Dietary fatty acid composition alters magnesium metabolism, distribution, and marginal deficiency response in rats*

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Abstract. Based on dietary intake recommendations, magnesium deficiency commonly occurs throughout the world. However, widespread pathological conditions induced by dietary magnesium deficiency have not been identified. This discrepancy may be caused by other dietary factors ameliorating or exacerbating the response to a marginal magnesium deficiency and/or the length of the deficiency. Thus, a study was performed to determine whether the n-6/n-3 fatty acid composition of the diet affects the response to marginal magnesium deprivation, and whether the effect was dependent upon the length of deprivation. Weanling female rats were fed diets containing 250 mg/kg magnesium in a factorial arrangement with dietary variables of supplemental magnesium at 0 or 250 mg/kg (total of 250 or 500 mg/kg) and fat sources of 75 g/kg corn oil or 65 g/kg fish (menhaden) oil plus 10 g/kg linoleic acid. After 8 and 12 weeks on their respective diets, each rat was placed in a metabolic cage for a 16-hour collection of urine. After 13 weeks, the rats were anesthetized with ether for the collection of plasma and organs. Marginal magnesium deficiency was confirmed by decreased urinary excretion and femur, tibia and vertebrae concentrations of magnesium. Dietary oil influenced the effect of marginal magnesium deficiency on magnesium metabolism, distribution and oxidative stress indicators. Fish oil, but not corn oil, significantly decreased urinary magnesium excretion and increased kidney magnesium concentration. Femur magnesium was significantly decreased by marginal magnesium deficiency in rats fed fish oil but not in rats fed corn oil, and liver magnesium concentration was decreased by fish oil. Marginal magnesium deficiency increased plasma extracellular superoxide dismutase and cysteine (component of glutathione) in rats fed corn oil but not in rats fed fish oil. Urinary prostaglandin E2 excretion was significantly decreased by marginal magnesium deficiency at 8 weeks, but not at 12 weeks; an increase between weeks 8 and 12 in marginally magnesium-deficient rats fed fish oil caused this change in significance. The findings show that the dietary fatty acid composition affects the response of rats to marginal magnesium deprivation. The findings also indicate that dietary or physiological factors affecting oxidative stress could affect the response to marginal magnesium deficiency, and that a response to a dietary change that takes time to develop, such as an increase in dietary n-3 fatty acids, may result in signs of marginal deficiency being different over time.

Key words: magnesium, n-3 fatty acids, n-6 fatty acids, oxidative stress, fish oil

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Based on dietary intake recommendations, subclinical or marginal magnesium deficiency commonly occurs throughout the world. The U.S. Food and Nutrition Board [1] set the Estimated Average Requirement (EAR) for magnesium at 255-265 mg/day for females and 330-350 mg/day for males. In the U.S., NHANES 2005-2006 data indicated that usual magnesium intakes from food of about 60% of all adults do not meet the EAR [2]. In addition, it is estimated that about 10% of adults older than 19 years have magnesium intakes from food and water that are only about 50% [2] of the U.S. Recommended Dietary Allowance (RDA) of 310-320 mg/day for females and 400-420 mg/day for males [1]. Yet, widespread pathological conditions induced by magnesium deficiency have not been identified. An expert consultation for the Food and Agriculture Organization/World Health Organization concluded that evidence was lacking for nutritional magnesium deficiency occurring with the consumption of diets supplying a range of magnesium intakes sometimes considerably less than the RDA for the United States and Canada, and the Recommended Nutrient Intake (RNI) for the United Kingdom [3]. However, numerous epidemiological and correlation studies suggest that a low magnesium status is associated with several pathological conditions including atherosclerosis, heart arrhythmias, hypertension, osteoporosis, and diabetes mellitus [4-7]. Supplementation trials and clinical studies have been inconsistent in showing that dietary magnesium deficiency alone is involved in these conditions. This inconsistency may be caused by other dietary factors ameliorating or exacerbating the response to a marginal or subclinical magnesium deficiency, and the length of the marginal deficiency, which, based on some experiments with rats, may have a lower limit near 50% of the requirement [8, 9].

