Dietary magnesium deficiency decreases plasma melatonin in rats

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Abstract. It has been postulated that Mg depletion is associated with decreased melatonin. Exogenous magnesium (Mg) has been found to increase the activity of serotonin N-acetyltransferase, an enzyme in the pathway for melatonin synthesis; but no data have been found on the effect of Mg deficiency on plasma melatonin. This pilot study examined the effect of a dietary Mg deficiency on plasma melatonin in male, Sprague-Dawley rats. Weanling rats were placed on a Mg-deficient (150 ppm) or a Mg-adequate (1000 ppm) diets for four weeks, after which they were sacrificed 4, 5 or 7 hours into the dark cycle. Plasma was assayed for melatonin concentrations. A significant decrease (p = 0.0101) occurred in mean (± SEM) plasma melatonin levels of the Mg-deficient animals (50 ± 6.4 pg/mL) when compared to the Mg-adequate animals (75 ± 6.6 pg/mL). There was no obvious phase shift in the melatonin profile of the Mg-deficient animals when compared to the Mg-adequate animals.

Keywords: magnesium deficiency, melatonin

Melatonin, the principal hormone secreted by the pineal gland, usually peaks 4-6 hours after the onset of darkness, which is usually around 02:00-04:00 hours in humans [1, 2]. Then, levels steadily decline during the second half of the night. Melatonin is synthesized from serotonin [3] in the pineal gland by a series of responses which result in the synthesis of the enzyme, serotonin N-acetyltransferase (NAT). NAT catalyzes the N-acetylation of serotonin, which is then methylated by hydroxyindole O-methyltransferase to become melatonin [4]. NAT activity increases nocturnally, with its peak activity being greater at night than during the day [5]. Mg deficiency causes sleep disturbances in rats [6, 7]. It has been postulated that Mg depletion influences biorhythms and would be associated with decreased melatonin and sleep disorders [8, 9]. Morton and James [10] found that NAT activity was increased in rats injected with Mg. In addition, Mg increased NAT activity in vitro in cultured pineal glands [10], suggesting that the site of action of Mg is in the pineal gland rather than at some other site in the body. This implies that a Mg deficiency might result in decreased NAT activity and a corresponding decrease in melatonin production. The purpose of this pilot study was to explore the effect of a dietary Mg deficiency on melatonin production in male, Sprague-Dawley rats and to ascertain whether or not a phase shift occurred with decreased Mg intake.

Materials and methods

Animals and study design

Thirty-nine male, weanling Sprague-Dawley rats (original stock from Sasco, St. Louis, MO) were born and raised at our facility. Litters were cut to eight pups. At approximately 3 weeks of age, weanling pups were randomly assigned to either a chronic Mg deficient diet (150 ppm Mg, n = 20) or a Mg adequate diet (1000 ppm Mg, n = 19). Each dietary group of
animals was then randomly distributed into three subgroups containing six or seven animals each (adequate groups 4, 5, 7 and deficient groups 4, 5, 7).

Following weaning, the animals were housed individually in hanging, stainless steel cages with wire mesh bottoms and fronts. Animals were maintained on a reversed light cycle of 14 h light: 10 h dark with lights off at 0500 h. The temperature of the animal room was maintained at 22 ± 2°C. The animals were permitted to consume both diet and distilled water ad libitum. Weights of the animals were measured three times per week. Procedures were approved by the Brigham Young University Institutional Animal Care and Use Committee.

Diet

The diets were based on the AIN-93G purified diet for rodents [11] with minor modifications. A 1:1 mixture of corn oil and canola oil was used for the oil. Methionine (3 g/kg diet) was used to supplement the sulfur amino acids. Magnesium was added to the diets from a premix made with powdered sugar.

Blood collection

After being fed the diets for four weeks, the animals were sacrificed by decapitation. Animals from groups 4, 5, and 7 were sacrificed four, five, and seven hours into the dark phase, respectively. These times correspond with 02:00 h, 03:00 h, and 05:00 h in a typical melatonin rhythm. Melatonin activity is suppressed in the presence of white light, so the animals were sacrificed under a low intensity red, photographic safe light.

Trunk blood was collected into sodium heparin tubes and centrifuged at 4°C within 20 minutes of collection. The plasma was then dispensed into microcentrifuge tubes and frozen at -20°C until analysis.

Blood analyses

Samples were analyzed for melatonin content using an enzyme-linked immunosorbent assay (ELISA) developed by Bühlmann Laboratories, Schünenbuehch, Switzerland (American Laboratory Products Co., Windham, NH).

Plasma Mg concentrations were determined using flame atomic absorption spectrophotometry (Perkin-Elmer model 306, Norwalk, CT). In addition, samples of the diets were analyzed to confirm dietary Mg levels. The analyzed levels of the Mg deficient (150 ppm) and the Mg adequate (1000 ppm) diets were 182 ± 28 and 984 ± 59 ppm Mg, respectively (mean ± SD).

Statistical analyses

Dietary groups were compared by ANOVA using SAS (SAS 9.1, Cary NC). For terms that were significant, pos hoc Tukey-Kramer tests were used. The model consisted of dietary Mg and sacrifice times. P values < 0.05 were considered significant.

