Dietary phytic acid improves serum and hepatic lipid levels in aged ICR mice fed a high-cholesterol diet

Sung-Hyen Lee, Hong-Ju Park, Hye-Kyung Chun, So-Young Cho, Hyun-Jin Jung, Soo-Muk Cho, Dae-Yong Kim, Min-Soo Kang, Hyun Soon Lillez

Abstract

Aging is a multifactorial phenotype. Several clinical conditions directly related to lipid metabolism that induces hypertriglyceridaemia, hypercholesterolaemia, and cardiovascular disease occur during aging. Attention has been focused on possible intervention strategies to control serum lipid levels. Phytic acid is a plant component in most grains that is the main source of energy intake for the aged, and its antioxidant and antinutrient effects have been reported. However, its effect on lipid metabolism in the aged model has not been evaluated. This study was carried out to investigate the effect of phytic acid on serum and hepatic lipid levels in aged mice. A total of 40 aged ICR male mice were fed purified diets supplemented with 0% (P0), 0.5% (P5), 1.0% (P10), and 1.5% (P15) sodium phytate for 12 weeks. There were no significant differences in food intake, body weight, and organ weight among the experimental groups. The concentrations of the serum low-density lipoprotein cholesterol, hepatic triacylglycerol, and total cholesterol, and the apparent absorption rates of total lipid and cholesterol were lower in the P15 group than in the P0 group. Serum high-density lipoprotein cholesterol levels of all groups fed phytate-containing diets were higher than that of the P0 group. The severity of fatty liver decreased as phytate percentage in the diet increased. The amounts of fecal total lipid, triacylglycerol, and total cholesterol were higher in the P10 and the P15 groups. These results suggested that phytate affect the serum and hepatic lipid levels in aged mice by increasing their fecal lipid content. Consuming phytate-rich foods may reduce serum and hepatic lipid levels in the aged.

Keywords: Phytic acid; Aged mice; Serum; Liver; Lipid level

1. Introduction

Population aging was one of the most distinctive demographic events of the 20th century [1]. The worldwide prolongation of mean life expectancy has resulted in a rapid increase in the size of the elderly population leading to an increased incidence of age-related diseases (e.g., atherosclerosis). Aging is a multifactorial phenomenon and there are several clinical conditions directly related to lipid metabolism that contribute to hypertriglyceridaemia, hypercholesterolaemia, and cardiovascular disease during aging. Attention has focused on possible interventions to control the levels of serum lipids, such as triacylglycerol, total cholesterol, and low- (LDL) or high-density lipoprotein (HDL) cholesterol [2,3]. The HDL has long been known to be antiatherogenic [4]. This atheroprotective effect is attributed to the role played by HDL in the reverse transport
of cholesterol and to their antioxidant properties [5]. The search for therapeutic agents that improve blood lipid profiles has been of great interest [6-8].

Phytic acid is a plant component that exists in most grains and legumes, which are the main source of energy intake for the elderly, and is the subject of considerable research because of its antinutrient, antioxidant, and anticancer properties in growing or adult models [9-11]. Its hypoglycemic and lipid lowering effects in diabetic mice has been reported [12,13], but reports regarding its effects on lipid metabolism in the aged rodent model have been rather scarce [14,15]. The hypothesis of the current study was that phytate would favorably reduce lipid levels in the aged rodent model. Therefore, we determined the effect of a diet containing phytate on parameters of lipid metabolism, that is, serum and hepatic lipids, fecal lipids, and lipid absorption ratio in the aged mice fed test diets without or with dietary phytate.

2. Methods and materials

2.1. Animals and feeding studies

A total of 60 young (4-week-old) ICR male mice purchased from the Laboratory Animal Center (Daehan Biolink Ltd, Daejon, South Korea) were housed in stainless steel wire cages and maintained on a 12-hour light/dark cycle in a temperature-controlled environment (22°C) with free access to Standard Laboratory Diet (Harlan Teklad, Madison, WI) and water ad libitum for 14 months. Forty aged (15-month-old) ICR male mice were used in this study. All mice were individually housed in stainless steel wire cages in the same controlled environment and were divided into 4 groups of mice with similar weight. Animal care and experiments were carried out according to the approved guidelines established by the Rural Resources Development Institute experimental animal care committee, Gweonson-gu Suwon, South Korea.