A review by Schwartz in 1988 [10] found that high dietary calcium, phosphorus, and protein enhanced the susceptibility to magnesium deficiency, and that a diet high in saturated fats and cholesterol precipitated magnesium deficiency. More recently, high dietary sucrose and fructose have been found to increase indicators of chronic inflammation and oxidative stress in magnesium-deficient rats [11-13]. These findings support the conclusion by Vormann et al. [8] that, based on changes in vitamin E, iron, and malondialdehyde concentrations in tissues, mild magnesium deficiency can be compensated and might not lead to pathological conditions. They also suggested that a mild magnesium deficiency might contribute to pathological biochemical effects if another pathologic factor, such as increased oxidative stress, is present.

A factor that can influence the presence of chronic inflammation and oxidative stress is the dietary composition of fatty acids. A high intake of n-3 long-chain polyunsaturated fatty acids (PUFAs) relative to n-6 PUFAs has been found to decrease the production of inflammatory eicosanoids (prostaglandins, thromboxanes, leukotrienes, and other oxidized derivatives), cytokines, and reactive oxygen species [14]. However, some studies have found a high peroxidation index and oxidative stress with high doses of n-3 PUFAs in some dietary conditions [15, 16].

Only a few studies have determined whether magnesium deficiency affects fatty acid metabolism. These studies indicate that the effect of severe magnesium deficiency may be different than a marginal deficiency on fatty acid metabolism. A severe deficiency (30 mg Mg/kg diet) of short duration (8 days) increased oleic and linoleic acids and decreased stearic and arachidonic acids in plasma of weanling rats [17]. In addition, very low magnesium impaired the conversion of linoleic acid to arachidonic acid in cultured arterial cells [18]. Severe magnesium deficiency (10% or less the requirement) also decreased sphingomyelin in serum [19] and erythrocyte membranes [20]. The change in membrane lipids apparently affected membrane fluidity. In contrast to the severe deficiency findings, more moderate magnesium deficiency (110 mg Mg/kg diet) of relatively long duration (14 weeks) increased the tissue levels of docosahexaenoic acid in rats [21]. In addition, the amount of eicosanoids (e.g., prostaglandin E2, thromboxanes, prostacyclin) from arachidonic acid was increased in plasma and tissues of rats fed about 73 mg Mg/kg diet for 12 weeks [22]. Whether dietary n-6/n-3 fatty acid composition affects the response to magnesium deprivation, particularly marginal deprivation as would be found in humans, apparently has not been determined.

Another factor that can affect the appearance of biochemical and physiological changes in response to magnesium deficiency is the length of time an organism is deprived. For example, Rude et al. [23] found that changes in serum parathyroid hormone, 1, 25-hydroxyvitamin D3, and osteocalcin in rats fed 10% of their magnesium requirement were different at 2, 4 and 6 months. Rayssiguier and Larvor [24] found that kidney magnesium concentration changed from day 10 to day 30 of magnesium deprivation. These studies also found that
some signs of magnesium deficiency became more severe over the course of the deprivation. Plannells et al. [25] found that calcium balance became more positive and phosphorus balance more negative during weeks 8-10 compared to weeks 5 and 6 in rats fed about 10% of their magnesium requirement. Biochemical changes over time in response to a marginal magnesium deprivation apparently have received limited attention.

The objective of the experiment described in the following was to determine whether dietary n-6/n-3 fatty acid composition may be a factor in the inconsistent response to marginal magnesium deprivation (50% of requirement). Also, urinary variables were measured at different times to determine whether the characteristics of the response to marginal magnesium deficiency may be dependent upon the length of deprivation.

