High Dietary Manganese Lowers Heart Magnesium in Pigs Fed a Low-Magnesium Diet

ABSTRACT Young pigs were fed a diet moderately high or low in manganese (Mn) (0.95 ± 0.10 mmol Mn/kg, n = 8 or 0.040 ± 0.003 mmol Mn/kg, n = 6) and deficient in magnesium (Mg) (4.1 mmol Mg/kg) for 5 wk. All eight pigs consuming the high Mn diet died following convulsive seizures, whereas only two of six died in the group fed low Mn. In an attempt to determine the cause of death, a subsequent study examined the interactive effect of deficient dietary Mg and Mn on the tissue distribution of Mg and Mn. Pigs were individually fed, for 5 wk, diets that contained: 4.1 mmol Mg/kg and 36.0 μmol Mn/kg, 4.1 mmol Mg/kg and 0.91 mmol Mn/kg, 4.1 mmol Mg/kg and 0.91 mmol Mn/kg with added ultratrace minerals, or 41.1 mmol Mg/kg and 0.91 mmol Mn/kg, and ultratrace minerals. Liver and skeletal muscle Mn concentrations were significantly elevated by increased dietary Mn. Increased dietary Mn did not affect heart Mn, but heart Mg concentrations were significantly depressed by high, as compared to low, dietary Mn (38.7 ± 3.3 vs. 32.7 ± 2.6 mmol Mg/kg). These data suggest high dietary Mn may exacerbate Mg deficiency in heart muscle and thus may be a complicating factor in the deaths observed in Mg-deficient pigs. J. Nutr. 130: 2032–2035, 2000.

KEY WORDS: manganese • magnesium • toxicity • heart • swine

Although manganese (Mn) is an essential component of several enzymes involved in carbohydrate, lipid and protein metabolism, it also is a toxic element (Khan et al. 1997). Acute Mn toxicity in humans is characterized by a crippling neurological disorder resembling Parkinson’s disease in humans (Chandra et al. 1974, Cotzias 1958). Although Mn toxicity also has been reported in animals (Grummer et al. 1950, Khan et al. 1997), Leibholz et al. (1962) fed growing swine 72.8 mmol Mn/kg diet without mortality or severe morbidity. Grummer et al. (1950) fed growing swine up to 9.1 mmol Mn/kg diet and observed “retarded appetite and growth especially during the latter part of the trial,” but no deaths. However, Grummer et al. (1950) did speculate that Mn may cause some symptoms of toxicity at concentrations between 0.91 and 1.82 mmol Mn/kg diet.

Magnesium (Mg) is present in soft tissues and bone, and functions as a component or activator of enzymes involved in cellular respiration (Wacker and Parisi 1968a, b, c). Mg deficiency, as a consequence of various factors such as low dietary intake and inhibition of absorption by fats, has been linked to an increased risk of ischemic heart disease (IHD), irreversible cardiac failure (Altura et al. 1981, Wu et al. 1992) and reduced coronary blood flow as a result of coronary vasospasm (Altura and Altura 1990, 1996, Chipperfield and Chipperfield 1978, Johnson et al. 1979, Karppanen et al. 1978, Raab 1969). IHD is a pathophysiologic state caused by vessel constriction or blockage that results in inadequate blood flow to the myocardium. Epidemiological research suggests Mg deficiency as a risk factor for several cardiovascular disorders, such as myocardial infarction and congestive heart failure in addition to IHD (Altura and Altura 1985, Eisenberg 1992, Rasmussen 1993, Seelig 1989).

There are several possible points of interaction between Mn and Mg. The body can replace Mn with Mg with similar efficiency in Mn-activated proteins (Wapnir 1990). Similarly, Mn can occupy Mg allosteric sites in Mg-activated proteins, such as the sarcoplasmic reticulum calcium ATPase (Chiesi and Inesi 1981). Mn-supplemented diets increased Mg excretion through the urine in rats (Gaillard et al. 1996), and Mg deficiency decreased the Mn absorption and retention in rats (Sanchez-Morito et al. 1999). Scheuhammer and Cherian (1983) reported that Mn decreases Mg concentrations in both heart and bone by an undefined mechanism. Sanchez-Morito et al. (1999) found that feeding rats a diet deficient in Mg increased urinary and fecal Mn excretion. They also observed greater Mn retention in skeletal muscle, heart and kidney in Mg-deficient rats as compared to control.

