Polyunsaturated Fats Attenuate the Dietary Phytol–Induced Increase in Hepatic Fatty Acid Oxidation in Mice

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ABSTRACT The effects of dietary phytol and the type of dietary fat on hepatic fatty acid oxidation were examined in male ICR mice. Mice were fed diets containing 0 or 5 g/kg phytol and 100 g/kg palm, safflower, or fish oil for 21 d. Among the groups fed phytol-free diets, the activities and mRNA abundance of various enzymes involved in fatty acid oxidation were greater in mice fed fish oil than in those fed palm or safflower oil. Dietary phytol profoundly increased the activities and mRNA abundance of hepatic fatty acid oxidation enzymes in mice fed palm oil. However, safflower and fish oils, especially the latter, greatly attenuated the phytol-dependent increase in hepatic fatty acid oxidation. The hepatic concentration of phytanic acid, a metabolite of phytol that is the ligand and activator of retinoid X receptors and peroxisome proliferator-activated receptors, was higher in mice fed fish oil than safflower or palm oil, and in those administered safflower oil than palm oil. The hepatic mRNA abundance of sterol carrier protein-2, a lipid-binding protein involved in phytol metabolism, was inversely correlated with the hepatic concentration of phytanic acid. We demonstrated that polyunsaturated fats attenuate the enhancing effect of dietary phytol on hepatic fatty acid oxidation. Dietary fat–dependent changes in the hepatic phytanic acid concentration cannot account for this phenomenon. J. Nutr. 136: 882–886, 2006.

KEY WORDS: • phytol • phytic acid • dietary fat • fatty acid oxidation

Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a branched-chain fatty alcohol; it is abundant in nature as a part of the chlorophyll molecule. After its release from chlorophyll, phytol is converted into phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) in mammals (1–3). The breakdown of phytol to phytanic acid takes place as follows: first, alcohol dehydrogenase converts phytol into the aldehyde; second, the aldehyde is converted to phytanic acid by the action of fatty aldehyde dehydrogenase (aldehyde dehydrogenase family 3 member A2, ALDH3a2) (1). Phytanic acid is activated, becoming pristanoyl-CoA, and the double bond in phytanoyl-CoA is reduced by the action of phytanoyl-CoA reductase (3) to yield pristanoyl-CoA. Phytanic acid undergoes α-oxidation to produce pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) via the following steps (1,2). Phytanoyl-CoA is converted into 2-hydroxyphytanoyl-CoA by phytanoyl-CoA hydroxylase. The reaction, catalyzed by 2-hydroxyphytanoyl-CoA lyase, in turn converts 2-hydroxyphytanoyl-CoA into pristanal, which is metabolized to pristanic acid by ALDH3a2. Pristanic acid is activated, becoming pristanoyl-CoA, and is degraded by the peroxisomal β-oxidation pathway.

The potential role of PPAR and RXR in regulating lipid metabolism was clearly shown (4,5). Upon the binding of the ligands to these nuclear receptors, heterodimerization of PPAR with RXR occurs, and the heterodimer binds to a specific cis-acting DNA response element termed the peroxisome proliferator response element (PPRE) and activates the various genes involved in the regulation of lipid metabolism. Phytanic acid, a phytol metabolite, is a ligand that activates retinoid X receptor (RXR) α (6–10), β (6,8,11), and γ (6,7), and peroxisome proliferator-activated receptor (PPAR) α (9,10,12–15), δ (9,14,15), and γ (9,14,15). There is information (8) to suggest that another phytol metabolite, pristanic acid, also acts as an activator of PPAR and RXR. However, phytol itself is virtually ineffective in activating these nuclear receptors (6,7,15). Therefore, it is expected that after conversion into its metabolites, dietary phytol would activate the genes targeted by the PPAR-RXR heterodimer in animals. In fact, it was demonstrated that consumption of phytol increased the hepatic concentration of phytanic and pristanic acids in mice (16), and this was associated with an upregulation of the expression of PPAR/RXR-dependent genes including those for liver-type fatty acid-binding protein (12,13) and various enzymes involved in fatty acid oxidation (12,16).