Materials and methods

Experimental design

Female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 39-55 g were randomly assigned to four groups of 10 with mean weights about 46 g. The rats were fed diets in a factorial arrangement with the variables being magnesium at 250 or 500 mg and either 75 g/kg corn oil or 65 g/kg fish (menhaden) oil plus 10 g/kg linoleic acid. Linoleic acid was added with fish oil to assure that the diet provided the linoleic acid requirement of 6 g/kg set for rats by the National Research Council [26]. The n-6/n-3 fatty acid ratio of the diet with corn oil was about 44 and with fish oil was less than 0.5. The basal diet, which met all the nutrient requirements set by the National Research Council [26] except magnesium (250 mg/kg), is shown in table 1. To make diets containing 500 mg/kg magnesium, 0.42 g of MgO replaced 0.42 g sucrose per kg diet. The diets were not pelleted and were stored at 16°C in tightly capped plastic containers.

Five days during weeks 5 and 10 of the experiment, food intake was determined. During weeks 8 and 12 of the experiment, rats were placed individually in metabolic cages with free access to deionized water but not food while urine was collected for 16 hours in plastic test tubes kept on ice. The urine was stored at -70°C until analysis. After 13 weeks on their respective diets, rats were weighed and anesthetized with ether for the collection of blood from the vena cava with a heparin-coated syringe and needle. After euthanasia by decapitation, the heart, kidneys, liver, and spleen were collected, blotted dry, weighed, and stored at -70°C until analyzed. The right femur and tibia and 4th lumbar vertebra with flesh removed, and plasma (obtained by centrifugation) were collected and stored at -70°C until analyzed.

Animal handling

The rats were housed individually in stainless steel cages in a room maintained at 23°C and 50% humidity with a normal 12-hour light and dark cycle. Food was provided in plastic food cups and deionized water (Super Q, Millipore, Bedford, MA) in plastic water bottles with metal stainless steel tubes. Absorbent paper under the wire mesh cages was changed daily. Rats were weighed and provided clean cages weekly.

### Table 1. Composition of basal diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin-free casein</td>
<td>165.00</td>
</tr>
<tr>
<td>Sucrose</td>
<td>359.08</td>
</tr>
<tr>
<td>Fructose</td>
<td>100.00</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>150.00</td>
</tr>
<tr>
<td>Cellulose (alphacel)</td>
<td>80.00</td>
</tr>
<tr>
<td>Oil†</td>
<td>75.00</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>5.00</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.00</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>0.50</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>5.00</td>
</tr>
<tr>
<td>MgO†</td>
<td>0.42</td>
</tr>
<tr>
<td>Mineral mix-1*</td>
<td>33.25</td>
</tr>
<tr>
<td>Mineral mix-2*</td>
<td>18.00</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>5.00</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.75</td>
</tr>
<tr>
<td>Total</td>
<td>1,000.00</td>
</tr>
</tbody>
</table>

† Oil is a dietary variable; 75 g/kg of corn oil or 65 g/kg fish oil plus 10 g/kg linoleic acid; 0.014 g/kg TBHQ added to diet to prevent oxidation or rancidity of oils, especially fish oil. † Add 0.42 g of MgO to make a magnesium-adequate diet with 500 mg Mg/kg; reduce sucrose accordingly.

‡ Mineral mix – 1 (in g): CaCO₃, 18.75; NaCl, 12.5; and KCl, 2. ‡ Mineral mix – 2 (in mg): KH₂PO₄, 8800; CuSO₄·5H₂O, 20; MnSO₄·4H₂O, 165; ZnO, 15; KI, 0.5; (NH₄)₂MoO₄·4H₂O, 0.3; Na₂SeO₃, 0.6; Na₂HASO₄·7H₂O, 0.5; H₃BO₃, 6; Cr(C₂H₃O₂)₃·3H₂O, 2; NaF, 6; Na₂SiO₃·9H₂O, 100; NH₄VO₃, 0.5; Fe(SO₄)₂, 155, and sucrose, 8728.6. † Vitamin mix (in mg): dl-α-tocopherol acetate (500 IU/g), 72; retinyl palmitate (500,000 IU/g), 8; vitamin D₃ (400,000 IU/g), 2.5; phylloquinone, 0.2; biotin, 1; folic acid, 1; niacin, 30; calcium pantothenate, 16; riboflavin, 6; thiamine, 6; pyridoxine·HCl, 8; vitamin B₁₂ (0.1% in mannitol), 50; and sucrose, 4,799.3.
The Animal Care Committee of the Grand Forks Human Nutrition Research Center approved the study, and lawfully acquired animals were maintained in accordance with National Institute Health guidelines for the care and use of laboratory animals.