Table 1
Composition of the experimental diets fed to mice (g/kg diet)

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P5</th>
<th>P10</th>
<th>P15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>570.8</td>
<td>571</td>
<td>572.1</td>
<td>573.2</td>
</tr>
<tr>
<td>Phytate a</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>CaCO3</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>17.4</td>
<td>12.2</td>
<td>6.1</td>
<td>0</td>
</tr>
<tr>
<td>Casein</td>
<td>140</td>
<td>140</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Soy bean oil</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Lard</td>
<td>75</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>α-Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>AIN-93 mineral mix b (Ca, P-free)</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>AIN-94 vitamin mix c</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

* Phytic acid, sodium phytate from corn (Sigma).
* AIN-93M mineral mixture (ICN, Aurora, Ohio).
* AIN-93VX vitamin mixture (ICN, Ohio).

Composition of diets is shown in Table 1. P0 (control) was formulated using casein and corn starch with no added phytate, whereas P5, P10, and P15 were supplemented with 0.5%, 1.0%, and 1.5% sodium phytate (Sigma, St Louis, Mo), respectively, in the basal diet (Table 1). The P5 diet group corresponded to a diet with a phytate content equivalent to normal human consumption [16]. The P10 and P15 diets contained 2 and 3 times more of phytate than the P5 diet, respectively. The fat content in the diets was adjusted to 15% by addition of corn oil and lard [12]. All diets were adjusted to contain identical amounts of nutrients except phytate so that the effect of increasing amounts of phytate could be evaluated. Diets and deionized water were provided ad libitum for 12 weeks. Records of daily feed intake and weekly body weight changes of individual mice were maintained throughout the experiment.

2.2. Analytic procedures

The aged mice were fasted overnight and mice were euthanized before blood collection from the eye vein after 12 weeks of diet feeding [13]. Serum was prepared by centrifugation (3000 rpm, 10 minutes) after standing for 30 minutes at room temperature. Liver, kidney, heart, spleen, and epididymal fat pads were obtained. The liver samples were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Sections (5 μm thick) were stained with haematoxylin and eosin, and were histologically examined [17].

Composition of diets in Table 1. P0 (control)

Table 2
Food intake, final body weight, and food efficiency ratio of the mice

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P5</th>
<th>P10</th>
<th>P15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>2.5 ± 0.1 NS</td>
<td>2.4 ± 0.1</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>41.3 ± 0.6 NS</td>
<td>41.7 ± 0.7</td>
<td>41.3 ± 0.5</td>
<td>41.7 ± 0.7</td>
</tr>
<tr>
<td>FER a</td>
<td>2.3 ± 0.3 NS</td>
<td>2.3 ± 0.5</td>
<td>2.1 ± 0.3</td>
<td>2.6 ± 0.3</td>
</tr>
</tbody>
</table>

* Food efficiency ratio = weight gain (g)/Food intake (g) × 100.

Values are means ± SEM; NS indicates not significant.

P0 (0% phytic acid), P5 (0.5% phytic acid), P10 (1.0% phytic acid), P15 (1.5% phytic acid).

Composition of diets is shown in Table 1. P0 (control) was formulated using casein and corn starch with no added phytate, whereas P5, P10, and P15 were supplemented with 0.5%, 1.0%, and 1.5% sodium phytate (Sigma, St Louis, Mo), respectively, in the basal diet (Table 1). The P5 diet group corresponded to a diet with a phytate content equivalent to normal human consumption [16]. The P10 and P15 diets contained 2 and 3 times more of phytate than the P5 diet, respectively. The fat content in the diets was adjusted to 15% by addition of corn oil and lard [12]. All diets were adjusted to contain identical amounts of nutrients except phytate so that the effect of increasing amounts of phytate could be evaluated. Diets and deionized water were provided ad libitum for 12 weeks. Records of daily feed intake and weekly body weight changes of individual mice were maintained throughout the experiment.

2.2. Analytic procedures

The aged mice were fasted overnight and mice were euthanized before blood collection from the eye vein after 12 weeks of diet feeding [13]. Serum was prepared by centrifugation (3000 rpm, 10 minutes) after standing for 30 minutes at room temperature. Liver, kidney, heart, spleen, and epididymal fat pads were obtained. The liver samples were fixed in 10% neutral buffered formalin and processed for paraffin embedding. Sections (5 μm thick) were stained with haematoxylin and eosin, and were histologically examined [17].

