Effects of a high-calcium diet on serum insulin-like growth factor-1 levels in magnesium-deficient rats

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Abstract. In order to clarify the effects of a high-calcium (Ca) diet on bone formation in magnesium (Mg)-deficient rats, this study focused on the effects of a high-Ca diet on serum insulin-like growth factor-1 (IGF-1) levels. Male rats were randomized by weight into four groups, and fed one of four experimental diets containing two different Mg concentrations (0.05% (normal-Mg) or Mg-free (Mg-deficient)), and two different Ca concentrations (0.5% (normal-Ca) or 1.0% (high-Ca)) for 14 days. Serum concentrations of osteocalcin and IGF-1 were significantly lower in rats fed the Mg-deficient diet than in rats fed the normal-Mg diet. On the other hand, dietary Ca concentration had no significant influence on serum concentrations of osteocalcin and IGF-1. This study suggested that: 1) a high-Ca diet has no preventive effects on the decreased bone formation seen in Mg-deficient rats; and 2) a high-Ca diet does not enhance serum IGF-1 levels in Mg-deficient rats. Moreover, unchanged serum IGF-1 concentrations may contribute to the decreased bone formation seen in Mg-deficient rats receiving a high-Ca diet.

Key words: high-calcium diet, bone formation, serum insulin-like growth factor-1, magnesium-deficient rats

Magnesium (Mg) plays an important role in bone growth, and a large number of studies have shown that Mg deficiency impairs bone growth. Epidemiological studies have confirmed a relationship between dietary Mg intake and bone mineral content and/or bone mineral density (BMD) [1, 2]. In experimental studies, Mg deficiency has been shown to result in decreased BMD and bone strength in rats [3, 4], and rats fed a Mg-deficient diet show decreased bone formation and increased bone resorption [5, 6].

Calcium (Ca) intake is known to be important in the maintenance of bone mass and the prevention of fractures [7, 8]. Hence, we examined the effects of a high-Ca diet on bone metabolism in Mg-deficient rats, and consequently found that a high-Ca diet had no preventive effects on the decreased bone formation seen in Mg-deficient rats [9, 10]. This result was very interesting, as bone growth is generally believed to be maintained by high Ca intake. We thus decided to examine more closely the reasons why the decreased bone formation in Mg-deficient rats was not prevented by consumption of a high-Ca diet.

Here, we focused on insulin-like growth factor-1 (IGF-1). IGF-1 is produced by osteoblasts in bone, and is an important factor in the development and growth of bone [11-14]. Furthermore, our previous study [15] reported that serum IGF-1 concentrations are lower in rats fed a Mg-deficient diet, thus suggesting that the decreased bone formation under Mg deficiency is caused by decreased serum IGF-1 levels. We also hypothesized that the effects of a high-Ca diet on decreased bone formation seen in Mg-deficient rats...
formation in Mg-deficient rats may be related to changes in serum IGF-1 levels. Accordingly, this study investigated the effects of a high-Ca diet on serum IGF-1 levels in Mg-deficient rats.

Materials and methods

Four-week-old male Wistar rats (Charles River Laboratories Japan, Kanagawa, Japan) were housed in individual, stainless-steel wire-mesh cages. During the experiment, cages were located in a room with controlled lighting with a 12-h light:dark cycle (light, 0700-1900 h), a temperature of 22 ± 1°C and relative humidity of 60-65%. Experimental diets were based on the AIN-93G diet [16]. The four experimental diets contained two different Mg concentrations (0.05% (normal-Mg) or Mg-free (Mg-deficient)) and two different Ca concentrations (0.5% (normal-Ca) or 1.0% (high-Ca)). Ca concentrations in the experimental diets were adjusted using calcium carbonate.

Before the study period, there was a four-day acclimation period, during which all rats were given free access to the normal-Mg diet containing normal-Ca and deionized water. After the acclimation period, rats were randomly divided into four groups of six rats, with each group having a similar mean body weight. Each group was fed one of the four experimental diets differing in Mg and Ca concentrations for 14 days. Rats fed the other experimental diets were fed the mean weight of food consumed by the rats fed the Mg-deficient diet containing high-Ca on the previous day. Rats were given free access to deionized water. On the last three days of the experimental period, rats were housed individually in stainless-steel metabolic cages. Feces were collected from each rat, and were analyzed to determine the apparent mineral absorption. At the end of the experimental period, rats were sacrificed under diethyl ether, and blood was collected for analyses. The present study was approved by the Animal Use Committee at the Ibaraki Christian University, and all animals were maintained in accordance with the university’s guidelines for the care and use of laboratory animals.

