Quantitative and Qualitative Changes in Phospholipid in the Intestine of the Gerbil and the Development of Lipodystrophy

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ABSTRACT Female gerbils fed a diet containing 20% coconut oil develop an intestinal lipodystrophy that is not seen in animals fed a diet containing 20% safflower oil or a diet of 20% coconut oil supplemented with 0.1% inositol. The coconut oil diets contained 1.5% safflower oil to prevent essential fatty acid deficiency. The level of inositol in the intestinal tissue of animals fed the coconut oil diet not supplemented with inositol has been shown to be decreased. Phospholipid analyses of the intestinal tissue were undertaken to determine if this decrease in total inositol was reflected in a decrease in phosphatidylinositol or resulted in an altered phospholipid pattern. No difference in the phosphatidylinositol level was seen between animals fed 20% coconut oil with and without inositol supplementation (ng P/gut section), although animals fed coconut oil diets had lower levels of phosphatidylinositol than animals fed safflower oil diets. Fatty acid analyses of total phospholipid and phosphatidylinositol in gut tissue revealed that animals which developed the lesion had an altered phosphatidylinositol fraction with a depressed level of arachidonic acid and an elevated level of oleic acid. This suggests that the development of the intestinal lipodystrophy may be correlated with qualitative rather than quantitative changes in phosphatidylinositol. J. Nutr. 109: 2146-2151, 1979.

INDEXING KEY WORDS inositol • gerbil • phosphatidylinositol • arachidonic acid

Hegsted et al. (1) have reported the development of an intestinal lipodystrophy in 10 to 20 days in the female gerbil (Meriones unguiculatus) fed a diet containing 10 to 20% coconut oil which was not seen in animals fed diets containing 10 to 20% safflower oil. The addition of inositol at 50 to 100 mg/kg diet prevented the lipid accumulation (2). The male gerbil seldom developed the lesion and this was attributed to the high rate of inositol synthesis in the testis (3) since castrated males were not protected (4).

Microbiological determination of the total inositol content of the small intestine indicated that the feeding of coconut oil diets depleted the tissue of inositol in both the male and female gerbil but that females had lower inositol levels than the males fed the safflower oil diet as well as the coconut oil diet (2). Feeding triglycerides (4) rich in saturated fatty acids, unsupplemented with inositol, showed that diets rich in lauric acid (12:0) gave the maximum lipid accumulation followed by capric (10:0) and myristic (14:0) acids. Increasing chain length decreased the de-
posit of gut lipid, and unsaturated triglycerides containing oleic acid (18:1) and linoleic acid (18:2) gave no lipid accumulation. Tricaprylin (8:0) and tristearin (18:0) also produced no lipid deposit, possibly because of the portal transport of the former and poor absorption of the latter.

Phospholipids (PL), particularly phosphatidylcholine (PC), are important components of lipoproteins, and while phosphatidyl inositol (PI) does not constitute much of this lipoprotein phospholipid, a possible role for PI might be foreseen in the formation and extrusion of lipoproteins. That PI may have a role here is suggested by the considerable data showing rapid PI turnover in a number of tissues during the packaging and extrusion of many different types of secretory products (5–7). In addition, inositol has been shown to have lipotropic action in the liver (8–10).

The phospholipids of the gerbil gut have been measured in this study in order to determine if the decreased total inositol levels seen in the coconut oil fed animals were reflected in decreased levels of PI and whether the presence or absence of inositol might affect other phospholipid levels. A drop in the level of PI or of another phospholipid could help explain the development of the inositol deficiency. Since dietary fatty acids have been shown to affect the susceptibility to the lesion, the fatty acid pattern of the total phospholipid class and PI alone were also analyzed.