Biochemical analyses
Commerically available kits were used to determine plasma cholesterol (kit #80015, Raichem, San Diego, CA), triglycerides (kit #80009, Raichem, San Diego, CA), phospholipids (kit # 990-54009E, Wako Chemical, Richmond, VA) and C-reactive protein (kit # 042-CRPR-25E, Alpco Diagnostics, Salem, NH), and urine creatinine (Creatinine reagent #83069, Raichem, San Diego, CA), prostaglandin E2 (kit #900-001, Assay Designs, Ann Arbor, MI) and citrate (Cat #10 139 076 035, R-Biopharm/Boehringer Mannheim, Marshall, MI). Extracellular superoxide dismutase (ECSOD) activity was determined by assaying the inhibition of acetylated cytochrome c reduction at pH 10.0, as previously described [27, 28]. Plasma glutathione and cysteine were determined by using the HPLC method of Durand et al. [29].

Magnesium analyses
Magnesium was determined by using inductively coupled argon plasma emission spectroscopy (ICAPES) (Optima 3100 XL, Perkin-Elmer, Shelton, CT) that employed a Gem cone nebulizer with a cyclonic spray chamber and an alumina injector tube. Magnesium was measured by using line 279.077 nm with a limit of quantification of 0.659 μg/mL. Magnesium was determined in urine diluted 1:5 with deionized water. Seronorm normal urine (SERO, Billingstad, Norway) was used as the quality control standard; analyzed value obtained was 71 ± 0.8 μg/mL vs a certified value of 70.1 ± 2.5 μg/mL. Femurs, tibias and vertebrae (cleaned to the periosteal surface with cheesecloth) and kidneys, livers and hearts were lyophilized and then subjected to a wet-ash, low-temperature digestion in Teflon tubes [30] before analysis. Standard reference material (National Institute of Standards and Technology, Gaithersburg, MD) #1577b bovine liver was used as the quality control standard; mean analyzed value for all magnesium determinations was 591 ± 31 μg/g vs certified value of 600 ± 15 μg/g.

Statistical analysis
Data were statistically compared by using two-way analysis of variance (SAS/STAT, Version 9.2, SAS Institute, Inc., Cary, NC) followed by Tukey’s contrasts when appropriate. Values more than two standard deviations from the mean were considered outliers and not included in the analyses. A p value of ≤ 0.05 was considered significant.

Results
Statistically, neither marginal magnesium deficiency nor a difference in dietary n-3/n-6 fatty acids affected the final weights of the rats (table 2). However, the rats fed the fish oil diet with adequate magnesium appeared to weigh more than any other group (275 g vs 244-246 g; Mg × oil, p < 0.06). Organ weight/body weight ratios were significantly affected by the difference in dietary n-6/n-3 fatty acids but not by marginal magnesium deficiency (table 2). Both liver weight/body weight and spleen weight/body weight ratios were significantly higher, and the quality control standard; analyzed value obtained was 71 ± 0.8 μg/mL vs a certified value of 70.1 ± 2.5 μg/mL. Femurs, tibias and vertebrae (cleaned to the periosteal surface with cheesecloth) and kidneys, livers and hearts were lyophilized and then subjected to a wet-ash, low-temperature digestion in Teflon tubes [30] before analysis. Standard reference material (National Institute of Standards and Technology, Gaithersburg, MD) #1577b bovine liver was used as the quality control standard; mean analyzed value for all magnesium determinations was 591 ± 31 μg/g vs certified value of 600 ± 15 μg/g.