Blood samples were centrifuged, and supernatants were used as serum samples. Ca and Mg levels in serum were measured with Calcium E and Magnesium B (Wako Pure Chemical Industries, Osaka, Japan), respectively. Osteocalcin in serum was measured with the Osteocalcin rat ELISA system (GE Healthcare Japan, Tokyo, Japan). C-terminal telopeptide of type I collagen (CTx) in serum was measured with a Rat/Laps™ EIA (Immunodiagnostic Systems Ltd., UK). IGF-1 in serum was measured with a Rat/Mouse IGF-1 ELISA (Immunodiagnostic Systems Ltd., USA). Feces were dried and pulverized with a mill. Samples of experimental diet and feces were ashed at 550°C for 48 h in a muffle furnace, and minerals were extracted in 1 mol/L of HCl for analysis. Ca and Mg levels were determined by atomic absorption spectrophotometry (ANA-182; Tokyo Photo Electric, Tokyo, Japan). The apparent absorption of Ca and Mg was calculated as follows: apparent absorption (mg/day) = intake − fecal excretion.

Results

Of the rats fed the normal-Ca diet, final body weight was significantly lower in rats fed the Mg-deficient diet than in rats fed the normal-Mg diet (table 1). Serum Ca concentrations among rats fed the Mg-deficient diet were significantly higher in rats fed the high-Ca diet than in rats fed the normal-Ca diet. Serum Mg concentrations were significantly lower in rats fed the Mg-deficient diet than in rats fed the normal-Mg diet, irrespective of dietary Ca concentration. The apparent Ca absorption was significantly higher in rats fed the high-Ca diet than in rats fed the normal-Ca diet. The dietary Mg concentration had no significant influence on apparent Ca absorption. The apparent Mg absorption was significantly lower in rats fed the Mg-deficient diet than in rats fed the normal-Mg diet, irrespective of dietary Ca concentration. Serum osteocalcin concentrations were significantly lower in rats fed the Mg-deficient diet than in rats fed the normal-Mg diet. Serum CTx levels in rats fed the Mg-deficient diet were significantly higher than in rats fed the normal-Mg diet. Dietary Ca concentrations also had no
Table 1. Body weight, serum mineral concentrations, apparent mineral absorption, biochemical markers of bone turnover and serum IGF-1 concentrations in rats fed on experimental diets1.

<table>
<thead>
<tr>
<th></th>
<th>Normal-Mg</th>
<th>Mg-deficient</th>
<th>Two-way ANOVA2</th>
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<tbody>
<tr>
<td></td>
<td>Normal-Ca</td>
<td>High-Ca</td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>116.2 ± 3.8</td>
<td>117.9 ± 6.5</td>
<td>117.1 ± 5.6</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>195.0 ± 2.7a</td>
<td>189.5 ± 6.7ab</td>
<td>184.4 ± 6.7b</td>
</tr>
<tr>
<td>Ca in serum (mmol/L)</td>
<td>2.34 ± 0.14a</td>
<td>2.42 ± 0.08a</td>
<td>2.36 ± 0.11a</td>
</tr>
<tr>
<td>Mg in serum (mmol/L)</td>
<td>0.78 ± 0.04a</td>
<td>0.63 ± 0.07b</td>
<td>0.24 ± 0.04c</td>
</tr>
<tr>
<td>Apparent Ca absorption (mmol/day)</td>
<td>1.16 ± 0.11a</td>
<td>1.39 ± 0.20b</td>
<td>1.08 ± 0.14a</td>
</tr>
<tr>
<td>Apparent Mg absorption (mmol/day)</td>
<td>0.229 ± 0.013a</td>
<td>0.226 ± 0.006a</td>
<td>0.024 ± 0.001b</td>
</tr>
<tr>
<td>Osteocalcin in serum (μg/L)</td>
<td>101.8 ± 10.5a</td>
<td>108.2 ± 14.6a</td>
<td>68.8 ± 15.4b</td>
</tr>
<tr>
<td>CTx in serum (μg/L)</td>
<td>33.3 ± 3.7a</td>
<td>37.7 ± 12.5ab</td>
<td>51.6 ± 12.7bc</td>
</tr>
<tr>
<td>IGF-1 in serum (nmol/L)</td>
<td>156.2 ± 10.3a</td>
<td>164.8 ± 15.0a</td>
<td>136.2 ± 22.1b</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 6 per group.  
2 Significant effect (p < 0.05): Mg = effect of dietary Mg concentration; Ca = effect of dietary Ca concentration; Mg×Ca = effect of interaction.  
a,b,cValues with different superscript letters are significantly different (p < 0.05).

Significant influence on the biochemical markers of bone turnover. Dietary Mg concentration had a significant influence on serum IGF-1 levels. Serum IGF-1 concentrations were significantly lower in rats fed the Mg-deficient diet than in rats fed the normal-Mg diet. On the other hand, dietary Ca concentration had no significant influence on serum IGF-1 levels.

Discussion

Bone metabolism is characterized by the formation of new bone and the resorption of old bone, and this is an important factor in the determination of bone quality. It is known that Mg deficiency adversely affects bone metabolism. Previous studies [5, 6, 15] observed that decreases in bone formation and increases in bone resorption in rats fed a Mg-deficient diet, which suggests that Mg deficiency impairs bone growth by uncoupling bone formation and bone resorption.