In the present investigation we report that Mn-deficient pigs had greater mortality when fed moderately high, as compared to low dietary Mn; however, Mn concentrations were well below previously reported toxic levels. A follow-up investigation was performed to more carefully examine the causes of death of pigs fed high Mn.

MATERIALS AND METHODS

Animals and diets
All studies were approved by the Animal Care and Use Committee of the Grand Forks Human Nutrition Research Center, and pigs

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2 To whom correspondence should be addressed.

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Manganese and Magnesium in Pig Hearts

Table 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Study 1 Low Mn</th>
<th>Study 1 High Mn</th>
<th>Study 2 Low Mn</th>
<th>Study 2 High Mn</th>
<th>Study 2 Low Mn + UT</th>
<th>Study 2 High Mn + UT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet2</td>
<td>1000.0</td>
<td>999.5</td>
<td>1000.0</td>
<td>999.5</td>
<td>998.5</td>
<td>996.5</td>
</tr>
<tr>
<td>Mn Premix3</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Ultratrace premix4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mg Oxide</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

1 Mn, manganese; Mg, magnesium; UT, ultratrace.
2 Basal diet expressed in g/kg diet: ground corn, 500 g; corn starch, 138.6 g; sucrose, 128 g; sunflower oil, 40 g; casein protein, 160 g; mineral premix, 1.4 g (provided per kg diet; zinc as zinc acetate, 65.5 mg; copper as copper sulfate, 4.0 mg; potassium as potassium iodate, 43.4 g; selenium as sodium selenate, 0.2 mg); vitamin premix, 2 g (provided per kg diet; retinol, 7 mg; cholecalciferol, 1 mg; D,L-α-tocopherol acetate, 44 mg; menadione, 0.1 mg; folic acid, 0.6 mg; nicotinic acid, 25 mg; d-pantothenic acid, 18 mg; riboflavin, 6 mg; thiamin, 2 mg; pyridoxine, 3 mg; cyanocobalamin, 30 mg); choline bitartrate, 1.3 g; CaHPO4, 11.3 g; NaCl, 2.6 g; KH2PO4, 4.2 g; CaCO3, 9.2 g; iron premix, 1.4 g (provided per kg diet; iron, 34.7 mg).
3 Mn premix expressed in g/kg mix: (50 g Mn/kg): Mn sulfate monohydrate, 307.6 g; corn starch, 692.4 g.
4 Ultratrace mineral mix supplied the following per kg diet: copper as copper carbonate, 2.3 mg; chromium as chromium potassium sulfate, 0.1 mg; silicon as sodium silicate, 5 mg; boron as boric acid, 0.5 mg; fluorine as sodium fluoride, 1 mg; nickel as nickel carbonate, 0.5 mg; lithium as lithium chloride, 0.1 mg; molybdenum as ammonium molybdate, 0.27 mg; vanadium as ammonium vanadate, 0.13 mg; selenium as sodium selenate, 0.05 mg.

Statistical analyses

Mortality data from study 1 were analyzed by a chi-square test. Data from study 2 were analyzed by one-way ANOVA for effect of diet on tissue Mn and Mg concentrations (SAS; SAS Institute, Cary, NC). Data were considered different if P < 0.05. Contrasts between treatments were determined by Tukey's Studentized Range Test.

RESULTS

Study 1. All pigs fed the high-Mn diet died before the end of the study (2 moribund pigs were euthanized), but only 2 fed the low-Mn diet died (P = 0.003). The pigs that died were observed paddling with their feet while lying on their sides, convulsing and hemorrhaging from the nose and mouth prior to death; pathology included severe, necrotizing myocarditis. An initial veterinary pathologist diagnosis was death from Mulberry Heart Disease (MHD) as a result of Se deficiency. Livers from the pigs contained 50.3 ± 1.4 nmol α-tocopherol/g and 21.5 ± 1.2 nmol selenium (Se)/g; normal ranges are between 42–63.4 and 12.5–31.5 nmol/g for α-tocopherol and Se, respectively. Subsequently pigs were injected with Se-enriched (BoSe; Shering-Plough, Union, NJ). The deaths continued after the pigs were given supplemental vitamin E and Se injections.

