Liver Phospholipids of Rats Fed a Choline-Deficient Diet Supplemented With Choline or Methionine

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ABSTRACT

The low amount of arachidonic acid in the total phospholipids in the liver of rats fed a standard type of choline-deficient diet was corrected by either choline or methionine, which also increased food intake. Choline increased the content of this fatty acid in the phosphatidyl ethanolamine but not in the phosphatidyl choline. Methionine increased both the amount of phosphatidyl choline and its content of arachidonic acid.

INTRODUCTION

Phosphatidyl choline was shown to be derived through the sequential methylation of phosphatidyl ethanolamine in the liver of choline-deficient rats (1-5). A decrease in the total amount of phosphatidyl choline was accompanied by a reduced level of arachidonic acid in phosphatidyl ethanolamine (6). It seemed possible that such phospholipid alterations might depend upon the effectiveness of the methylation of some species of phosphatidyl ethanolamine to form phosphatidyl choline rather than on a deficiency of exogenous choline itself.

Besides being deficient in choline, the test diets were also low in methionine, attained by the use of soya or peanut proteins (1-6). To ascertain if the relevant dietary inadequacy was that of methyl groups, methionine was added to a choline-deficient diet and its effectiveness in correcting the phospholipid metabolism compared to that of supplementary choline.

EXPERIMENTAL PROCEDURES

Male Wistar rats weighing 68 to 81 g, from Woodlyn Farms, Guelph, Ontario, were divided into three similar groups on the basis of body weight. One group received the choline-deficient diet previously described (6), and the other groups received this diet supplemented with either 1% choline bitartrate (General Biochemicals) or 0.5% L-methionine (Nutritional Biochemicals). After two weeks the livers were obtained from the nonfasted rats, the lipids extracted, and the total fat and lipid phosphorus determined. The phospholipids were precipitated in acetone saturated with MgCl₂, individual phospholipids separated by thin layer chromatography and the methyl esters of their fatty acids gas-chromatographed (6,7). To distinguish 20:5 ω3 from 20:5 ω6, relative retention times were employed (8). Methyl arachidate (Applied Science Laboratories) was used as an internal standard to quantitate the phospholipid fatty acids.

RESULTS

As shown in Table I, the addition of either choline or methionine to the choline-deficient diet improved the weight gain of rats, and methionine also significantly increased the food intake, liver weight and lipid phosphorus level. The total fat accumulated in the liver of the deficient rats was lowered to a greater extent by choline than by methionine.

The amounts of saturated and monoenoic fatty acids in the liver phospholipids were

<table>
<thead>
<tr>
<th></th>
<th>Food intake, grams per two weeks</th>
<th>Body weight, g</th>
<th>Liver weight, g</th>
<th>Total lipid P, mg/liver</th>
<th>Total lipid, mg/liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choline-deficient</td>
<td>128 ± 9a</td>
<td>99 ± 6</td>
<td>5.75 ± 0.41</td>
<td>3.75 ± 0.26</td>
<td>919 ± 123</td>
</tr>
<tr>
<td>+ choline</td>
<td>143 ± 24</td>
<td>128 ± 4b</td>
<td>6.51 ± 0.28</td>
<td>4.51 ± 0.26</td>
<td>233 ± 9b</td>
</tr>
<tr>
<td>+ methionine</td>
<td>173 ± 5b</td>
<td>141 ± 5b</td>
<td>7.26 ± 0.33c</td>
<td>4.90 ± 0.29c</td>
<td>382 ± 30b</td>
</tr>
</tbody>
</table>

aMean ± standard error of mean for seven rats.
bDifferent from the unsupplemented choline-deficient group at P < 0.01.
cDifferent from the unsupplemented choline-deficient group at P < 0.05.
FIG. 1. Amounts of polyunsaturated fatty acids in the total phospholipids of the seven rats in each group fed the unsupplemented choline-deficient diet (D), that diet supplemented with choline (C) or with methionine (M). Values shown, means ± standard errors of the mean.

FIG. 2. Liver lipid phosphorus in sphingomyelin (SPH), phosphatidyl choline (PC), a mixture of phosphatidyl serine and phosphatidyl inositol (PS + PI) and phosphatidyl ethanolamine (PE) in three rats randomly selected from each of the groups fed the unsupplemented choline-deficient diet (D), that diet supplemented with choline (C) or with methionine (M).

Methionine increased this fatty acid in both phospholipids. Docosahexaenoic acid was relatively high in phosphatidyl ethanolamine when arachidonic acid was low. This increased amount of the ω3 polyenoic acid was not transferred to phosphatidyl choline.

**DISCUSSION**

Methionine was more effective than choline in increasing the amount of phosphatidyl choline, but, as found by others (9,10), not in reducing the total lipids in the liver. Since the direct route for the synthesis of phosphatidyl choline from CDP-choline and diglyceride involves fatty acids which more closely resemble those of the accumulated liver fat than those derived from phosphatidyl ethanolamine, Yamamoto (10) suggested that the direct route was more important in the removal of liver triglycerides.

It is well established that methyl groups are involved in the conversion of phosphatidyl ethanolamine to phosphatidyl choline (11-13,3). Although more methyl groups were supplied here by 1% choline than by 0.5% methionine, the latter appeared to be more effective in correcting the phospholipid alterations produced by the basal choline-deficient diet. In agreement with earlier work which indicated that the methyl groups of choline were not labile (9), Weingold (14) demonstrated their lack of utilization in the methylation of phosphatidyl ethanolamine. Also, the results of Haggard and Parks (15) suggested that...