This study examined serum osteocalcin levels as the biochemical marker of bone formation, and showed decreases in bone formation in rats fed the Mg-deficient diet. We speculate that reduction of IGF-1 secretion contributed to the decrease in bone formation under Mg deficiency, since serum IGF-1 levels in this study were decreased in rats fed the Mg-deficient diet; this hormone plays an important role in the development and growth of bone [11-14].

Our previous study [10] investigated the effects of a high-Ca diet on bone turnover in Mg-deficient rats, and found that a high-Ca diet reduces the mineral apposition rate and surface referent bone formation rate in Mg-deficient rats. This study also showed that dietary Ca concentration had no effect on serum osteocalcin levels. These observations suggest that a high-Ca diet is unable to prevent bone impairment in Mg deficiency. High-Ca intake is generally known to increase BMD and bone mineral content [7, 8]; thus, it was noteworthy that our previous studies [9, 10] and this study showed that, despite the administration of a high-Ca diet, bone formation was not increased in Mg-deficient rats. We believe that serum IGF-1 levels may, at least partially, account for the decreased bone formation in Mg-deficient rats with administration of a high-Ca diet. As mentioned above, several studies [11-14] have demonstrated that IGF-1 plays an important role in bone formation. In vitro, IGF-1 reportedly stimulates the proliferation of osteoblast-like cells and bone collagen synthesis [11, 12]. In adult ovariectomized rats, IGF-1 treatment stimulates bone formation and enhances bone volume.
High-Ca diet and serum IGF-1 levels

[13]. IGF-1-deficient mice showed a reduction of bone development [14]. We observed decreases in serum IGF-1 concentrations in rats fed the Mg-deficient diet, and that the decrease in serum IGF-1 concentrations suppresses bone formation under Mg deficiency. In addition to this observation, the present study showed that a high-Ca diet had no effect on serum IGF-1 concentrations in rats fed the Mg-deficient diet. This indicates that a high-Ca diet does not enhance serum IGF-1 levels in Mg-deficient rats. Based on the present serum IGF-1 levels, we believe that unchanged serum IGF-1 levels contribute to the decreased bone formation seen in Mg-deficient rats receiving a high-Ca diet.

Dorup et al. [17] reported that serum IGF-1 concentrations were reduced in rats fed the Mg-deficient diet, while following supplementation with Mg, serum Mg concentrations normalized within one week, and serum IGF-1 also reached control levels after two weeks. These results suggest that IGF-1 secretion may be affected by serum Mg levels. In this study, serum Mg levels in rats fed the Mg-deficient diet were unchanged by the high-Ca diet. This suggests that lowered serum Mg levels may contribute to the inhibition of serum IGF-1 levels under Mg deficiency. Moreover, we speculated that the unchanged serum IGF-1 levels in Mg-deficient rats receiving a high-Ca diet might be explained by parathyroid hormone (PTH) and 1,25(OH)2-vitamin D3. Previous studies observed that PTH and 1,25(OH)2-vitamin D3 enhanced IGF-1 production [18-20]. Although serum PTH and 1,25(OH)2-vitamin D3 levels were not measured in this study, these hormones were probably decreased in the Mg-deficient rats by administration of the high-Ca diet, as serum Ca levels in Mg-deficient rats were elevated by the high-Ca diet. Mg deficiency and increased serum Ca levels reduce serum levels of PTH and 1,25(OH)2-vitamin D3.

This study found increases in bone resorption in rats fed the Mg-deficient diet. Mechanisms of increased bone resorption induced by Mg deficiency have been discussed in previous studies [6, 15, 21]. Substance P, tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) were enhanced in Mg-deficient rats, which suggests that increased substance P, TNF-α and IL-1 stimulate the increases in osteoclasts activity and bone resorption with Mg deficiency [6, 21]. Our previous study [15] also suggested that the high bone resorption in Mg-deficient rats was the result of an increase in receptor activator of nuclear factor-kappa B ligand (RANKL) secretion. Thus, cytokines (substance P, TNF-α and IL-1) and RANKL are important factors in the bone resorption seen with Mg deficiency. We observed that a high-Ca diet had no preventive effects on the increased bone resorption seen in Mg-deficient rats. This result may be explained by cytokines and RANKL. In other words, a high-Ca diet does not affect the cytokines and RANKL in Mg-deficient rats, and consequently, high bone resorption resulting from Mg deficiency was not prevented by administration of a high-Ca diet. However, further studies are needed to elucidate the details of the effects of a high-Ca diet on cytokines and RANKL under Mg deficiency.

In conclusion, we examined the effects of a high-Ca diet on serum IGF-1 levels and bone formation in Mg-deficient rats. Serum IGF-1 levels and bone formation were decreased in rats fed the Mg-deficient diet. In addition, serum IGF-1 levels and bone formation in Mg-deficient rats were not enhanced by administration of a high-Ca diet. These results suggest that: 1) a high-Ca diet has no influence on serum IGF-1 concentrations and bone formation in Mg-deficient rats; and 2) unchanged serum IGF-1 concentrations contribute to the decreased bone formation seen in Mg-deficient rats receiving a high-Ca diet.

Disclosure


References


