Effects of dietary ascorbic acid supplementation on lipid peroxidation and the lipid content in the liver and serum of magnesium-deficient rats

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Abstract. We investigated the effects of ascorbic acid (AsA) supplementation on lipid peroxidation and the lipid content in the liver and serum of magnesium (Mg)-deficient rats. Eighteen 3-week-old male Sprague-Dawley strain rats were divided into 3 groups and maintained on a control diet (C group), a low-Mg diet (D group), or a low-Mg diet supplemented with AsA (DA group) for 42 d. At the end of this period, the final body weight, weight gain, and serum Mg concentrations were significantly decreased in the Mg-deficient rats. Further, dietary AsA supplementation had no effect on the growth, serum Mg concentration, Mg absorption, and Mg retention. The serum concentration of AsA was significantly lower in the D group than in the C group but was unaltered in the DA group. The levels of phosphatidylcholine hydroperoxide (PCOOH) in the serum and of triglycerides (TGs) and total cholesterol (TC) in the serum and liver were significantly higher in the D group than in the C group. The serum PCOOH, liver TG, and liver TC levels were decreased in the DA group. These results indicate that Mg deficiency increases the AsA requirement of the body and that AsA supplementation normalizes the serum levels of PCOOH and the liver lipid content in Mg-deficient rats, without altering the Mg status.

Key words: magnesium deficiency, ascorbic acid, phosphatidylcholine hydroperoxide, lipids

Several researchers have reported that magnesium (Mg) deficiency increases the iron (Fe) [1, 2], triglyceride (TG) and total cholesterol (TC) contents in the liver [3] and decreases the Mg [4-6] and high-density lipoprotein (HDL)-cholesterol (HDL-C) levels in serum [3]. We previously found that Mg-deficient rats exhibit increased levels of phosphatidylcholine hydroperoxide (PCOOH), a primary product of lipid peroxidation in biological membranes, in plasma and several tissues [7]. Further, Hus et al. [8] reported that Mg-deficient rats exhibit reduced concentrations of ascorbic acid (AsA) in the liver. AsA has been known to play important roles in many biochemical reactions. Antioxidants and antioxidant enzymes are the primary elements involved in defence responses that protect organisms from oxidative damage, and AsA is an important biological antioxidant [9]. AsA and α-tocopherol synergistically react with organic free radicals; the antioxidant properties of these compounds are known to be responsible for their biological activity. These facts suggest that reduced concentrations of AsA also influence lipid peroxidation in Mg-deficient rats. In this study, hypothesizing that Mg deficiency may increase the requirement of AsA, we investigated the effects of dietary AsA supplementation on lipid peroxidation and the lipid content in the liver and serum of Mg-deficient rats.
Materials and methods

Eighteen 3-week-old male Sprague-Dawley rats (Clea Japan, Tokyo, Japan) were housed individually in stainless-steel cages at 22°C, under a 12-h light/12-h dark cycle. The Tokyo University of Agriculture Animal Use Committee approved the study, and the animals were maintained in accordance with the university guidelines for the care and use of laboratory animals. All the rats were fed a control diet, formulated on the basis of the AIN-93G diet [10], over a 2-d acclimatization period. After this period, the rats were randomly assigned to 3 experimental groups, each containing 6 rats, and were maintained on one of the following 3 diets: the control diet (C group); a low-Mg diet containing 0.008% Mg (D group), and a low-Mg diet supplemented with 0.03% AsA (DA group). The rats in the C and DA groups were pair-fed their respective diets with the rats in the D group. All the rats were provided free access to deionized water. Faeces and urine samples were collected from all the rats for 3 d prior to the analysis of Mg. The rats were maintained on the different diets for 42 d, following which they were sacrificed, and blood and liver samples were collected for analyses. The blood samples were centrifuged, and the supernatants were used as serum samples. The liver was perfused with cold 0.9% NaCl solution and resected.

To measure the Mg levels, the urine samples, micropulverized faecal samples, and liver samples were collected from all the rats for 3 d prior to the analysis of Mg. The rats were maintained on the different diets for 42 d, following which they were sacrificed, and blood and liver samples were collected for analyses. The blood samples were centrifuged, and the supernatants were used as serum samples. The liver was perfused with cold 0.9% NaCl solution and resected.

