MAGNESIUM STATUS AFFECTS NUTRIENT METABOLISM.

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Magnesium (Mg) is essential for a diverse range of physiological functions and is involved in a variety of the body's biochemical processes. Mg deficiency, either from inadequate intake or excess excretion, is often suspected to be associated with the development of many symptoms and diseases. The nutritional and physiological importance of Mg has already been well established. In 1952, a positive correlation between the rates of death caused by apoplexy and consumption of hard local drinking water was reported by Kobayashi. Numerous subsequent investigations confirmed that consumption of hard local drinking water decreases hypertension and/or cardiovascular diseases. Mg was finally confirmed to be the most effective element for preventing these diseases. Recently, many studies and meta-analyses, have proved the above finding. Other researchers observed that Mg intake was inversely related to the prevalence of metabolic syndromes. Thus, dietary Mg is important for preventing various lifestyle-related diseases such as hypertension, cardiovascular diseases, and diabetes. Further, a number of studies have reported the effects of Mg deficiency on carbohydrate, lipid, protein, vitamin and mineral metabolism. In the present study, we investigated that the effects of dietary-Mg deficiency on proteins, lipids, ascorbic acid and mineral metabolism including kidney calcification and bone loss in rodents. Furthermore, we performed transcriptome analysis to comprehensively understand the effects of dietary-Mg deficiency in rat liver by using DNA microarray.

1. Magnesium deficiency and protein metabolism

With regard to growth in rats, final body weight and food intake is decreased due to a Mg-deficient diet. We have reported reductions in the levels of serum total protein and albumin in rats that were fed the Mg-deficient diet. Rico et al. also reported that the serum total protein level is decreased in Mg-deficient rats. These findings suggested that the Mg-deficient diet influences not only mineral metabolism but also protein metabolism. Although several studies have reported the effects of both dietary Mg and protein levels on protein metabolism, the effects of the Mg-deficient diet alone on protein metabolism remain unclear. Further studies are needed to elucidate the effect of a Mg-deficient diet on protein metabolism. Previous studies have shown that impairment of mineral utilization in Mg-deficient rats is reversed by dietary Mg supplementation. Based on these findings it can be inferred that dietary Mg supplementation reverses the alteration of protein utilization in Mg-deficient rats. However, the effect of dietary Mg supplementation on the alteration of protein utilization in Mg-deficient rats has not yet been assessed.

In this study, we investigated the effect of the dietary Mg level on protein utilization, particularly with reference to the effect of dietary Mg supplementation on the Mg-deficient diet-induced alteration of protein utilization, by assessing nitrogen (N) balance and serum protein levels.

Male Wistar rats were fed a control diet based on the AIN-93G formula (control group) and a Mg-deficient diet (Mg-deficient group) for 28 days. After 28 days, the diet of half of the Mg-deficient group (recovery group) was changed from the Mg-deficient diet to the control diet for either 7 or 14 days.

After 28 days, final body weight, weight gain and food efficiency were significantly decreased due to the Mg-deficient diet. Apparent Mg absorption, Mg retention and serum Mg levels were also significantly decreased due to the Mg-deficient diet. Furthermore, the Mg-deficient group showed a significant increase in urinary nitrogen (N) excretion and significant decreases in N retention and serum albumin level. At day 7 and 14 after changing the Mg-deficient diet to the control diet, apparent Mg absorption, Mg retention and serum Mg levels were significantly increased in the recovery group as compared with those in the Mg-deficient group. However, with regard to final body weight, weight gain and food efficiency, no significant differences were observed between the Mg-deficient group and the recovery group. At day 14 after changing the diet, urinary N excretion was significantly decreased and N retention was significantly increased in the recovery group as compared with the Mg-deficient group. At day 7 and 14 after changing the diet, the serum albumin level was also significantly increased in the recovery group as compared with that in the Mg-deficient group. These results suggest that (1) the Mg-deficient diet depresses protein utilization; (2) the Mg-deficient diet-induced impairment of protein utilization is reversed by dietary Mg supplementation and (3) the Mg-deficient diet-induced growth retardation is not completely reversed after 14 days of Mg supplementation.

2. Magnesium deficiency and lipid metabolism

Several studies have provided evidence that Mg deficiency can affect the metabolism of membrane lipids associated with tissue injury. Recent investigations have shown that Mg deficiency increases the susceptibility of tissues to peroxidation by a mechanism possibly involving elevated levels of thiobarbituric acid-reactive substances (TBARS). However, the specific mechanism by which Mg deficiency can result in oxidative injury remains to be determined.

The aim of this study was to clarify the effect of a Mg-deficient diet on phosphatidylcholine hydroperoxide (PCOOH) and phosphatidylethanolamine hydroperoxide (PEOOH) concentrations, the primary peroxidation products of phospholipid components of the membrane, and its relationship to changes in tissue distribution of minerals such as Mg, calcium (Ca), iron (Fe) and copper (Cu).

