of venous blood were obtained from gallbladders from 4 different animals after instillation of 1.2 µCi of [¹⁴C]linoleic acid. We found 1372 (721–2049) dpm per 10 µl of serum. When the proteins in a 10-µl aliquot of this serum were separated by cellulose acetate electrophoresis, all of the radioactivity migrated with the albumin fraction. When separated by disc gel electrophoresis, the radioactivity was found to be homogenously distributed throughout the albumin fraction. After separation of the lipids by thin layer chromatography with the solvent system containing chloroform/methanol/water (65 : 25 : 4, by vol.) all of the radioactivity migrated near the solvent front. When separated with the solvent system containing light petroleum/diethyl ether/acetic acid (80 : 20 : 1, by vol.), over 80% of the radioactivity migrated with unesterified fatty acid.

6 h after instillation of 1 µmol [¹⁴C]linoleic acid, 16.6 ± 4% of the radioactivity was recovered in the gallbladder wall, whereas after instillation of the same quantity of [¹⁴C]stearic acid, 7.2 ± 1.2% of the radioactivity was in the gallbladder wall (Table I). The distribution of this radioactivity recovered in various lipid classes extracted from the gallbladder wall is presented in Table III. For the linoleic-acid instilled animals, significantly more of the radioactivity (49.6 ± 3.8%) was present in the triacylglycerol than for the stearic acid instilled animals (23.2 ± 6.6%). On the other hand, the stearic-acid instilled animals incorporated a significantly greater portion of the radioactivity into sphingomyelins (10.9 ± 1.0%) and unesterified fatty acids (10.4 ± 4.5%) than for the linoleic acid instilled animals (<3% for both sphingomyelin and un-
### TABLE III

**DISTRIBUTION OF RADIOACTIVITY IN LIPIDS EXTRACTED FROM GALLBLADDER WALL 6 h AFTER INSTILLATION OF [\(^{14}\text{C}\)]LINOLEIC OR [\(^{14}\text{C}\)]-STEARIC ACID**

1 µmol of [\(^{14}\text{C}\)]linoleic or [\(^{14}\text{C}\)]stearic acid (400000—500000 dpm) in 1 ml of guinea pig bile containing bromosulfophthalein (100 µg/ml) was instilled into the gallbladder for 6 h. Values represent mean ± S.D.; there were 6 animals in each group. N.S., not significant.

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Radioactivity in lipid class</th>
<th>Amount of lipid class</th>
<th>Specific activity of lipid class</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Linoleic (%)</td>
<td>Stearic (%)</td>
<td>Linoleic (µmol)</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>49.6 ± 3.8</td>
<td>23.2 ± 6.6</td>
<td>0.53 ± 0.31</td>
</tr>
<tr>
<td>Phosphatidylcholine</td>
<td>26.8 ± 2.9</td>
<td>22.4 ± 5.5</td>
<td>0.59 ± 0.20</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>10.2 ± 0.7</td>
<td>14.2 ± 2.9</td>
<td>0.50 ± 0.07</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>2.5 ± 0.6</td>
<td>10.9 ± 1.0</td>
<td>0.26 ± 0.10</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>2.1 ± 0.5</td>
<td>10.4 ± 4.5</td>
<td>—</td>
</tr>
</tbody>
</table>

a The gallbladder wall contained 16.6 ± 4.0% of the radioactivity instilled (Table I).

b The gallbladder wall contained 7.2 ± 1.2% of the radioactivity instilled (Table I).

c Determined by the Student's *t* test.

d The quantity of unesterified fatty acid was too small to accurately measure by quantitative gas liquid chromatography.
esterified fatty acid). The total quantities and the specific activities of the lipid classes found in the gallbladder wall are also given in Table III. No statistically significant difference was found for the amounts (µmol) of triacylglycerol, phosphatidylcholine, phosphatidylethanolamine and sphingomyelin between the linoleic-acid instilled animals and the stearic acid instilled. *P* values in all cases were greater than 0.2. The quantity of unesterified fatty acid in an 0.1-ml aliquot of the gallbladder extract was too small (<1.0 nmol per 0.1 ml of extract) to measure by quantitative gas-liquid chromatography with a flame ionization detector and therefore the specific activity of the fatty acid portion could not be calculated. Thus, the observed differences in the distribution of radioactivity in the lipid classes and the specific activities indicate that a greater incorporation of linoleic acid into triacylglycerol occurs as compared to stearic acid and, on the other hand, there is greater incorporation of stearic acid into sphingomyelins.