Table 3
Comparison of the liver, kidney, heart, spleen, and epididymal fat pad weights (g) of mice

<table>
<thead>
<tr>
<th></th>
<th>P0</th>
<th>P5</th>
<th>P10</th>
<th>P15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>1.81 ± 0.08 NS</td>
<td>1.83 ± 0.07</td>
<td>1.69 ± 0.07</td>
<td>1.76 ± 0.08</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.60 ± 0.02 NS</td>
<td>0.60 ± 0.02</td>
<td>0.61 ± 0.02</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>Heart</td>
<td>0.19 ± 0.01 NS</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.09 ± 0.01 NS</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Epididymal fat pad</td>
<td>0.48 ± 0.06 NS</td>
<td>0.37 ± 0.04</td>
<td>0.47 ± 0.08</td>
<td>0.41 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

P0 (0% phytic acid), P5 (0.5% phytic acid), P10 (1.0% phytic acid), P15 (1.5% phytic acid).
via the Friedewald formula, which is reliable when triacylglycerol levels are lower than 400 mg/dL [20].

Hepatic and fecal total lipid, triacylglycerol, and total cholesterol were measured by using established methods [21-25]. Fecal samples were collected over the final 4 days of the exposure period, freeze-dried, and ground before analysis. Total lipid was extracted by the method of Folch et al [23] and was gravimetrically determined. Triacylglycerols were measured using the method described by Biggs et al [24] and cholesterol was determined using the method described by Zlatkis et al [25]. The apparent total lipid absorption rate (%) was defined as: (daily ingested total lipid – daily fecal total lipid excretion) × 100% / daily ingested total lipid. The apparent absorption rate of total cholesterol was calculated accordingly.

2.3. Statistical analysis

Data analyses were performed using SPSS software (SPSS 10.0 for Windows; SPSS, Chicago, IL). All data were expressed as means ± SEM. Analysis of variance was used to test for differences between the groups. Duncan’s multiple range test was used to determine the significance of differences among the means at $P < .05$ [26].

3. Results

3.1. Feed intake, body weight change, and organ and fat weights

Table 2 shows food intake and food efficiency ratio of the 4 groups fed the diets for 12 weeks. There were no significant differences in feed intakes, final body weights, and feed efficiency ratio among all groups. Liver, kidney, heart, spleen, and epididymal fat pad weights were similar among the 4 groups (Table 3).

3.2. Serum triacylglycerol, total cholesterol, LDL cholesterol, and HDL cholesterol

Fig. 1 presents the serum lipid profiles of the 4 experimental groups fed diets with 0%, 0.5%, 1.0%, and 1.5% phytate. Serum triacylglycerol levels were not significantly ($P < .05$) different between groups supplemented...
with or without phytate. In contrast, serum concentrations of total cholesterol were lower in the P5, P10, and P15 groups as compared with the P0 group and a significant \((P < .05)\) difference was found in the P10 group. Serum LDL cholesterol levels were lower in the groups fed phytate, and a significant difference from control (P0) was shown in P15 with a trend toward decreasing levels with increasing phytate concentrations. Serum HDL cholesterol levels were significantly \((P < .05)\) higher in the 3 groups fed phytate than in the P0 group fed no phytate.

3.3. Total lipid, triacylglycerol, and total cholesterol levels in the liver

Fig. 2 shows the hepatic lipid levels of aged mice fed diets supplemented with or without phytate. Total lipid, triacylglycerol, and total cholesterol levels in the liver were reduced in mice fed phytate diets as compared to the P0 group fed diet without phytate. Hepatic total lipid level was significantly \((P < .05)\) lower in all groups fed diets supplemented with phytate compared with that of the control group (P0). Hepatic triacylglycerol level was significantly \((P < .05)\) lower in the P10 and P15 groups supplemented with 1.0% and 1.5% of phytate than that of the P0 group. Hepatic total cholesterol level was significantly \((P < .05)\) reduced in the P15 group compared with the P0 group.

Histologic differences in the liver were found in the experimental groups (Fig. 3). Compared to the P0 group, the severity of fatty liver decreased as phytate was given to mice. These findings reflect the hepatic lipid profiles, which showed lower values of total lipid, triacylglycerol, and total cholesterol in the phytate groups as compared to the control group (PO) not fed phytate.