Fat type is also crucial to lipid metabolism. Dietary fats rich in (n-3) fatty acids such as fish, linseed, and perilla oils (17–21), and borage oil rich in γ-linolenic acid (22,23) increase the
activities and mRNA abundance of hepatic fatty acid oxidation enzymes. Here, PUFA or some of their metabolites (prostanoids) similar to phytol metabolites are considered natural ligands and hence activators of PPAR (24–26) and RXR (27–29). It is therefore possible that the simultaneous ingestion of phytol and PUFA affects hepatic fatty acid metabolism differently than the physiological activities of individual compounds through the intricate interaction of phytol metabolites and PUFA with nuclear receptors. With this understanding, we examined the interaction of phytol and different fats, either palm oil (a saturated fat) or fats rich in (n-6) (saflower oil) or (n-3) (fish oil) PUFA, in affecting hepatic fatty acid oxidation in mice in the present study.

MATERIALS AND METHODS

Animals and diets. Male ICR mice purchased from Charles River Japan, Kanagawa, Japan, at 5 wk of age were housed individually in a room with controlled temperature (20–22°C), humidity (55–65%), and lighting (lights on from 0700 to 1900) and fed a commercial nonpurified diet (Type NMF, Oriental Yeast). After 7 d of acclimation, the mice were divided into 6 groups with the same mean body weights (31.5–32.8 g) and were fed purified experimental diets containing 0 or 5 g/kg phytol (Sigma-Aldrich) and 100 g/kg of palm, saflower, or fish oil for 21 d. The basal composition of the purified experimental diets was (g/kg): casein, 200; dietary fat, 100; cellulose, 20; mineral mixture (30), 35; vitamin mixture (30), 10; DL-methionine, 3; choline bitartrate, 2, and sucrose to 1 kg. Fatty acid compositions of dietary fats analyzed by GLC in our laboratory are listed in Table 1. Phytol replaced sucore in the experimental diets. Mice had free access to the diets and water during the experimental period. This study was approved by the review board of animal ethics of our institute and we followed the institute’s guidelines in the care and use of laboratory animals.

Enzyme assays. At the end of the experimental period, the mice were killed by bleeding from the inferior vena cava under diethyl ether anesthesia. The livers were then quickly excised, and frozen in liquid nitrogen. Liver tissue was homogenized in 6.0 mL of a solution containing 0.25 mol/L sucrose, 1 mmol/L EDTA, and 3 mmol/L Tris-HCl (pH 7.2). The activities of various enzymes involved in hepatic fatty acid oxidation were measured spectrophotometrically using the whole-liver homogenate stored at –40°C for up to 10 d as an enzyme source as detailed previously (17,18,20,21). Carnitine palmitoyltransferase activity was analyzed in the presence of detergent (triton X-100).

RNA analysis. Hepatic RNA was extracted (31), and purified with an RNaseasy Mini Kit (QIAGEN). mRNA abundance was analyzed by quantitative real-time PCR, as detailed previously (32). mRNA abundance was calculated as a ratio to the β-actin mRNA abundance in each cDNA sample and expressed as a percentage, assigning a value of 100 for mice fed a phytol-free diet containing 100 g/kg palm oil. Nucleotide sequences for forward and reverse primers, and probes to detect mRNAs for various enzymes involved in hepatic fatty acid oxidation and peroxin 11r and cytochrome P450 IV A-1 were reported elsewhere (32). Nucleotide sequences for forward and reverse primers, and a probe to detect mRNA of phytanoyl-CoA hydroxylase were as follows: 5′-GATGCGAAGCGGCAGATCTGTTT-3′, 5′-TIGAGTTTGTGTCGACCGATC-3′, and 5′-CTTCCACCTCCTGCTCATCATC-3′, respectively. Primers and probes supplied by Applied Biosystems (Assay on Demand Products™) were used to measure mRNA abundance of ALDH3a2, 2-hydroxysteroyl-CoA lyase and sterol carrier protein-2 (SCP2).