Results

The mean (± SEM) plasma melatonin concentration in the Mg deficient animals (50 ± 6.4 pg/mL, n = 20) was significantly (p = 0.0101) lower, by a third, than in the adequate Mg group (75 ± 6.6 pg/mL, n = 19). The median and variance of these data are in figure 1. If all the animals had been sacrificed at four hours into the dark phase, the significance between the two levels of dietary Mg would have increased.

There was no statistical difference between the two diet groups (p = 0.0501) at the sacrifice times for the plasma melatonin, but a strong trend was evident (table 1). The lack of significance among the six groups was attributed to the small number of animals (n = 6 or 7) in each diet group.

The weaning weights were 56 and 57 grams and the sacrifice weights were 241 and 245 grams in the Mg deficient and Mg adequate animals, respectively. There were no significant differences in the weights at the time of weaning or at the time of sacrifice between the two groups.

![Figure 1. Box plots of the plasma melatonin levels in the Mg adequate and deficient groups. The bottom and top of the box plots are the 25th and 75th percentiles. The median is the horizontal line within the box. The adjacent lines demonstrate the variance of the data. Plasma melatonin was significantly lower in the Mg deficient animals when compared to the Mg adequate animals (p = 0.0101).](image-url)
The mean plasma Mg concentration of the Mg deficient animals (0.52 ± 0.01 mmol/L) was 57% of that of the Mg adequate animals (0.91 ± 0.01 mmol/L) (p < 0.0001).

Discussion

This pilot study supports that Mg deficiency results in decreased plasma melatonin. It is unclear if Mg deficiency induces phase shifts. The results in this study showed a trend to support that no phase shifts occurred with Mg deficiency, but another study with larger numbers of animals in the groups is needed to confirm or disprove this observation.

Because only plasma melatonin was measured, it is unclear whether the decreased melatonin was due to decreased synthesis of the hormone or increased destruction. A future study needs to measure pineal melatonin, which would definitely assess melatonin synthesis activity.

The Mg concentration of the control diet was about twice that recommended for rodents in the AIN-93G diets [11]. Stock diets usually contain more than four times the recommended Mg concentration. In spite of these large differences, the plasma melatonin levels of our controls were comparable to serum melatonin levels in rats fed a stock diet [12].

Plasma Mg levels decreased significantly in the Mg deficient animals. However, the data on the growth of the animals indicates that the deficiency was moderate. If the deficiency had been more severe, it would have influenced growth. This makes the results more applicable to humans because of the rarity of severe Mg deficiency in humans.

Because this was a pilot study, it has limitations. No previous studies were found that could be used to calculate the number of animals needed in each group to obtain statistical power. There were enough animals to determine that Mg nutritional status affected plasma melatonin, but there were not enough animals in the groups that were sacrificed at the different time periods. There was a strong trend that no phase shift had occurred and that the dietary Mg affected the plasma melatonin levels at the sacrifice times. Further work is needed to either confirm or refute these results.

Another limitation is that these weanling rats were fed a Mg deficient diet for only four weeks. A longer feeding time, a less severe deficiency, or a different age when the Mg deficient diet was started might have influenced the plasma melatonin levels differently.

The results in this study may have implications in humans. In the National Health and Nutrition Examination Survey, 1999-2000, the median daily Mg intake was 215 mg/d and 296 mg/d for females and males, respectively, that were 20 to 39 years [13]. These intakes fall short, by more than 100 mg, of the 1997 Recommended Dietary Allowance (RDA) for Mg which is 320 mg/d for adult females and 420 mg/d for adult males [14]. If these median intakes were low enough to decrease plasma melatonin levels, much of the population might realize significant benefits from increased Mg intake from food or supplements.

Elderly people are at increased risk for developing Mg deficiency for a variety of reasons. In the Netherlands, the elderly have a reduced dietary intake of Mg [15]. Mean Mg intake decreases after age 60 in the U.S. [13]. The elderly also experience a decrease in Mg absorption with increasing age [16] and an increase in urinary Mg excretion [15]. Studies in rats [17, 18] and humans [19-22] indicate that circulating melatonin levels decline with age. In addition, diminished melatonin secretion may be associated with an acceleration of the aging process [23] because of a lesser ability to combat oxidative stress [24]. There may be a relationship between a marginal Mg nutritional status of the elderly and the decreased plasma melatonin levels seen in many of these individuals.

Sleep disturbances occur with Mg deficiency in rats [6, 7] and melatonin promotes the onset of sleep and improves the duration and quality of sleep in humans [2]. Decreased melatonin synthesis occurs in patients with coronary artery disease [25-31] and Mg deficiency has been implicated in development of coronary artery disease [32-35]. Whether Mg deficiency is a contributor to the decrease in melatonin

Table 1. Plasma melatonin at 4, 5, and 7 hours after initiation of darkness.

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<tr>
<th></th>
<th>4 h</th>
<th>5 h</th>
<th>7 h</th>
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<tr>
<td>Mg deficient</td>
<td>32 ± 12</td>
<td>64 ± 11</td>
<td>52 ± 11</td>
</tr>
<tr>
<td>Mg adequate</td>
<td>64 ± 12</td>
<td>80 ± 11</td>
<td>71 ± 12</td>
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Mean ± SEM. n = 6 or 7 animals in each group.
synthesis in coronary artery disease is unknown and needs to be investigated.

**Conclusion**

Moderate dietary Mg deficiency in young male rats resulted in a significantly decreased plasma melatonin level in comparison to the Mg adequate animals. This Mg deficiency did not cause an obvious phase shift in the melatonin profile.

**References**