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Table 2. Effect of dietary magnesium, oil, and their interaction on final weights, food intakes, and organ weight/body ratios.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight, g</th>
<th>Food Intake, g/d</th>
<th>Organ weight, g/body weight, 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Final</td>
<td>Week 5</td>
<td>Week 10</td>
</tr>
<tr>
<td>Mg, mg/kg</td>
<td>Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>Corn 246 ± 6</td>
<td>15.5 ± 0.4</td>
<td>15.0 ± 0.4</td>
</tr>
<tr>
<td>250</td>
<td>Fish 245 ± 7</td>
<td>14.6 ± 0.5</td>
<td>13.6 ± 0.5</td>
</tr>
<tr>
<td>500</td>
<td>Corn 244 ± 8</td>
<td>15.2 ± 0.7</td>
<td>15.1 ± 0.5</td>
</tr>
<tr>
<td>500</td>
<td>Fish 275 ± 11</td>
<td>15.1 ± 0.6</td>
<td>15.3 ± 0.6</td>
</tr>
</tbody>
</table>

Analysis of Variance – p values
Magnesium 0.09 0.90 0.09 0.18 0.43 0.37 0.50
Oil 0.08 0.40 0.25 0.80 0.001 0.03 < 0.0001
Mg × Oil 0.06 0.48 0.13 0.20 0.97 0.57 0.41

Values are means ± SEM for groups of 10. Dietary oil was either corn oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg.
heart weight/body weight ratio was lower in rats fed fish oil instead of corn oil. Neither dietary magnesium nor oil affected kidney weight/body weight ratio.

A difference in dietary n-6/n-3 fatty acids also affected plasma lipids (table 3). Feeding fish oil instead of corn oil decreased plasma cholesterol and phospholipid concentrations; marginal magnesium deficiency did not affect these plasma lipids. Neither dietary oil nor magnesium significantly affected plasma triglycerides (data not shown).

Dietary fish oil instead of corn oil decreased the inflammatory biomarker C-reactive protein; marginal magnesium deficiency did not affect this variable (table 3). The effect of a difference in dietary n-6/n-3 fatty acids on oxidative stress indicators was influenced by dietary magnesium (table 3). Extracellular superoxide dismutase was significantly higher when dietary corn oil instead of fish oil was fed to marginally magnesium-deprived rats; this difference was not seen in magnesium-adequate rats. Plasma glutathione was increased by marginal magnesium deprivation in rats fed fish oil, but not in rats fed corn oil. Cysteine, which is a component of glutathione, was significantly increased in plasma by marginal magnesium deficiency in rats fed corn oil, but not in rats fed fish oil.

Table 4 shows that the rats fed the low magnesium diet had lower magnesium status. The concentration of magnesium in femur, tibia and vertebra were decreased and in kidney was increased in rats fed the low magnesium diet. The difference in dietary n-6/n-3 fatty acids influenced the response to the low magnesium diet. The decrease in femur magnesium and increase in kidney magnesium induced by the magnesium deprivation was more marked in rats fed fish oil. Feeding fish oil instead of corn oil decreased the concentration of magnesium in liver.

Table 3. Effect of dietary magnesium, and their interaction on plasma lipids and indicators of oxidative stress.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Oil</th>
<th>Cholesterol, mg/dL</th>
<th>Phospholipids, mg/dL</th>
<th>ECSOD, U/mL</th>
<th>CRP, μg/mL</th>
<th>Glutathione, μM</th>
<th>Cysteine, μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg, mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>Corn</td>
<td>65 ± 3</td>
<td>124 ± 3</td>
<td>153 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>269 ± 14</td>
<td>15.2 ± 1.2</td>
<td>283 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>250</td>
<td>Fish</td>
<td>36 ± 2</td>
<td>74 ± 5</td>
<td>117 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>213 ± 15</td>
<td>18.4 ± 1.6</td>
<td>244 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>Corn</td>
<td>58 ± 3</td>
<td>126 ± 8</td>
<td>122 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>267 ± 11</td>
<td>15.7 ± 1.3</td>
<td>233 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>500</td>
<td>Fish</td>
<td>36 ± 3</td>
<td>73 ± 3</td>
<td>130 ± 12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>203 ± 16</td>
<td>13.6 ± 1.0</td>
<td>240 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Analysis of variance – p values

| Magnesium | 0.28 | 0.95 | 0.29 | 0.71 | 0.12 | 0.002 |
| Oil       | < 0.0001 | < 0.0001 | 0.10 | 0.0002 | 0.68 | 0.07 |
| Mg × Oil  | 0.27 | 0.70 | 0.01 | 0.78 | 0.05 | 0.009 |

Values are means ± SEM for groups of 10. Means without a common letter are significantly different (p < 0.05). Dietary oil was either corn oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg.