Analysis of hepatic phytanic acid. Liver lipids were extracted and purified (33). The concentration of phytanic acid in the liver lipid extract was analyzed by GLC using a 1.4BDS capillary column (40 m × 0.25 mm, Shimadzu) and heicenoisanoic acid as an internal standard. Phytanic acid methyl ester as a reference standard was purchased from Sigma-Aldrich.

Statistical analysis. StatView for Macintosh (SAS Institute) was used for statistical analyses. D’Agostino-Pearson’s test was used to inspect the constancy of the variance of the observations. If variances were heterogeneous, they were transformed logarithmically. The transformations were successful in rendering the variance of the observations constant, and hence the transformed values were used for subsequent statistical evaluations. The data for the 2-way classification were analyzed using a 2-way ANOVA. If no significant interaction existed, the Tukey-Kramer post-hoc test was conducted to detect differential effects of palm, saflower, and fish oils. When the interaction was significant, the data were reanalyzed with a 1-way ANOVA and the post-hoc test. The data for the one-way classification were analyzed using a 1-way ANOVA and the post-hoc test. Differences were considered significant when P < 0.05.

RESULTS

Animal growth, tissue weights. Dietary phytol but not fat type reduced growth and body weight at the end of the study (Table 2). Phytol also decreased food intake. Food intake was lower in mice fed fish oil than in those fed palm oil (P = 0.018). A significant interaction between dietary fat type and phytol occurred for liver weight. Relative liver weight was higher in mice fed the phytol-containing diet only when the fat source was palm oil (fat source × phytol, P < 0.05). In mice fed phytol-free diets, relative liver weight was significantly higher in mice administered fish oil than in the other groups.

Activities of hepatic enzymes involved in fatty acid oxidation. Among mice fed phytol-free diets, peroxisomal palmitoyl-CoA oxidation rates and activities of various enzymes involved in fatty acid oxidation were higher in mice fed fish oil than in those fed palm or saflower oil; the activities did not differ between the latter 2 groups (Table 3). When the dietary fat source was palm oil, these variables for mice fed the phytol-containing diet were 1.4- to 6.5-fold those for mice fed the phytol-free diet. However, the extent of the increases was attenuated in mice given saflower or fish oil, especially in the latter (fat type × phytol, P < 0.05). The values for mice fed the phytol-containing diets were 1.2- to 3.0-fold and 1.0- to 1.2-fold those of mice fed phytol-free diets when the dietary fat sources were saflower and fish oils, respectively. In fact, the 2-way ANOVA showed significant interactions of 2 dietary factors, i.e., fat and phytol, for the activities of various enzymes.

mRNA abundance of hepatic fatty acid oxidation enzymes. The analyses of the activities of hepatic fatty acid oxidation enzymes indicated that dietary (n-6) and (n-3) PUFA strongly counteract the effect of phytol in increasing hepatic fatty acid oxidation. To confirm whether this effect could be observed
Effect of dietary phytol and fat type on body weight, weight gain, food intake, and relative liver weight of mice