Weanling (4-week-old) male Wistar rats were divided into 2 groups of 6 each, and pair-fed either a Mg-deficient diet (D: 0.006% Mg) or a control diet (C: 0.05% Mg). These diets were prepared according to the AIN-76 formulation, and slightly modified.

It is known that Fe, bound in an active form, is involved in the formation of oxygen free radicals in reactions such as the Fenton and Harber-Weiss reactions. The Fe concentration in the liver increased as a result of Mg deficiency. Two possible mechanisms exist by which this occurs: 1) a low intestinal Mg concentration due to intake of a Mg-deficient diet may cause an increase in intestinal Fe absorption, as it has been shown that Fe uptake by duodenum fragments is inhibited in the presence of Mg, and 2) Mg deficiency increases hemolysis, thus liberating Fe from hemoglobin. Several researchers have shown that Fe concentrations are correlated with an increase in malondialdehyde (MDA) and TBARS in the liver. In this study, we observed that the concentrations of Fe and PCOOH, the primary peroxidation products of the phospholipid components of the membrane, were elevated in the liver of rats fed a Mg-deficient diet. A significant positive correlation between Fe and PCOOH concentrations in the liver ($r=0.837$, $P=0.001$) was evident. In the heart, a significant positive correlation between Fe and PCOOH concentrations ($r=0.780$, $P=0.003$), and between Ca and PCOOH ($r=0.633$, $P=0.027$), was evident. Numerous experimental, epidemiological and clinical studies have pointed out that Mg deficiency contributes to the development of many cardiovascular diseases. Under conditions of Mg deficiency, the intracellular Ca concentration is elevated and the increase in Ca can enhance lipid peroxidation. In the present study, an increased PCOOH concentration in the aorta was also demonstrated. We did not measure mineral concentrations in the aorta, however, our findings that the concentrations of Ca, Fe and Cu in the RBC membrane and the concentration of Cu in plasma were elevated suggest that oxidation of the mucus membrane of the aorta might occur. In our previous study,

the concentration of PCOOH was found to be increased in the liver of rats with iron deficiency and this was associated with accumulation of hepatic Cu. In this study, a significant correlation between PCOOH and Cu concentrations in the liver ($r = 0.579$, $P = 0.048$) was evident. It is also known that free radical formation is markedly influenced by the presence of Cu ions (Cu^{2+}). Gueux et al. reported that hypertriglyceridemia observed in rats with Mg deficiency was associated with a significant increase in levels of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL). The results concerning copper-induced lipid peroxidation in vitro, expressed in terms of conjugated dienes and TBARS content, indicate that VLDL + LDL particles from rats with Mg deficiency are more susceptible to oxidative damage than the lipoproteins from control rats. In this study, a significant correlation between Cu and PCOOH concentrations in the RBC ($r=0.784$, $P=0.008$) and in the plasma ($r = 0.907$, $P = 0.0001$) was evident. In the RBC, the PEOOH concentration was also elevated. The PEOOH concentrations in other tissues did not change as a result of Mg deficiency. This might be due to increased concentrations of Ca, Fe and Cu, which are involved in the formation of oxygen free radicals, in the RBC membrane in the rats with Mg deficiency as compared to the control rats. We have reported that a magnesium-deficient diet induces renal structural injury, nephrocalcinosis, and diminished kidney function in rats. Thus, we hypothesized that the concentration of PCOOH might be elevated in the kidney under conditions of Mg deficiency. However, the kidney did not show high PCOOH or PEOOH levels, whereas renal Ca and Cu concentrations were elevated in rats fed the Mg deficient diet as compared to the control rats. In the kidney under conditions of Mg deficiency, Ca does not exist in its free form, but rather as calcium phosphate or as some other complex involving proteins or glucoproteins. Cu might bind to metallothionein. In such cases, Ca or Cu might not promote an increase in PCOOH and PEOOH concentrations. However, further studies should be carried out. Another approach for assessment of lipid peroxidation under conditions of Mg deficiency is based on demonstration of a depressed antioxidant defense system. The levels of activity of the antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPX) are decreased in animals with Mg deficiency. In this study, neither the level of SOD activity nor the level of GPX activity was elevated in plasma, liver, heart nor kidney in rats fed the Mg-deficient diet. 8-hydroxy-deoxiguanosin (8-OH-dG) is known as a marker of oxidative DNA damage. The concentration of 8-OH-dG in the urine of the rats fed the Mg-deficient diet was found to be 2-fold higher than that in the control group. This result may indicate that DNA damage had occurred in the Mg-deficient tissues, although we should measure the tissue levels of 8OH-dG as well.

3. Magnesium deficiency and ascorbic acid.

Hus et al. 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 defense responses that protect organisms from oxidative damage, and AsA is an important biological antioxidant. 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.

Eighteen 3-week-old male Sprague-Dawley strain rats were divided into 3 groups and maintained on a control diet based on the AIN93-G formula (C group), a low-Mg diet (D group), or a low-Mg diet supplemented with AsA (DA group) for 42 d.

