Content of phospholipids in human diets studied by the duplicate-portion technique 

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1. The phospholipid intake by eight healthy women was studied by the duplicate-portion technique, i.e. chemical analyses of duplicates of the food consumed. Their intake of phospholipids was 1.5-2.5 mmol lipid-phosphorus/d, which corresponds to 0.023-0.059 mmol lipid-P/g dietary fat and 0.24-0.45 mmol lipid-P/MJ respectively.

2. Choline phosphoglyceride constituted 480-700 mmol/mol and ethanolamine phosphoglyceride 170-240 mmol/mol total lipid-P. Most of the choline in nature occurs as choline phospholipids, the intake of which ranged between 0.91-1.85 mmol/d or 0.15-0.33 mmol/MJ.

3. Palmitate, stearate, oleate, and linoleate were the four major fatty acids both in choline phosphoglyceride and ethanolamine phosphoglyceride. Significant amounts of highly unsaturated, C18 and C20 fatty acids were also observed. It was calculated that 13-33 mg/g total dietary fatty acids was consumed as phospholipids. Together with bile phospholipids, dietary phospholipids and their degradation products may be important in the digestion and absorption processes.

EXPERIMENTAL

The main dietary lipid is triacylglycerol but the quantity and composition of less-abundant acyl lipids, such as phospholipids, are not much studied. Most available information concerns individual foodstuffs whereas information on the polar lipid composition in human diets, mixed and prepared for consumption is almost completely lacking. Together with bile phospholipids dietary phospholipids may have important functions in the intestinal lumen (Borgström, 1976) and may influence, for example, triacylglycerol release from the intestine (O’Doherty et al. 1973), and the absorption of cholesterol (Rodgers & O’Connor, 1975). Bile and dietary phospholipids may be metabolized in the same way (Arnesjö et al. 1969) although a difference has been proposed (Boucrot & Clement, 1971). The consumption of phospholipids is also relevant for the provision of dietary choline, most of which is lipid-bound.

The intake of phospholipids by healthy free-living subjects has been studied. This study was part of a project designed to assess dietary intake by chemical analysis of so-called duplicate portions (Borgström et al. 1975; Borgström et al. 1979). This methodological approach is especially advantageous for components which are not listed in customary food composition tables, such as polar lipids.

The subjects studied were eight healthy women, aged 34-69 years, who formed part of a reference group in a study on dietary intake in relation to blood pressure. Other aspects of their dietary intake are reported elsewhere (Thulin et al. 1980). The nutrient intake agreed well with the pattern normally encountered in Sweden. Each subject collected six daily portions as described previously (Thulin et al. 1980). The portions were homogenized as described previously (Borgström et al. 1975) and 2-5 g of the homogenate was extracted for 1 h with 5 vol. chloroform-methanol (1:2, v/v). The mixture was filtered and the extraction was repeated with 3 vol. chloroform-methanol (1:1, v/v). Enough water to give a value for water-methanol of 1:1 was added to the combined extracts and the chloroform
Fig. 1. Thin-layer chromatography of non-polar dietary lipids isolated from six daily portions from subject 6. The reference substances to the left (R) are (a) cholesterol oleate, (b) triolein, (c) oleic acid and (d) cholesterol. Between the reference and the six samples one fifth and one half the amount of the A sample was applied. The developing solvent was diethyl ether–petroleum ether–acetic acid (30:70:1, v/v). Charring with 0.6% K$_2$Cr$_2$O$_7$ in 55% H$_2$SO$_4$. Charring with 0.6% K$_2$Cr$_2$O$_7$ in 55% H$_2$SO$_4$.

layer was recovered after phase separation. After drying and weighing, total lipids were dissolved in chloroform and separated into a neutral lipid fraction and a polar fraction by column chromatography on silicic acid (< 200 mesh; Mallinckrodt). The first eluent was chloroform (20 ml/g silicic acid) and the second eluent chloroform-methanol (1:1, v/v) plus methanol (each 7–10 ml/g silicic acid). The phosphorus content in the polar fraction was determined according to Chen et al. (1956). Phospholipids were separated by thin-layer chromatography on silica gel H with chloroform–methanol–acetic acid–water (65:25:4:4, v/v) as the developing solvent. The phospholipid fractions were either eluted with chloroform–methanol–acetic acid–water (50:39:1:10, v/v) (Åkesson et al. 1970) or directly measured by P determination (Rouser et al. 1966). Some of the isolated fractions were further purified by thin-layer chromatography with chloroform–methanol–concentrated ammonia (65:35:8, v/v) as the developing solvent. Fatty acid analysis by gas–liquid chromatography of methyl esters was performed as described previously (Åkesson et al. 1970). The components were identified by comparing the retention times with those of authentic standards.
Analysis of dietary polar lipids

Fig. 2. Thin-layer chromatography of polar dietary lipids isolated from six daily portions from subject 6. The reference to the left (R) is rat liver phospholipid: (a) cardiolipin, (b) ethanolamine phosphoglyceride, (c) choline phosphoglyceride and (d) sphingomyelin. The developing solvent was chloroform-methanol-acetic acid-water (65:25:4:4, v/v). Charring with 0-6% K$_2$Cr$_2$O$_7$ in 55% H$_2$SO$_4$.