3.4. Total lipid, triacylglycerol, and total cholesterol levels excreted in feces

Fig. 4 presents the amount of lipid excreted in feces of aged mice fed diets supplemented with or without phytate. Total lipid, triacylglycerol, and total cholesterol levels in feces were significantly \((P < .05)\) increased in the groups supplemented with 1.0% and 1.5% phytate compared with the P0 group.

Dietary phytic acid reduced the amount of total lipid and cholesterol absorbed in aged mice (Fig. 5). The apparent absorption ratio of total lipid was significantly \((P < .05)\) lower in the P15 group than that of the P0 group. The apparent absorption ratio of total cholesterol was significantly \((P < .05)\) reduced in the P5, P10, and P15 groups as compared with the P0 group (Fig. 5).

4. Discussion

A number of studies have demonstrated that higher serum cholesterol levels are associated with an increase in subsequent morbidity and mortality due to coronary heart disease [22,23].
Parallel to changes in serum cholesterol levels, coronary heart disease mortality has also declined among US adults [24]. High levels of total cholesterol and LDL cholesterol are major risk factors for cardiovascular diseases, whereas increased HDL cholesterol is associated with a decrease in cardiovascular disease risk [25]. It is well known that HDL cholesterol plays an important role in the transport of cholesterol from the periphery to the liver by the “reverse cholesterol transport” pathway. However, most drugs used in the management of hypercholesterolemia decrease both total cholesterol and HDL cholesterol. In this study, serum total cholesterol and LDL cholesterol were lower in the aged mice fed diets supplemented with 1.0% or 1.5% phytate of the diet as compared to the P0 group fed a diet without phytate. Moreover, the serum HDL cholesterol levels were higher in the P5, P10, and P15 groups than in the P0 group. This result is consistent with a previous report that serum HDL cholesterol was higher in animals fed a phytate diet as compared with a control diet without phytate [13]. Our study showed that 1.0% or 1.5% phytate dietary supplementation effectively decreased the levels of serum total cholesterol and LDL cholesterol, but increased HDL cholesterol. These results indicate that higher doses of phytate supplementation confer more beneficial effects in the management of an aged rodent model of hypercholesterolemia.

Hepatic total lipid, total cholesterol, and triacylglycerol levels were also effectively decreased in the mice groups given P10 or P15 diets as compared with the P0 diet. The observed increase in serum HDL cholesterol in the P10 and P15 groups may be related to decreased serum cholesterol and subsequent reduced hepatic cholesterol synthesis through depression of HMG CoA reductase [26]. Convincing experimental evidence has been presented for depressing increases in hepatic lipids and in the hepatic activities of lipogenic enzymes by dietary phytate in the growing animal [27]. The beneficial effect of dietary phytate on the prevention of fatty liver in the aged mice may be mediated through the reduction of hepatic lipogenesis [28]. Elevated triacylglycerols have also been reported to increase the incidence of coronary heart disease [29]. In our study, 1.0% and 1.5% phytate dietary supplementation significantly ($P < .05$) reduced hepatic triacylglycerol levels and significantly ($P < .05$) increased fecal triacylglycerol levels. It appears that hepatic triacylglycerol concentrations in the groups fed diets supplemented with phytate decreased by increasing the fecal excretion of triacylglycerol. Interestingly, the hepatic lipid levels decreased as the levels of phytate in the diet increased.

These results demonstrate that serum and hepatic lipid profiles in the aged mice were clearly improved by dietary phytate in the range of 1.0% rather than 0.5% of diet. The reducing effect of phytate on serum and hepatic total cholesterol was partially explained by an effect of increasing fecal cholesterol or bile acids [30]. Therefore, phytate may be a beneficial dietary supplement in the management of aged hyperlipidemia in patients and in the prevention of related diseases. In contrast, many studies suggested that phytate decreased mineral bioavailability in growing animals because of its strong chelating ability [31,32]. Therefore, further studies are needed to investigate the action of phytate supplementation exceeding 1.0% of the diet on mineral bioavailability in the aged model.

Acknowledgment

This study was supported by a Biogreen 21 Project grant from the Rural Development Administration of Korea. The authors thank Margie Nichols for her critique and editing of the manuscript.

References


Fig. 5. Effects of dietary phytic acid on apparent absorption ratio of total lipid and total cholesterol in the aged mice. Values are means ± SEM (n = 10). Values with different letters are significantly different at $P < .05$, as assessed by Duncan’s multiple range test.