To measure the Mg levels, the urine samples, micropulverized faecal samples, and liver samples were collected from all the rats for 3 d prior to the analysis of Mg. The rats were maintained on the different diets for 42 d, following which they were sacrificed, and blood and liver samples were collected for analyses. The blood samples were centrifuged, and the supernatants were used as serum samples. The liver was perfused with cold 0.9% NaCl solution and resected.

Liver lipids were extracted using a mixture of chloroform and methanol (in a volume ratio of 2:1), by the method described by Folch et al. [14]. The TG and TC concentrations in the liver and serum were measured using enzymatic colorimetric methods, with the triglyceride E-test and cholesterol C-test Wako kits (Wako Pure Chemical Industries, Osaka, Japan).

Each result was expressed as the mean ± SE for each group comprising 6 rats. One-way analysis of variance (ANOVA) was performed, followed by Fisher’s protected least square difference (PLSD) test to determine whether the differences among the groups were significant. The differences were considered significant at p < 0.05.

Results

The final body weight and weight gain were significantly lower in the D and DA groups than in the C group but did not differ between the former 2 groups (table 1). The apparent absorption, retention, and serum concentrations of Mg were significantly lower in the D and DA groups than in the C group (table 2). The serum AsA levels were significantly lower in the D group than in the C and DA groups but did not differ between the latter 2 groups (table 3). The serum PCOOH levels were significantly elevated under conditions of Mg deficiency but were suppressed by dietary AsA supplementation (table 3). The TG and TC concentrations in the liver were significantly higher in the D group than in the C and DA groups, while those in the serum were significantly higher in the D and DA groups than in the C group but not differ between the former 2 groups (table 3).

Discussion

Mg deficiency reduced the final body weights of the animals in the present study, consistent with previ-

Table 1. Changes of body weight and food intake in control rats (C), Mg-deficient rats (D) and Mg-deficient rats supplemented with AsA (DA).

<table>
<thead>
<tr>
<th>Group</th>
<th>C (g)</th>
<th>D (g)</th>
<th>DA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>42.07 ± 1.61</td>
<td>43.27 ± 1.22</td>
<td>44.45 ± 1.18</td>
</tr>
<tr>
<td>Final body weight</td>
<td>268.36 ± 3.03a</td>
<td>227.78 ± 3.08b</td>
<td>228.02 ± 3.39b</td>
</tr>
<tr>
<td>Weight gain</td>
<td>226.29 ± 2.28a</td>
<td>184.50 ± 2.15b</td>
<td>183.57 ± 2.40b</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>12.99 ± 0.29</td>
<td>13.01 ± 0.19</td>
<td>13.05 ± 0.25</td>
</tr>
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</table>

Data are presented as the means ± SE for each group of six rats.

a, b Values with different superscript letters are significantly different (p < 0.05).
Dietary Mg deficiency is reported to affect the growth of rats via a reduction in the protein utilization [6]. The apparent Mg absorption and retention and the serum Mg levels decreased in the Mg-deficient rats in the present study. These results were consistent with those of previous studies [5, 6]. However, AsA supplementation did not affect the absorption, retention, and serum concentrations of Mg in the Mg-deficient rats.

Hsu et al. [8] reported that Mg-deficient rats exhibit a reduced capacity to utilize glucuronolactone or gulonolactone for the synthesis of AsA in the liver. In the present study, we observed reduced AsA levels in the rat sera. It has been suggested that Mg deficiency interferes with AsA synthesis and increases the AsA requirement of the body. We have previously reported that Mg-deficient rats exhibit elevated serum levels of PCOOH [7]. Similar to the results of our previous study, those obtained in the present study revealed that the serum PCOOH levels were elevated in Mg-deficient rats. Glutathione catalyzes the conversion of dehydro-AsA to AsA, and Mg deficiency affects the metabolism of glutathione [15]. The reduced serum levels of AsA that are induced by Mg deficiency may in turn increase the PCOOH levels, in association with an impairment in the glutathione metabolism; however, we did not analyse the glutathione levels of the rats in the present study. Nevertheless, dietary AsA supplementation restored the serum levels of AsA and suppressed the increase in the PCOOH concentrations induced by Mg deficiency.