ECSOD: extracellular superoxide dismutase; CRP: C-reactive protein; U: units.

Table 4. Effect of dietary magnesium, oil, and their interaction on indicators of magnesium status.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Magnesium, mg/kg</th>
<th>Oil</th>
<th>Femur, mg/g</th>
<th>Tibia, mg/g</th>
<th>Vertebra, mg/g</th>
<th>Vertebral Kidney, μg/g</th>
<th>Liver, μg/g</th>
<th>Heart, μg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium</td>
<td>250</td>
<td>Corn</td>
<td>2.79 ± 0.05&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.62 ± 0.04</td>
<td>2.58 ± 0.05</td>
<td>821 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>771 ± 12</td>
<td>920 ± 12</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>Fish</td>
<td>2.54 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.64 ± 0.07</td>
<td>2.55 ± 0.08</td>
<td>856 ± 23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>744 ± 22</td>
<td>914 ± 7</td>
</tr>
<tr>
<td>500</td>
<td>250</td>
<td>Corn</td>
<td>2.93 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.02 ± 0.06</td>
<td>2.88 ± 0.03</td>
<td>805 ± 8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>766 ± 12</td>
<td>920 ± 8</td>
</tr>
<tr>
<td>500</td>
<td>Fish</td>
<td>3.16 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.93 ± 0.05</td>
<td>2.83 ± 0.03</td>
<td>751 ± 14&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>686 ± 16</td>
<td>938 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance – p values

| Magnesium | 0.0004 | < 0.0001 | < 0.0001 | 0.0004 | 0.07 | 0.22 |
| Oil       | 0.90   | 0.53      | 0.45      | 0.53   | 0.003 | 0.52 |
| Mg × Oil  | 0.02   | 0.35      | 0.83      | 0.006  | 0.11 | 0.21 |

Values are means ± SEM for groups of 10. Means without a common letter are significantly different (p < 0.05). Dietary oil was either corn oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg.
Neither dietary oil nor magnesium deprivation affected heart magnesium concentration. Both magnesium deprivation and the difference in dietary n-6/n-3 fatty acids affected the urinary excretion of magnesium (table 5). As expected, magnesium deprivation decreased urinary magnesium excretion. Feeding fish oil instead of corn oil also decreased the urinary excretion of magnesium.

Two other urinary metabolites that have been reported elsewhere to be affected by the dietary variables were affected here. However, the effects of the dietary treatments changed over time. Prostaglandin E2 was significantly decreased by the magnesium deprivation at 8 weeks, but not at 12 weeks; a significant increase (p < 0.02) between 8 and 12 weeks in magnesium-deprived rats fed fish oil was the major reason for this change in significance. Urinary citrate excretion was significantly higher in rats fed fish oil instead of corn oil at 8 weeks, but there was only a tendency for this increase (p < 0.10) at 12 weeks. Magnesium deprivation tended to decrease urinary citrate excretion at 12 weeks (p < 0.07). These changes appeared to be the result of a small non-significant difference between 8 and 12 weeks in urinary citrate excretion by rats fed the magnesium-adequate corn oil diet. If both urine collections were combined for statistical comparisons, then magnesium deprivation (p < 0.03) and corn oil instead of fish oil (p < 0.009) decreased urinary citrate excretion.

**Discussion**

The decreases in urinary excretion of magnesium and the concentration of magnesium in femur, tibia and vertebra indicate that the rats fed the diet containing 250 mg/kg magnesium had a decreased magnesium status that could be considered marginally magnesium-deficient. This conclusion is supported by the finding that rats fed a similar amount of magnesium (208 mg/kg diet) for 30 days showed signs of magnesium deficiency, including reduced tissue concentrations of magnesium [8]. However, dietary oil apparently influenced several of the responses to marginal magnesium deficiency, which have been inconsistently reported elsewhere.