**Discussion**

In the present study, we find that the rate of disappearance of linoleic acid from the in situ guinea pig gallbladder is greater than that of stearic acid. At the end of 24 h, the total absorption of these fatty acids by the gallbladder is complete, linoleic acid is absorbed within 12 h and stearic acid is absorbed by 24 h. Differences were also observed in the metabolism of the two fatty acids by the gallbladder wall and in the rate of $^{14}$C$\text{CO}_2$ formation.

The precise mechanism for the absorption of fatty acid by the gallbladder epithelial cell is not known. For the intestine, earlier studies suggested that fatty acid absorption by the epithelial cell involves an initial step of passive diffusion [19]. However, more recent studies [20–22] indicate that this process in the intestine is more complex. Ockner et al. [11,21] reported that differences in the absorption of palmitic and linoleic acid by the intestine are due to the specificity of a fatty acid-binding protein present in the cytosol of the mucosal cell, which preferentially binds unsaturated long chain fatty acids. It is not known whether the gallbladder epithelial cell contains a fatty acid-binding protein, since its presence in the gallbladder epithelium has not been demonstrated.

After entrance of absorbed fatty acid into the intestinal epithelium, esterification of the fatty acid with triacylglycerol formation occurs [23]. The triacylglycerol is then transported from the intestine via the lymphatics. Our studies with the gallbladder reported here and in a previous publication [3] suggest that the absorbed long chain fatty acid passes through the gallbladder epithelial cell and enters the capillary system. All the fatty acid in the venous effluent is bound to albumin. The fatty acid is then transported via the portal venous system to be metabolized with a major portion of the radioactivity isolated as expired CO$_2$.

The major portion of radioactivity present in the gallbladder wall after instillation of $^{14}$C-labelled fatty acid is incorporated into complex lipids: triacylglycerol, phosphatidylcholine, phosphatidylethanolamine and sphingomyelins. Although all the mechanisms involved in the metabolism of fatty acids by the gallbladder mucosa are not known, we have recently reported on one mecha-
nism whereby fatty acid is incorporated into phosphatidylcholine [8]. The gallbladder mucosa contains the enzyme lysophosphatidylcholine acyltransferase which catalyzes the transfer of fatty acid from acyl-CoA to lysophosphatidylcholine to form phosphatidylcholine. Furthermore in the present study, we find that a greater portion of stearic acid than of linoleic acid is incorporated into sphingomyelins. This is characteristic of sphingolipids in general, since naturally occurring sphingomyelins normally contain only long chain-saturated fatty acids [24]. Therefore, the gallbladder appears to contain a mechanism for the metabolism of the fatty acid that enters the absorptive cell which, at least in part, parallels the intestinal epithelial cell.

The concentration of fatty acid used in our experiments (1 μmol/ml) is very high and under physiological conditions guinea pig bile contains much lower concentrations of fatty acid. Human bile, however, does contain measurable quantities of unesterified fatty acid [25,27]. Our experimental model has been limited to the higher concentrations and differences in absorption and metabolism of an unsaturated vs. a saturated fatty acid at lower concentrations remain to be studied.

Studies performed by Blomstrand and Ekdahl [10] on gallbladder bile from patients with obstructed cystic ducts show an increase in the relative portion of saturated fatty acid, while the portion of unsaturated fatty acid decreases. Further, Misra [26] found that the relative portion of stearic acid in gallbladder bile was as high as 22% of the total fatty acids, while in hepatic bile from the same patients, it was only 2–4% of the total. Our findings with the guinea pig suggest that the increase in saturated fatty acids reported for gallbladder bile is due to a decreased absorption by the gallbladder of the saturated fatty acid and a preferential absorption of the unsaturated fatty acids.

Acknowledgement

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