MATERIALS AND METHODS

These studies were conducted on young female gerbils housed in group cages of five and fed their respective diets ad libitum. The rats used in the study were young adult female Sprague-Dawley which had been raised on a stock diet. The purified diets used in this study contained (in %): casein, 15; dextrose, 54.2; fat, 20; salt mix, 5; cellulose, 5; choline chloride, 0.3; complete vitamin mix (2), 0.5. Inositol when provided was at 0.1% by weight. All diets contained 1.5% safflower oil to protect against essential fatty acid deficiency. Therefore, a diet described as containing 20% coconut oil contained 18.5% coconut oil and 1.5% safflower oil. The diets were fed for 14 days. After an overnight fast the animals were killed and the duodenum and jejunum of the small intestine (approximately 10 cm) were removed, washed, blotted dry and weighed. The sample was homogenized and the lipid extracted by the method of Bligh and Dyer (11). Neutral lipid was separated from the phospholipids by thin layer chromatography on silica gel H. The developing solvent was hexane:diethyl ether:18 N acetic acid (70:30:1). The phospholipid band was scraped and extracted with two washes of 10 ml of chloroform:methanol:water (16:7:1). An aliquot of this extract was taken for methylation of the phospholipid fatty acids according to the method outlined by Cohen and Derksen (12) with the addition of 10 µg of heneicosanoic acid (21:0) as an internal standard. The remaining phospholipid extract was separated into the individual phospholipids by thin layer chromatography using silica gel H according to the method of Kaulen (13) using 0.26% (NH₄)₂SO₄ in the silica gel. Phospholipids were identified with appropriate standards and Rhodamine 6-G. Phosphatidylinositol was scraped from the plates and the methylation procedure for the fatty acids was carried out using 10 µg of heneicosanoic acid (21:0) as internal standard. The methylated fatty acids from the phospholipid class and PI were separated by gas liquid chromatography on 10% SP-222 PS with a carrier gas flow rate of 20 ml/minute through a 1,830-mm x 2-mm glass column operated at 195°. Peak areas were quantified by an electronic digital integrator and normalized against the internal standard. Quantitation of the individual phospholipids was done by phosphorus determination using the method of Bartlett (14).
TABLE 1

<table>
<thead>
<tr>
<th>Diet</th>
<th>Gut weight</th>
<th>Gut fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>% wet wt</td>
</tr>
<tr>
<td>Coconut oil — inositol</td>
<td>1.30 ±0.13*</td>
<td>15.97 ±2.83*</td>
</tr>
<tr>
<td>Coconut oil + inositol</td>
<td>0.92 ±0.09*</td>
<td>3.40 ±0.57*</td>
</tr>
<tr>
<td>Safflower oil — inositol</td>
<td>0.80 ±0.06*</td>
<td>3.21 ±0.94*</td>
</tr>
<tr>
<td>Safflower oil + inositol</td>
<td>0.70 ±0.08*</td>
<td>3.44 ±0.48*</td>
</tr>
</tbody>
</table>

1 Values represent mean ±sd for five animals per group; means within a column not sharing a common superscript letter differ significantly at P < 0.05 (Tukey's t-test) (15).

RESULTS

Quantitation of phospholipids. Table 1 shows the gut weight and % fat (% wet weight) found in the gerbils fed the different diets. An increase in weight and % fat was seen in the group fed the coconut oil without inositol (Coc — I) as described previously (1, 2). The total phospholipid levels were elevated in the gerbils fed the Coc — I diets (table 2) and this increase was due mainly to higher levels of phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Phosphatidylinositol, total phospholipid, and phosphatic acid (PA) levels were higher in the animals fed the coconut oil diet compared to the animals fed the safflower oil diet. The total PL, PC, PE and PA of the Coc — I group was statistically higher than those of the animals fed the coconut oil diet + inositol (Coc + I), but there was no difference in PI levels between these groups.

When PI and total PL were represented as µg P/g wet weight (table 2), the data were modified due to the increase weight of fat found in the Coc — I group. The total phospholipid/g of tissue was higher in the animals fed the safflower oil diet + inositol (Saf + I) with little difference seen in the other groups. On a per gram basis the animals supplemented with inositol had slightly higher values of PI but no difference was seen between the animals fed the safflower oil diet without inositol (Saf — I) and Coc — I group.

Fatty acid analysis of phospholipid and phosphatidylinositol. No information is available on the fatty acid pattern of the phospholipids in the intestine of the gerbil.

TABLE 2

<table>
<thead>
<tr>
<th>Diet</th>
<th>PC</th>
<th>PI</th>
<th>PE</th>
<th>PA</th>
<th>Total</th>
<th>µg lipid phosphorus/g of gut</th>
<th>µg lipid phosphorus/g wet wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coc - 1</td>
<td>235.0 ±26.9*</td>
<td>41.7 ±7.3*</td>
<td>102.0 ±14.4*</td>
<td>30.9 ±5.0*</td>
<td>571.2 ±50.9*</td>
<td>36.1 ±3.6*</td>
<td>502.4 ±18.0*</td>
</tr>
<tr>
<td>Coc + 1</td>
<td>184.9 ±42.0*</td>
<td>37.5 ±7.7*</td>
<td>47.3 ±15.3*</td>
<td>19.5 ±1.9*</td>
<td>409.4 ±71.3*</td>
<td>45.8 ±8.3*</td>
<td>513.9 ±94.0*</td>
</tr>
<tr>
<td>Saf - 1</td>
<td>142.3 ±19.8*</td>
<td>27.4 ±7.1*</td>
<td>65.6 ±11.7*</td>
<td>15.9 ±2.0*</td>
<td>358.8 ±35.3*</td>
<td>38.1 ±7.6*</td>
<td>500.7 ±15.0*</td>
</tr>
<tr>
<td>Saf + 1</td>
<td>204.5 ±26.7*</td>
<td>25.3 ±5.0*</td>
<td>43.9 ±5.1*</td>
<td>11.5 ±2.7*</td>
<td>367.5 ±40.7*</td>
<td>41.2 ±9.1*</td>
<td>600.8 ±91.0*</td>
</tr>
</tbody>
</table>