RESULTS

Lipid classes in the diet

Total dietary lipid was separated into a non-polar and a polar fraction by column chromatography, and the fractions were analysed by thin-layer chromatography. The non-polar fraction contained mainly triacylglycerol with smaller amounts of compounds with the chromatographic mobilities of diacylglycerol, sterols and unesterified fatty acid.

The intake of phospholipids was 1.5–2.5 mmol/d, determined from the content of lipid-P in the polar fraction (Table 1). The intake in relation to fat and energy was 0.023–0.059 mmol lipid-P/g fat and 0.24–0.45 mmol lipid-P/MJ respectively. The energy intake was 3.7–10.1 MJ/d and fat intake 38–107 g/d. For one subject, the daily variation in phospholipid intake was investigated (Table 2). Total phospholipid intake varied approximately twofold. It could be calculated that 13–33 mg/g total dietary fatty acids were consumed as phospholipids. Although the contribution by phospholipids for most dietary fatty acids is small, their role may be greater for fatty acids enriched in phospholipids, such as long-chain, highly-unsaturated acids (see p. 228).
Table 1. Intake of individual phospholipids

(The values represent the relative distribution of phosphorus mmol/mol among different phospholipids separated by thin-layer chromatography. A pooled sample from six daily portions was analyzed for each person)

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Lipid fraction</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent front</td>
<td>84</td>
<td>38</td>
<td>38</td>
<td>13</td>
<td>62</td>
<td>31</td>
<td>31</td>
<td>69</td>
<td>43</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Ethanolamine phosphoglyceride</td>
<td>22</td>
<td>23</td>
<td>23</td>
<td>3</td>
<td>11</td>
<td>22</td>
<td>11</td>
<td>22</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Choline phosphoglyceride</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>Sphingomyelin</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>18</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Total lipid-P (mmol/d)</td>
<td>2.35</td>
<td>1.62</td>
<td>2.32</td>
<td>1.54</td>
<td>1.84</td>
<td>2.44</td>
<td>1.84</td>
<td>2.44</td>
<td>1.84</td>
<td>2.44</td>
</tr>
<tr>
<td></td>
<td>Total fat (g/d)</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
<td>1.54</td>
</tr>
</tbody>
</table>

A, B, are intermediate fractions between the compounds indicated.
Analysis of dietary polar lipids

Table 2. Phospholipids in individual daily portions from subject no. 6

<table>
<thead>
<tr>
<th>Day...</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total lipid-phosphorus (mmol/d)</td>
<td>1.4</td>
<td>1.8</td>
<td>3.2</td>
<td>2.6</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Lipid-P: mmol/g total fat</td>
<td>0.012</td>
<td>0.024</td>
<td>0.024</td>
<td>0.027</td>
<td>0.014</td>
<td>0.019</td>
</tr>
<tr>
<td>Lipid-P: mmol/MJ energy</td>
<td>0.14</td>
<td>0.22</td>
<td>0.29</td>
<td>0.31</td>
<td>0.15</td>
<td>0.19</td>
</tr>
</tbody>
</table>

The polar fraction was analyzed by thin-layer chromatography. Spots migrating as ethanolamine phosphoglyceride and choline phosphoglyceride were the major components but several unidentified spots were seen, especially close to the solvent front. Quantitative analysis showed that the choline phosphoglyceride fraction constituted 48% of total phospholipids and the ethanolamine phosphoglyceride fraction 17% of total phospholipids (Table 1). The intake of choline phospholipids, calculated from total lipid-P and the sum of the relative amounts of choline phosphoglyceride, sphingomyelin and lysophosphatidylcholine, ranged between 0.91 and 1.85 mmol/d or 0.15 and 0.33 mmol/MJ.

Fatty acid composition

The fatty acid composition of total polar lipids in the diet was similar for different persons (Table 3). Only the proportions of oleic acid and linoleic acid varied reciprocally. The fatty acid composition of dietary choline phosphoglyceride, as expected, resembled that of total polar lipids in several respects (Table 4). The proportions of palmitate and oleate were somewhat higher and that of linoleate lower than in total polar lipids. The ethanolamine phosphoglyceride fraction contained a higher proportion of linoleate than choline phosphoglyceride, and also a larger amount of total fatty acids than expected from the content of P. This fraction and the choline phosphoglyceride fraction (pooled sample from eight subjects) was therefore further purified by thin-layer chromatography. The purified choline phosphoglyceride contained 890 mg/g of the fatty acids in the starting fraction, the rest probably partly representing inositol phosphoglyceride. The purified dietary ethanolamine phosphoglyceride contained 530 mg/g fatty acids in the starting fraction. The rest was mainly recovered in a compound migrating as digalactosyl diacylglycerol. The fatty acid composition...
Table 4. Fatty acid composition (mg/g) of individual dietary polar lipids
(Mean values with their standard errors for eight subjects)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Choline phosphoglyceride fraction</th>
<th>Ethanolamine phosphoglyceride fraction</th>
<th>Choline phosphoglyceride</th>
<th>Ethanolamine phosphoglyceride</th>
<th>Diglycosyl diacylglycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>16:0</td>
<td>277</td>
<td>7</td>
<td>165</td>
<td>7</td>
<td>290</td>
</tr>
<tr>
<td>16:1</td>
<td>12</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>18:0</td>
<td>120</td>
<td>8</td>
<td>140</td>
<td>19</td>
<td>101</td>
</tr>
<tr>
<td>18:1</td>
<td>238</td>
<td>19</td>
<td>157</td>
<td>13</td>
<td>256</td>
</tr>
<tr>
<td>18:2</td>
<td>210</td>
<td>11</td>
<td>378</td>
<td>30</td>
<td>197</td>
</tr>
<tr>
<td>18:3+20:1</td>
<td>24</td>
<td>5</td>
<td>44</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>20:4</td>
<td>35</td>
<td>4</td>
<td>54</td>
<td>9</td>
<td>33</td>
</tr>
<tr>
<td>20:5</td>
<td>16</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>22:5</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>22:6</td>
<td>57</td>
<td>10</td>
<td>44</td>
<td>3</td>
<td>51</td>
</tr>
</tbody>
</table>