<table>
<thead>
<tr>
<th>Dietary phytol, g/kg</th>
<th>Palm oil</th>
<th>Safflower oil</th>
<th>Fish oil</th>
<th>Fat</th>
<th>Phytol</th>
<th>Fat × phytol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>40.0 ± 0.7</td>
<td>39.0 ± 1.0</td>
<td>41.6 ± 1.0</td>
<td>40.1 ± 0.7</td>
<td>41.3 ± 0.9</td>
<td>37.8 ± 1.0</td>
</tr>
<tr>
<td>Weight gain, g/20 d</td>
<td>8.0 ± 0.3</td>
<td>7.2 ± 0.8</td>
<td>9.8 ± 0.9</td>
<td>8.6 ± 0.6</td>
<td>9.5 ± 0.7</td>
<td>6.4 ± 0.9</td>
</tr>
<tr>
<td>Food intake, g/d</td>
<td>4.9 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>4.7 ± 0.2</td>
<td>4.2 ± 0.1</td>
</tr>
<tr>
<td>Liver weight, g/100 g</td>
<td>5.60 ± 0.13a</td>
<td>6.72 ± 0.12b</td>
<td>5.66 ± 0.07a</td>
<td>5.77 ± 0.24a</td>
<td>6.69 ± 0.19b</td>
<td>6.66 ± 0.21b &lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 7–8. Means in a row with superscripts without a common letter differ, P < 0.05.

at the level of mRNA expression, we measured mRNA abundance of various hepatic β-oxidation enzymes, cytochrome P-450 IV A1 involved in the ω-oxidation of fatty acids, and a peroxisomal membrane protein (peroxin 11α) induced by peroxisome proliferators (Table 4). Values are expressed as percentages with mice fed a palm oil diet free of phytol assigned a value of 100. Among the groups of mice fed phytol-free diets, the mRNA abundance of hepatic peroxisomal and mitochondrial fatty acid oxidation enzymes and of peroxin 11α and cytochrome P-450 IV A1 was higher in mice fed fish oil than in those fed palm and safflower oils. When the dietary fat was palm oil, the mRNA abundance of enzymes involved in fatty acid oxidation, peroxin 11α, and cytochrome P-450 IV A1 for mice given the phytol-containing diet was 1.4- to 3.0-fold, 1.8-fold, and 10.3-fold, respectively, that of mice fed the phytol-free diet. However, increases were greatly attenuated in mice given safflower and fish oils, especially that latter as indicated by significant interactions between dietary fat type and phytol for all of the variables.

Hepatic phytanic acid concentration and mRNA abundance of proteins involved in phytol metabolism. We showed that dietary fat type strongly affects the physiological activity of phytol in increasing hepatic fatty acid oxidation. There is a possibility that dietary fat type differentially affects phytol metabolism and hence caused this phenomenon. Therefore, we analyzed the hepatic concentration of phytanic acid, a metabolite of phytol, as well as the mRNA abundance of proteins involved in phytol metabolism. Phytanic acid was detectable in mice fed phytol-containing diets but not in the mice fed phytol-free diets. The hepatic concentration of phytanic acid was dependent on the type of dietary fat. The value was higher for mice fed fish oil (1.39 ± 0.30 μmol/g) than for mice given safflower oil (0.41 ± 0.12 μmol/g, P = 0.003) or palm oil (0.11 ± 0.05 μmol/g, P < 0.001). The difference between mice fed palm and safflower oils was also significant (P = 0.001). Among the various proteins involved in phytol metabolism, we analyzed the mRNA abundance of ALDH3a2, phytanoyl-CoA hydroxylase, 2-hydroxyphytanoyl-CoA lyase, and SCP2 in the present study. SCP2 is a peroxisomal lipid carrier involved in the peroxisomal metabolism of bile acids and branched-chain fatty acids (34). Among mice fed phytol-free diets, the mRNA abundance of ALDH3a2 and phytanoyl-CoA hydroxylase was higher in mice fed fish oil than in those fed palm but not safflower oil (Table 5). Phytol significantly increased these variables for mice given palm oil but not for the safflower and fish oil groups. Both fat type and phytol affected the mRNA abundance of 2-hydroxyphytanoyl-CoA lyase. The mRNA level was significantly higher in mice given fish oil than for those fed safflower oil but not palm oil. The level did not differ between mice fed safflower and palm oils. Fat type but not phytol affected the mRNA abundance of SCP2. The values were higher for mice given palm oil than for those fed safflower or fish oil. Also, the values were significantly higher for mice fed safflower oil than for those fed fish oil (P < 0.001). A significant inverse correlation (r = 0.527, P = 0.009) was observed between SCP2 mRNA abundance and phytanic acid concentration in the liver of mice fed 5 g/kg phytol diets containing various fats.