1 Values represent mean ±sd for five animals per group; means within a column not sharing a common superscript letter differ significantly at P < 0.05 (Tukey’s t-test) (15). 2 Numbers in parentheses represent % of total phospholipid content in sample. PC = phosphatidylethanolamine; PI = phosphatidylinositol; PE = phosphatidylcholine; PA = phosphatic acid.
and preliminary data indicated that they were quite different from that of the rat. It seemed useful to analyze the phospholipid fatty acids of the adult rat liver and gut for comparison with the adult gerbil, using animals that had been fed a stock diet in order to check our methods and provide base line data. The percent of arachidonic acid in the PL and PI fraction of the gerbil and rat liver and intestine are shown in table 3. The data indicate that in every case the gerbil had a lower level of arachidonic acid than the rat. The value reported by Holub and Kuksis (16) for the percentage of arachidonic acid in PI is included in parentheses for comparison, and agrees with our data.

Three dietary treatments were chosen in this investigation of fatty acid patterns of PL and PI in the gerbil gut. The Saf – I and Coc – I treatments were studied in order to determine if the dietary fatty acids would affect the fatty acid patterns of phospholipids. The Coc + I group was added to determine if the addition of inositol would significantly alter this fatty acid pattern.

Analysis of the fatty acids of the total phospholipids from animals fed the various diets showed significant differences in the percentage of oleic acid (18:1) and arachidonic acid (20:4) between dietary treatments (table 4). Animals fed coconut oil without inositol showed elevated levels of oleic acid compared to those fed Coc + I or Saf – I. Levels of arachidonic acid were lower in coconut oil fed animals compared to the safflower oil fed group, but no significant difference between the Coc – I and Coc + I groups was seen. Eicosatrienoic acid (20:3 w 9) was present in the coconut oil fed animals but not in the safflower oil fed animals.

The fatty acid pattern of PI (table 5) showed that the animals fed coconut oil without inositol had a significantly lower percentage of arachidonic acid compared to the Coc + I and Saf – I groups. The absolute arachidonic acid levels were also significantly decreased (data not shown). The arachidonic acid, expressed either as percent or as total amount, was higher in the Saf – I versus Coc + I group but the difference was not significant.

**DISCUSSION**

The development of the intestinal lipodystrophy in gerbils fed diets containing coconut oil without inositol, but not in the animals fed diets containing safflower oil,

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**TABLE 4**

<table>
<thead>
<tr>
<th>Diet</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:3</th>
<th>20:4</th>
<th>20:6</th>
<th>Total/gut section</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>µg</td>
</tr>
<tr>
<td>Coc – I</td>
<td>0.8±0.1</td>
<td>14.1±1.0</td>
<td>21.2±2.0</td>
<td>14.8±1.1</td>
<td>32.0±2.1</td>
<td>0.9±0.5</td>
<td>6.3±0.6</td>
<td>3.2±0.9</td>
<td>250.8±49.6</td>
</tr>
<tr>
<td>Coc + I</td>
<td>0.5±0.1</td>
<td>15.6±1.9</td>
<td>24.8±1.7</td>
<td>7.9±0.3</td>
<td>35.3±1.5</td>
<td>1.7±0.6</td>
<td>6.9±1.0</td>
<td>2.9±0.3</td>
<td>256.8±28.3</td>
</tr>
<tr>
<td>Saf – I</td>
<td>—</td>
<td>14.7±0.4</td>
<td>22.9±0.2</td>
<td>6.5±0.5</td>
<td>35.8±1.0</td>
<td>—</td>
<td>10.9±0.9</td>
<td>4.3±0.4</td>
<td>224.1±13.7</td>
</tr>
</tbody>
</table>

1 Values represent mean±so for five animals per group; means within a column not sharing a common superscript letter differ significantly at P < 0.05 (Tukey’s t-test) (15).