* Pooled sample representing eight subjects.

composition of this compound deviated distinctly from that of the phospholipids, since it contained 680 mg linoleic acid/g (Table 4). In comparison to choline phosphoglyceride, purified ethanolamine phosphoglyceride had a higher stearate:palmitate value and also contained larger proportions of highly unsaturated fatty acids. Since these acids are very minor components in dietary triacylglycerol, their main location in the diet may be the phospholipids. It could be calculated that the mean consumption of C 20:4 from ethanolamine phosphoglyceride was 0.09 mmol/d and from choline phosphoglyceride 0.08 mmol/d. The corresponding intake of C 22:6 was 0.07 and 0.13 mmol/d respectively. A nutritional role for such fatty acids has been suggested in brain development (Crawford et al. 1973) and also in prostaglandin synthesis (Dyerberg et al. 1978). Trans-isomers of octadecenoate constitute approximately 50 mmol/mol dietary octadecenoate (Åkesson et al. 1981), but the proportion of such isomers in dietary phospholipids has not been studied.

**DISCUSSION**

In the intestinal lumen dietary phospholipids are mixed with biliary phospholipids. The secretion rate of biliary phospholipids, mainly phosphatidylcholine, is in the range 9.2–23.1 mmol/d (Northfield & Hoffmann, 1975) or 4.4–5.0 g/d (5.7–6.5 mmol/d, Shioda et al. 1968). Since the diets studied by the present authors contained 1.5–2.5 mmol phospholipid/d it can be concluded that the bile is the dominating origin of lumen phospholipid under normal conditions. Phosphatidylcholine from both sources is probably metabolized in the same way (Nilsson, 1968; Arnesjö et al. 1969) although a heterogeneity has been suggested (Boucrot & Clement, 1971). A proposed enterohepatic circulation of biliary phosphatidylcholine has not been verified (Larsson & Nilsson, 1978).

Since phosphatidylcholine is the dominating source of choline in the diet, the present results also give information on choline intake. Choline is essential for several animals but the requirement in humans is not known (Lucas & Ridout, 1967; Griffith & Dyer, 1968). For infant formulas 7 mg choline/100 kcal (0.16 mmol/MJ) is recommended (Committee on Nutrition, American Academy of Pediatrics, 1976), and the present intake of choline phospholipids corresponded to 0.15–0.33 mmol/MJ.

Previous estimates of choline intake showed in three human rations as mean daily values
7 mmol phospholipid and 3.5 mmol choline, corresponding to 0.078 mmol lipid-P/g fat and 0.039 mmol choline/g fat (Ridout et al. 1952). An experimental diet for young men contained 150 mg choline/d (1.4 mmol; Connor Johnsson et al. 1945). Borglin (1947) calculated that an average Swedish diet would contain 500 mg choline/3450 kcal (4.8 mmol/d or 0.33 mmol choline/MJ). These values and those of Mudd & Poole (1975) are in the same range as those reported here.

Foodstuffs rich in phospholipids include egg yolk, meat, fish, dairy products and several grains (Lucas & Ridout, 1967), and one estimation indicated that half of the dietary choline originated from eggs (Borglin, 1947). In phosphatidylcholine from eggs (Noble & Moore, 1965) and also from milk (Jensen, 1973) oleate dominates over linoleate which is contrary to the situation for dietary phosphatidylcholine (Table 4). This finding indicated that other phosphatidylcholines rich in linoleate, such as soya-bean phosphatidylcholine, occur in the diet in addition to egg phosphatidylcholine.

Several aspects of dietary phospholipids in human nutrition require further elucidation. Detailed knowledge of the intake of different dietary lipids by persons on various regimens is one prerequisite. The duplicate-portion technique permits such analyses on free-living individuals and such dietary information can be related to the health status of the consumer. Previously, the intake of fatty acids and sterols was studied by the same technique (Borgström et al. 1975; Borgström et al. 1979). The present study further exemplifies the usefulness of this approach.

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