Study 2. After 5 wk, pigs fed diet 4 were significantly heavier (Table 2) than those given other treatments (P < 0.05). Mn concentrations in the liver and skeletal muscle were greater (P < 0.01) in pigs fed high-, as compared to low-, dietary Mn (Table 2). Increased dietary Mn did not affect heart Mn, but high Mn depressed heart Mg concentrations in pigs without ultratrace mineral supplementation (P < 0.01) (Table 2). Brain Mg concentrations were not significantly (P > 0.05) affected by dietary Mn, but were numerically lower in the pigs fed high-Mn without ultratrace minerals. Dietary Mn did not affect the Mg concentrations of any other tissues or fluids measured. Plasma Mg was increased (P < 0.001) by increased dietary Mg (Table 2).
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TABLE 2

Initial and final weights, tissue manganese and magnesium concentrations in swine fed diets high or low in manganese and moderately deficient in magnesium (study 2).1,2

<table>
<thead>
<tr>
<th></th>
<th>Low Mn Low Mg</th>
<th>High Mn Low Mg</th>
<th>High Mn Low Mg + UT</th>
<th>High Mn High Mg + UT</th>
<th>ANOVA (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>22.1 ± 0.9</td>
<td>22.3 ± 1.4</td>
<td>22.0 ± 1.5</td>
<td>22.2 ± 1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Final</td>
<td>46.3 ± 3.8a</td>
<td>47.9 ± 3.9a</td>
<td>43.8 ± 4.1a</td>
<td>55.1 ± 6.2b</td>
<td>0.05</td>
</tr>
<tr>
<td>Mn, nmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>23.6 ± 10.9</td>
<td>34.6 ± 5.4</td>
<td>32.7 ± 3.6</td>
<td>34.6 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Liver</td>
<td>156 ± 34a</td>
<td>240 ± 29b</td>
<td>238 ± 47b</td>
<td>254 ± 23b</td>
<td>34.5</td>
</tr>
<tr>
<td>Lung</td>
<td>18.2 ± 5.4</td>
<td>21.8 ± 7.3</td>
<td>20.0 ± 5.4</td>
<td>23.6 ± 5.4</td>
<td>7.3</td>
</tr>
<tr>
<td>Muscle</td>
<td>9.1 ± 1.8a</td>
<td>16.4 ± 1.8b</td>
<td>16.4 ± 1.8b</td>
<td>14.5 ± 1.8b</td>
<td>1.8</td>
</tr>
<tr>
<td>Bile</td>
<td>3.6 ± 1.8a</td>
<td>49.1 ± 5.4bc</td>
<td>83.7 ± 43.6c</td>
<td>40.0 ± 9.1b</td>
<td>0.001</td>
</tr>
<tr>
<td>Brain</td>
<td>30.9 ± 9.1</td>
<td>32.7 ± 3.6</td>
<td>34.6 ± 3.6</td>
<td>32.7 ± 7.3</td>
<td>NS</td>
</tr>
<tr>
<td>Mg, μmol/g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>38.7 ± 3.3b</td>
<td>32.7 ± 2.7a</td>
<td>38.1 ± 2.6ab</td>
<td>38.9 ± 3.3b</td>
<td>3.0</td>
</tr>
<tr>
<td>Liver</td>
<td>29.0 ± 3.8</td>
<td>30.2 ± 3.3</td>
<td>29.5 ± 5.2</td>
<td>29.4 ± 1.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Lung</td>
<td>16.5 ± 3.4</td>
<td>19.1 ± 4.9</td>
<td>20.0 ± 4.3</td>
<td>24.1 ± 6.8</td>
<td>5.0</td>
</tr>
<tr>
<td>Muscle</td>
<td>32.9 ± 6.0</td>
<td>35.2 ± 1.9</td>
<td>37.2 ± 3.4</td>
<td>36.5 ± 4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Brain</td>
<td>29.4 ± 1.6ab</td>
<td>25.2 ± 0.4a</td>
<td>27.0 ± 1.2ab</td>
<td>28.2 ± 1.5b</td>
<td>1.3</td>
</tr>
<tr>
<td>Serum</td>
<td>0.18 ± 0.03a</td>
<td>0.29 ± 0.03a</td>
<td>0.14 ± 0.09a</td>
<td>0.81 ± 0.15b</td>
<td>0.08</td>
</tr>
</tbody>
</table>

1 Values are means ± sd, n = 5.
2 n = 5 with exceptions of bile and serum samples in Low Mn, Low Mg and Low Mn, Low Mg + UT where n = 4. NS = not significant (P > 0.05). Values for the same variable not sharing a superscript are significantly different; P < 0.05.