It is well known that Mg deficiency is accompanied by an inflammatory syndrome characterized by release of inflammatory cytokines and acute phase proteins [16]. This acute phase response has been shown to be responsible for oxidative stress and lipid disturbances induced by Mg deficiency. In addition, Ikeda et al. reported that the serum concentration of IL6, an inflammatory cytokine that stimulates gene expression of acute phase proteins, has been shown to be higher in AsA deficient rats, and AsA deficiency causes changes similar to those that occur in the acute phase response [17]. Thus the possibility might exist that a less severe inflammatory response explains the beneficial effect of AsA supplementation in Mg deficient rats and that the inflammatory response in Mg deficient rats participates in AsA deficiency.

Mg deficiency increases the very low-density lipoprotein (VLDL)-cholesterol and LDL-cholesterol levels but decreases the HDL-cholesterol levels, because this condition decreases the activity of lecithin-cholesterol acyltransferase (LCAT) [18].

**Table 2.** Changes of Mg balance and serum Mg in control rats (C), Mg-deficient rats (D) and Mg-deficient rats supplemented with AsA (DA).

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>D</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent Mg absorption (mg/day)</td>
<td>6.48 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.82 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg retention (mg/day)</td>
<td>6.22 ± 0.184&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum Mg (mg/dL)</td>
<td>1.82 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.57 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.43 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Data are presented as the means ± SE for each group of six rats.<br><sup>a, b</sup> Values with different superscript letters are significantly different (p < 0.05).

**Table 3.** Changes of serum AsA, PCOOH, TG and TC and liver TG and TC in control rats (C) Mg-deficient rats (D) and Mg-deficient rats supplemented with AsA (DA).

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>D</th>
<th>DA</th>
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<tbody>
<tr>
<td>Serum AsA (mg/dL)</td>
<td>1.08 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.78 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.04 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Serum PCOOH (pmol/mL)</td>
<td>40.61 ± 3.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.94 ± 3.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.68 ± 4.36&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Serum TG (mg/dL)</td>
<td>122.16 ± 10.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>145.66 ± 11.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150.20 ± 15.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum TC (mg/dL)</td>
<td>93.25 ± 5.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104.64 ± 8.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.07 ± 4.70&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver TG (mg/g)</td>
<td>16.30 ± 1.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.91 ± 2.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.06 ± 0.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver TC (mg/g)</td>
<td>2.07 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.51 ± 0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.00 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

Data are presented as the means ± SE for each group of six rats.<br><sup>a, b</sup> Values with different superscript letters are significantly different (p < 0.05).
Moreover, Mg deficiency increases the percentage composition of triglycerides in VLDL, LDL, and HDL, and reduces that of proteins [19]. AsA plays an important role in cholesterol metabolism. Compared to normal guinea pigs, guinea pigs deficient in AsA are reported to exhibit significantly higher levels of cholesterol in the serum and liver; this is because cholesterol catabolism is significantly impaired in AsA-deficient animals, owing to a reduction in the 7α-hydroxylation of cholesterol [20]. Mg deficiency may secondarily induce a condition similar to that observed in AsA deficiency and may impair lipid metabolism. In the present study, we observed elevated serum concentrations of TG and TC in the Mg-deficient rats; however, dietary AsA supplementation did not affect these concentrations. Further, we measured the liver concentrations of TG and TC: these concentrations were elevated in the Mg-deficient rats but were normalized with AsA supplementation.

Although many studies have demonstrated that AsA exerts a cholesterol-lowering effect, its effects on cholesterol metabolism remain debated [21]. In a previous study on humans, no significant changes were observed in the plasma cholesterol or TG levels of hypercholesterolaemic subjects who had been receiving AsA (4 g/d) orally for 2 months. In our animal experiment, no significant changes were observed in the serum TC and TG levels after dietary AsA supplementation although the liver TG and cholesterol levels were reduced by AsA supplementation in Mg-deficient rats. This discrepancy may be attributable to differences in the feeding conditions, such as the dose of AsA, the dietary regimen and the age of the subjects.

In this study, we investigated the effects of dietary AsA supplementation on lipid peroxidation and the lipid content in the liver and serum of Mg-deficient rats. The elevated serum levels of PCOOH and TG and TC lipids were lowered by AsA supplementation. These results indicate that Mg deficiency increases the AsA requirement of the body and that dietary AsA supplementation can normalize the serum levels of PCOOH and the liver lipid content in the liver and serum of Mg-deficient rats, without altering the Mg status.

References