DISCUSSION

Metabolites of phytol such as phytanic and pristanic acids but not phytol itself are potent activators of RXR (6–11) and PPAR (9–15). It is therefore highly plausible that after dietary phytol is metabolized into phytanic acid, it activates genes targeted by PPAR-RXR. In fact, it was demonstrated that the

TABLE 3

Effect of dietary phytol and fat type on the activity of hepatic enzymes involved in fatty acid oxidation in mice

<table>
<thead>
<tr>
<th>Dietary phytol, g/kg</th>
<th>Palm oil</th>
<th>Safflower oil</th>
<th>Fish oil</th>
<th>2-Way ANOVA</th>
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<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
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<tr>
<td>Peroxisomal fatty acid oxidation</td>
<td>2.7 ± 0.2ab</td>
<td>13.1 ± 0.6c</td>
<td>3.8 ± 0.3a</td>
<td>9.2 ± 0.5c</td>
</tr>
<tr>
<td>Acyl-CoA oxidase</td>
<td>2.9 ± 0.3a</td>
<td>18.9 ± 1.2c</td>
<td>4.1 ± 0.4a</td>
<td>12.4 ± 0.8c</td>
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<tr>
<td>Carnitine palmitoyltransferase</td>
<td>5.0 ± 0.2ab</td>
<td>8.1 ± 0.2b</td>
<td>5.5 ± 0.4a</td>
<td>7.5 ± 0.6b</td>
</tr>
<tr>
<td>3-Hydroxyacyl-CoA dehydrogenase</td>
<td>349 ± 15ab</td>
<td>528 ± 15c</td>
<td>394 ± 16a</td>
<td>472 ± 15c</td>
</tr>
<tr>
<td>3-Ketoacyl-CoA thiolase</td>
<td>226 ± 11a</td>
<td>334 ± 9b</td>
<td>249 ± 12ab</td>
<td>270 ± 15b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 7–8. Means in a row with superscripts without a common letter differ, P < 0.05.
feeding of a laboratory diet supplemented with 5 g/kg phytol increases the expression of genes targeted by the PPAR-RXR heterodimer (10,11,16) in the liver of mice. In the present study, we observed that a purified diet containing 5 g/kg phytol strongly increased activities and mRNA abundance of enzymes involved in hepatic fatty acid oxidation when the dietary fat source was a saturated fat (palm oil). However, we unexpectedly found that the simultaneous ingestion of fat abundant in (n-6) or (n-3) PUFA, especially the latter, strongly attenuated the effect of phytol on the activities and mRNA expression of fatty acid oxidation enzymes. Drugs and natural compounds that induce hepatic fatty acid oxidation caused hepatomegaly due to a proliferation of mitochondria and peroxisomes (35,36). Consistent with these observations, phytol added to the palm oil diet but not the safflower or fish oil diet increased liver weight for mice in the present study.

Great diversity is characteristic of the B-oxidation pathway. Several enzyme species differing in substrate specificity located either in peroxisomes or in mitochondria are involved at each step of the B-oxidation cycle. The fatty acid oxidation enzyme activity measured in cell-free enzyme preparations therefore represents the sum of the activity of various enzymes under the given conditions of the enzyme assay. There are at least 3 enzymes possessing carnitine palmitoyltransferase activity (carnitine palmitoyltransferase I and II, and carnitine octanoyltransferase). Carnitine palmitoyltransferase I is unstable when released from its membrane environment, and freezing-thawing of mitochondria and addition of detergent inactivate the enzyme (37). Therefore, the carnitine palmitoyltransferase activity measured in the present study using freeze-thawed total homogenate as an enzyme source and in the presence of detergent may represent the sum of the activities of carnitine palmitoyltransferase II and carnitine octanoyltransferase.