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**TABLE 5**

<table>
<thead>
<tr>
<th>Diet</th>
<th>14:0</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:3</th>
<th>20:4</th>
<th>20:5</th>
<th>Total/gut section</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>µg</td>
</tr>
<tr>
<td>Coc – I</td>
<td>11.4±2.1</td>
<td>44.7±5.0</td>
<td>17.6±1.0</td>
<td>11.1±3.6</td>
<td>1.1±0.7</td>
<td>4.7±2.7</td>
<td>—</td>
<td>22.0±5.7</td>
<td></td>
</tr>
<tr>
<td>Coc + I</td>
<td>10.3±0.3</td>
<td>42.1±0.7</td>
<td>11.4±1.1</td>
<td>13.4±2.1</td>
<td>6.3±1.2</td>
<td>11.5±1.7</td>
<td>—</td>
<td>16.0±2.2</td>
<td></td>
</tr>
<tr>
<td>Saf – I</td>
<td>10.3±2.4</td>
<td>45.0±6.1</td>
<td>6.2±0.7</td>
<td>14.6±1.5</td>
<td>1.9±0.4</td>
<td>16.4±2.6</td>
<td>—</td>
<td>18.9±3.7</td>
<td></td>
</tr>
</tbody>
</table>

1 Values represent mean±so for five animals per group; means within a column not sharing a common superscript letter differ significantly at P < 0.05 (Tukey’s t-test) (15).
demonstrates an effect of dietary fat on the susceptibility of the animal to the inositol deficiency. The addition of inositol to the coconut oil diet protects against this effect of dietary fat. Earlier studies on choline deficiency had shown that saturated (17, 18) and medium-chain fatty acids (18, 19) caused more severe lipid accumulation in the livers of deficient rats. Changes in fatty acid composition of phospholipids have been reported in choline deficiency where total phospholipid and phosphatidylethanolamine showed decreased levels of arachidonic acid (20–22). A decrease in liver and serum phosphatidylcholine was also observed in choline-deficient rats (21–23) but no drop in arachidonic acid in phosphatidylcholine was seen in rats fed the choline-deficient diet for 14 days (22, 23). Since both choline and inositol are considered lipotropic factors, such observations on choline deficiency were of interest.

In the current studies, there was no difference in the total tissue content of PI between the Coc—I and the Saf—I fed animals. If the amount of PI present is expressed on a per gram weight basis, the addition of inositol to the diet did result in a slight increase in the level of PI. The data thus suggest that differences in PI content do not explain the differential effect of coconut oil and safflower oil on the development of the lesion with the low inositol diet. The possibility that the PI content of specific cellular fractions is affected remains to be investigated.

Previous work in which the total inositol content of the tissue was measured using a microbiological assay reported a difference in the total inositol content of the Saf—I and Coc—I fed groups (2). If this is correct, it would appear that the difference was a reflection of changes in the free inositol content and not of PI. The amount of PI in the tissue using an average of the Coc + I and Saf + I groups was calculated at 1.4 μmole PI/g wet weight and is similar to the 1.38 μmole wet weight obtained by Dittmer and Douglas from the rat small intestine (24).

Analysis of the fatty acid pattern of the PL and PI fraction from the small intestine showed that the level of arachidonic acid in PI was much lower in the group that developed the lipodystrophy. This decrease in arachidonic acid in PI was accompanied by an increase in oleic acid. Higher levels of oleic acid were also seen in the PL fraction of animals fed the coconut oil diet unsupplemented with inositol. The mechanism by which the inositol supplement resulted in a higher level of arachidonic acid in the PI fraction could have a number of explanations. In the presence of adequate inositol, new PI could be synthesized and turned over using a diglyceride backbone that does not contain arachidonic acid resulting in a lower turnover rate for the arachidonyl species of PI. Without adequate inositol, PI turnover may increase yielding no net change of the PI level, but the diglyceride backbone containing arachidonic acid may be lost for reutilization in new PI synthesis. Slower turnover rates have been reported for the arachidonyl species of PC and PE (25, 26). It is also possible that inositol stimulates the synthesis of polyunsaturated fatty acids, particularly from oleic acid and influences their incorporation into PI. An increase in both 20:3 and 20:4 was seen in the PI in animals fed coconut oil supplemented with inositol. Whether the increased level of 20:3 seen in the animals fed coconut oil was due to essential fatty acid deficiency is unknown. The diet contained 1.9% of the energy as essential fatty acids which is double the minimal requirement of the rat (27).

A specific species of PI might be important for lipid transport in the intestine. A well-known function for the arachidonoyl species of PI and PC is as fatty acid donors for prostaglandin synthesis (28, 29). Prostaglandin synthesis does take place in the intestines and is affected by the availability of its precursor essential fatty acids (28, 30) and dietary fat (31). Whether prostaglandins have an effect on lipid transport is not known.

This study, therefore, suggests that changes in the molecular species of PI in the Coc—I animals may offer a better explanation for the development of the lesion and the protective action exerted by inositol than changes in the level of phosphatidylinositol per se.
PHOSPHOLIPID CHANGES IN LIPODYSTROPHY IN GERBILS

LITERATURE CITED

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