The rats fed the magnesium-adequate, fish oil diet tended to weigh more than the other groups (Mg × oil, p < 0.06), which suggests that fish oil vs corn oil might increase weight gain of rats fed adequate magnesium, or that marginal magnesium deficiency might decrease weight gain in rats fed fish oil. In either case, the lack of a consistent significant effect of marginal magnesium deficiency on weight gain is consistent with depressed growth being found in some moderate magnesium deprivation studies but not in others. Marginal magnesium deficiency did not depress the weight of male rats whose dietary fat was palm oil [31], male rats whose dietary fat was groundnut/corn oil [32], male rats fed a high sucrose diet with soybean oil as the fat [21], or female rats fed a high sucrose diet with corn oil or soybean oil as the fat [33, 34]. In contrast to females, male rats fed a moderately magnesium-deficient diet with corn oil for 4-6 months exhibited slightly depressed weight [23]. Moderate magnesium deprivation also decreased weight in male rats fed a commercially available magnesium-deficient diet [8]. Severe magnesium deficiency usually depresses weight; much of this effect may be caused by reduced food consumption [8, 24].

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**Table 5. Effect of dietary magnesium, oil and their interaction on urinary excretion of magnesium, prostaglandin E₂ (PGE₂), and citrate.**

<table>
<thead>
<tr>
<th>Diet, Mg, mg/kg</th>
<th>Oil</th>
<th>Urine, 8 weeks</th>
<th>Urine, 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mg, mg/mmol Cr</td>
<td>PGE₂, μg/mmol Cr</td>
<td>Citrate, mg/mmol Cr</td>
</tr>
<tr>
<td>250</td>
<td>Corn</td>
<td>14.7 ± 2.8</td>
<td>0.93 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Fish</td>
<td>7.9 ± 1.5</td>
<td>0.73 ± 0.13</td>
</tr>
<tr>
<td>500</td>
<td>Corn</td>
<td>28.6 ± 3.3</td>
<td>1.30 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>Fish</td>
<td>20.3 ± 2.4</td>
<td>1.03 ± 0.12</td>
</tr>
</tbody>
</table>

**Values are means ± SEM for groups of 10.** Dietary oil was either corn oil at 75 g/kg or fish oil at 65 g/kg plus linoleic acid at 10 g/kg.
The increase in kidney magnesium found in marginally magnesium-deficient rats in the present experiment may seem unusual to those not familiar with the response of rats to magnesium deprivation. However, reports by other researchers indicate that kidney magnesium concentration can increase, not change, or decrease in magnesium deprivation depending upon diet composition and the severity of magnesium deficiency. Magnesium deprivation increased kidney magnesium concentrations in rats fed a diet high in fructose and with corn oil as the fat [35, 36]. Mild magnesium deprivation decreased kidney magnesium in male rats whose dietary fat was palm oil [31] and in severely deficient rats fed a high sucrose diet with corn oil as the fat [37]. In another study, severe magnesium deprivation depressed kidney magnesium at 10 days but not at 20 or 30 days [24].

In the present study, marginal magnesium deprivation did not significantly affect any organ weight/body weight ratio determined. This is not surprising because increased organ weight/body weight ratio, including for kidney [35, 37], liver [11], and spleen [11] usually is seen only when magnesium deprivation is severe enough to induce a significant weight reduction.

Marginal magnesium deprivation also did not significantly affect plasma lipids. This lack of an effect may have been caused by the length of deprivation. A review by Rayssiguier [38] indicated that a moderate magnesium deficiency of short duration increased plasma triglycerides, which also occurs in severe deficiency, but the increase was less intense in long-term experiments. Plasma cholesterol, which is not markedly affected by severe magnesium deficiency of short duration, is significantly increased in moderate deficiency of long duration. Additional time may have resulted in the higher plasma cholesterol in marginal magnesium-deficient rats fed corn oil becoming significant in the present experiment.