Studies showed that PUFAs are the ligands and activators of PPAR (24–26) and RXR (27–29). Therefore, it is possible that the consumption of a large amount of PUFAs interferes with the binding of phytanic acid, a metabolite of phytol, to these nuclear receptors and hence attenuates the dietary phytol-dependent increase in hepatic fatty acid oxidation despite the fact that the binding affinity for and hence the ability to activate PPAR (12,15) and RXR (11) are much lower with PUFA than with phytanic acid. On a related note, Lee and Hwang (38) reported that docosahexaenoic acid suppressed a PPAR agonist–induced transactivation of PPRE and DNA-binding of PPAR in colon tumor cells.

Alternatively, there is the possibility that polyunsaturated fats interfere with the phytol metabolism to produce active phytol metabolites responsible for inducing hepatic fatty acid oxidation.

### TABLE 4

<table>
<thead>
<tr>
<th>Dietary phytol, g/kg</th>
<th>Palm oil</th>
<th>Safflower oil</th>
<th>Fish oil</th>
<th>Fat</th>
<th>Phytol</th>
<th>Fat (\times) Phytol</th>
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</table>

**P**-value

| Peroxisomal proteins | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

| Mitochondrial proteins | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

### TABLE 5

<table>
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<tr>
<th>Dietary phytol, g/kg</th>
<th>Palm oil</th>
<th>Safflower oil</th>
<th>Fish oil</th>
<th>Fat</th>
<th>Phytol</th>
<th>Fat (\times) Phytol</th>
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</table>

**P**-value

| Aldehyde dehydrogenase family 3 member A2 | 0.001 | 0.001 | 0.001 |
| Phytanoyl-CoA oxidase | 0.001 | 0.001 |
| 2-Hydroxyphytanoyl-CoA lyase | 0.219 | 0.119 |
| Sterol carrier protein-2 | 0.001 | 0.001 | 0.001 |

1 Values are means ± SEM, n = 7–8. Means in a row with superscripts without a common letter differ, \(P < 0.05\).
oxidation and hence attenuate phytol-dependent changes in the metabolic activity. Unexpectedly, however, the hepatic phytanic acid concentration was much lower for mice fed phytol and palm oil in combination than for those fed phytol and safflower or fish oil in combination. Therefore, it is difficult to consider that alterations in the hepatic phytic acid concentration are responsible for the dietary fat–dependent changes in the activity of dietary phytol to upregulate hepatic fatty acid oxidation.

There is the possibility that alterations in the gene expression of proteins involved in phytol metabolism are responsible for dietary fat–dependent changes in the hepatic phytic acid concentration. There were no correlations between the abundance of mRNAs for ALDH3a2, phytanoyl-CoA hydroxylase, and 2-hydroxyphytanoyl-CoA lyase and the phytic acid concentration in the liver of mice fed phytol-containing diets. However, we found that SCP2 mRNA abundance was inversely correlated with the phytic acid concentration in the liver of mice fed phytol diets containing various fats. It was demonstrated that phytanic acid accumulated in the tissues of SCP2 null mice (34,39), indicating that this protein plays a crucial role in the degradation of phytic acid. Therefore, changes in SCP2 mRNA expression may account for the dietary fat–dependent changes in the hepatic phytic acid concentration.

In conclusion, we demonstrated that safflower oil rich in (n-6) fatty acids and fish oil rich in (n-3) fatty acids, especially the latter, compared with a saturated fat (palm oil), greatly attenuated the dietary phytol-dependent increase in the activities and mRNA abundance of enzymes involved in hepatic fatty acid oxidation in mice. We also showed that dietary fat type markedly modified the hepatic concentration of phytic acid, a metabolite of phytol that is a potent activator of RXR (8–11) and of PPAR (9,10,12–15). However, the changes in the hepatic phytic acid concentration cannot account for the modification by dietary fat type of the physiological activity of dietary phytol to increase hepatic fatty acid oxidation.

LITERATURE CITED