DISCUSSION

Although Mn toxicity in animals is well-established, we are unaware of any work that has reported deaths at the concentrations fed in this study. Mn requirements in young swine were formerly set as high as 0.36 mmol Mn/kg (NRC 1973). Leibholz et al. (1962) reported that “no toxicity symptoms were observed in pigs fed 72.8 mmol Mn/kg”; however, there was evidence of growth depression and stiffness. Leibholz et al. (1962) summarized that pigs have a high tolerance to Mn and that there exists a “considerable margin of safety between levels of Mn likely to be ingested in the diet and detrimental levels.” Likewise, Grummer et al. (1950) fed growing swine for 16 wk a corn-soybean-based diet and reported only moderate growth inhibition at Mn concentrations of up to 2.4 mmol Mn/kg. In the present study, pigs fed diets with 0.91 mmol Mn/kg and moderately deficient in Mg were dying after only 3 wk.

Mg is essential for energy metabolism and bone structure. Growing pigs require ~16.4 mmol Mg/kg per day to maintain maximal growth (NRC 1978). The dietary Mg intake at which acute deficiency results in death is conflicting. Mayo et al. (1959) reported fatal deficiency (intake at which half the animals on test died) within 21 d when pigs were fed 2.8 mmol Mg/kg diet. However, they also reported that other pigs fed 2.6 mmol Mg/kg diet for 42 d survived, although they developed severe deficiency symptoms (Mayo et al. 1959). In the present study, both the high- and low-Mn diets were formulated to contain 4.1 mmol Mg/kg diet. Because of the above reports, and because only two of six pigs died in the low-Mn group, as compared to all eight fed the high-Mn diet, we concluded that dietary Mg deficiency was not solely responsible for their deaths.

Symptoms of acute hypomagnesemia, or grass tetany in ruminants, include grinding of teeth, paddling of limbs and convulsions prior to death (Church 1988, Merck Veterinary Manual 1990). Church (1988) reported that despite the lack of characteristic postmortem lesions, subendocardial lesions may be caused by severe tetanic convulsions associated with Mg deficiency. Signs of Mg deficiency in pigs also include muscular twitching, reluctance to stand, loss of equilibrium and tetany followed by death (Mayo et al. 1959, Miller et al. 1965). Similar symptoms were observed in the present study.

A veterinary pathologist's diagnosis of MHD was deduced from both the sudden death and cardiac muscle degeneration observed in necropsy; however, subsequent treatment of all remaining pigs with supplemental vitamin E and Se prophylaxis failed to prevent mortality. Moreover, the liver α-tocopherol and Se concentrations were not deficient providing further evidence that MHD as a result of inadequate α-tocopherol and Se was not the cause of death. The veterinary pathology summary of pigs fed the high-Mn diets included “myodegeneration, necrosis and mineralization of heart,” “moderate myocardioyte necrosis” and “severe, multifocal, myocardial necrosis.” In contrast, pigs fed the low-Mn diet were reported to have “mild, nonsupportive myocarditis” and “mild myocardial fibrosis.” The heart tissue pathology of MHD includes “severe, necrotizing myocarditis” and “myocardial lesions,” symptoms that are also associated with Mg deficiency (Wutzten and Rozyczka 1975).

In the second study, brain Mg was numerically lower in the pigs fed high-Mn and low-Mg. We recognize the link between Mn toxicity and neurological disorders. Electroencephalograms were recorded for each pig, and the data will be presented elsewhere, although initial visual analysis revealed no major differences.

The reduction of Mg in the hearts of pigs fed high-Mn implicates Mn as a potential Mg antagonist in that organ. Heart Mg concentrations were increased by supplementation with ultratrace minerals. Ultratrace minerals included in the supplemental mix were chromium (Cr), fluorine (F), silicon (Si), nickel (Ni), lithium (Li), molybdenum (Mo), vanadium (V) and selenium (Se) and copper (Cu). Copper was supplied in both the ultratrace and mineral premix at 0.09 mmol/kg diet. The mechanism through which supplementation of these minerals increased the heart Mg concentration is unknown.
In humans, the link between hypomagnesemia in the heart and the increased risk of IHD as well as coronary vasospasm, cardiac dysrhythmia and altered cellular respiration has been well-documented (Altura and Altura 1985, Eisenberg 1992, Rasmussen 1993, Seelig 1989). If high dietary Mn displaces Mg from the heart, then people suffering from depressed Mg status could be at greater risk for Mn toxicity.

In conclusion, these data suggest that Mn exacerbates Mg deficiency and is responsible for a decrease in heart muscle Mg concentrations. This reduction of Mg concentrations in the heart may therefore be a contributing factor in the deaths observed in pigs fed high Mn.

**LITERATURE CITED**