Fish oil compared to corn oil appeared to have a more marked effect on magnesium metabolism. This observation is based on the findings that fish oil compared to corn oil, significantly decreased urinary magnesium excretion independent of magnesium intake and only fish oil significantly increased kidney magnesium concentration in marginally magnesium-deficient rats. In addition, fish oil compared to corn oil changed the distribution of magnesium in the body. Femur magnesium was significantly decreased by marginal magnesium deficiency in rats fed fish oil but not corn oil, and liver magnesium concentration was decreased by fish oil compared to corn oil. The reason for these changes is unclear but may have occurred because fish oil reduced magnesium absorption from the gastrointestinal tract, increased magnesium reabsorption at the kidney level, or changed magnesium utilization such that a different distribution in the body was needed.

One possible reason for the change in magnesium metabolism or utilization when fish oil vs corn oil was fed may be a change in lipid metabolism. The decreased plasma cholesterol and phospholipids exhibited by rats fed fish oil suggests that cell membrane lipid composition was changed, which could alter cellular physicochemical properties [39, 40]. This change may have influenced the conformation and function of membrane-bound proteins [41], including ion channels and transporters, and thus altered the cellular transport of magnesium. In addition, the turnover of arachidonic acid, a membrane phospholipid, most likely was affected by the change in dietary oil [14]. Additional indirect support for the suggestion that dietary oil changed cell membrane lipid composition or function in the present study is the finding of decreased urinary PGE2 excretion by magnesium-deficient rats at 8 weeks, which changed to an increased excretion in magnesium-deficient rats fed fish oil at 12 weeks. A change in cell membrane arachidonic acid would affect its release induced by magnesium deprivation [42], and its conversion into PGE2. In addition, a change in arachidonic acid may affect the distribution of magnesium because arachidonic acid apparently has a regulatory role in the Na+-dependent efflux of magnesium into cells [42].

Another possible mechanism through which dietary fatty acids may influence the response to a marginal magnesium deprivation is by altering the response to oxidative stress, which should have been increased by the feeding of a high fructose/sucrose diet in the present study. Increased oxidative stress exacerbates magnesium deprivation signs [11-13], but high intake of n-3 long-chain PUFAs as fish oil in the present study may have changed this response by further increasing [15, 16] or decreasing [14, 43] any oxidative stress caused by the high fructose/sucrose diet. Fish oil (high in n-3 PUFAs) decreased plasma CRP In the present study, which suggests that it decreased oxidative stress. In rats fed fish oil, plasma ECSOD and cysteine (a component of glutathione) were not affected by marginal magnesium deficiency and plasma glutathione was only mildly increased. In rats fed corn oil; marginal magnesium deficiency significantly increased plasma ECSOD and cysteine, and did
not affect plasma glutathione. These findings indicate a difference in oxidative stress and/or glutathione utilization or metabolism, which may have affected magnesium metabolism, utilization, and/or distribution, possibly through altering membrane fluidity [44].

The urinary excretion of PGE$_2$ data infer that the response to marginal magnesium deprivation changes over time and this may depend upon dietary fatty acid composition. A difference in urinary citrate excretion, which may occur in magnesium deficiency [45], may take extended time to develop; in the present study, a non-significant difference (p < 0.18) at 8 weeks approached significance (p < 0.07) at 12 weeks. The significant change in urinary PGE$_2$ excretion between weeks 8 and 12 in magnesium-deprived rats fed fish oil indicates that the response to fish oil in marginal magnesium deficiency apparently takes time to develop.

The findings in the present study support the contention that the response to marginal magnesium deprivation depends upon other dietary factors, including the fatty acid composition of the diet. The findings also suggest that dietary or physiological factors affecting oxidative stress could affect the response to marginal magnesium deficiency. Moreover, a response to a dietary change that takes time to develop, such as the response to an increase in dietary n-3 PUFAs, may result in signs of marginal deficiency being very different over time, or a low magnesium status may modify the response to the dietary change. These modifying factors may be a major reason for the difficulty in identifying signs of marginal dietary magnesium deficiency in humans, and in definitively concluding that inadequate dietary magnesium significantly contributes to the occurrence of chronic diseases such as atherosclerosis and osteoporosis.

References