Mg deficiency reduced the final body weights of the animals in the present study, consistent with previous studies. However, AsA supplementation did not affect the absorption, retention, and serum concentrations of Mg in the Mg-deficient rats. It was 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. 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. 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 analyze 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. 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 acyltranseferase (LCAT). Moreover, Mg deficiency increases the percentage composition of triglycerides in VLDL, LDL, and HDL, and reduces that of proteins. 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. 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 Triglyceride (TG) and Total cholesterol (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. 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. 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.

4. Magnesium deficiency and mineral metabolism

It is known that Mg deficiency affects the metabolism of several minerals. One of the typical effects of Mg deficiency on mineral metabolism is induction of kidney calcification (nephrocalcinosis). Rats fed a Mg deficient diet developed an increase in calcium (Ca) and phosphorus (P) concentrations in the kidney by chemical analysis, and nephrocalcinosis by histological examination. Our previous study observed that despite the administration of a Mg deficient diet, the kidney Ca and P concentrations、were not increased in male rats. We speculated that this result was due to the P concentration of the experimental diet based on the AIN-93G formula used in the previous study. In this study, male rats were fed modified AIN-93G diets containing two different Mg concentrations [0.5 g per kg diet (normal-Mg) or Mg-free (Mg-deficient)] and three different P concentrations [3(3-P), 5(5-P)or 7(7-P) g per kg diet]. By histological examination of the kidney, nephrocalcinosis was observed in rats fed the Mg deficient diet containing 3-P, while nephrocalcinosis appeared in rats fed the Mg deficient diet containing 5-P and 7-P. The degree of nephrocalcinosis was severe in rats fed the Mg deficient diet containing 7-P compared with rats fed the Mg deficient diet containing 5-P. These results demonstrated that the Mg deficient diet based on AIN-93G formula dose not induce nephrocalcinosis and that the Mg deficient diet based on AIN-93G formula with increased dietary P concentrations induces nephrocalcinosis in male rats. We suggest that the onset of nephrocalcinosis could depend on the dietary P concentration in male rats fed on a Mg deficient diet.

In contrast, bone mineral density (BMD) is decreased by Mg deficiency in rats. After 6 weeks of dietary treatment, the Mg-deficient diet decreased BMD and bone strength with decreasing serum osteocalcin as a marker of bone formation and increasing urinary deoxypyridinoline as a marker of bone resorption in weanling male rats compared to the control (normal Mg) rats.

5. DNA microarray analysis in magnesium deficient rat liver

Mg is involved in a variety of biochemical processes in the body. Mg deficiency often induces physiological and even pathophysiological disorders. Further, a number of studies have reported the effects of Mg deficiency on carbohydrate, lipid, protein, vitamin and mineral metabolism. However, comprehensive information remains unavailable on the relationship between Mg deficiency and nutrient metabolism studied under well-controlled and reproducible experimental conditions. We performed transcriptome analysis to comprehensively understand the effects of dietary-Mg deficiency in rat liver. Four-week-old male Wistar rats were dichotomized and fed a control diet containing 0.5 g Mg/kg (control group) or a Mg-deficient diet containing only 0.004 g Mg/kg (Mg-deficient (MD) group) for 4 weeks. During the feeding period, rats in both groups were pair fed to ensure that they consumed equal amounts of their respective diets. DNA microarray analysis demonstrated significant between-group differences with regard to various items. The MD diet led to the up- or down-regulation of 734 among 31099 genes. In the MD group, the genes associated with inflammatory and immune responses were generally up-regulated, while those associated with protein and amino acid metabolism, ion transport, and xenobiotic metabolism for detoxification were down-regulated. We then focused on individual metabolic pathways; the following results were obtained for the MD group. With regard to carbohydrate metabolism, the genes involved in the pentose phosphate pathway were up-regulated, whereas the genes encoding glycogen-synthetic enzymes were down-regulated. With regard to lipid metabolism, the genes encoding enzymes involved in fatty acid synthesis, long chain fatty acid transport, and cholesterol synthesis were all up-regulated, while those encoding enzymes that play a role in mitochondrial/peroxisomal β-oxidation and cholesterol absorption were down- regulated. With regard to protein metabolism, the genes related to proteolysis and ubiquitine-dependent protein catabolism were up-regulated, whereas those related to translation were down-regulated. In terms of amino acid metabolism, the genes involved in cysteine biosynthesis and glycine, cystine, and cysteine catabolism were up-regulated. All these data suggest that dietary Mg deficiency induces (1) a decrease in protein utilization; (2) changes in the overall hepatic gene expression, especially the expression of the genes involved in carbohydrate, lipid, and amino acid metabolism; and changes in even the transcription levels of these genes.

In the second study, gene expression showing the recovery by 7 day-feeding of a control diet from Mg deficiency, that appeared by 28 day-feeding of a Mg deficient diet (MD28), was discussed. Three rats were selected from those fed for 28 days on a control (C)diet and for 35days on the C diet (C35 group) from those fed likewise a Mg-deficient diet (MD35 group) and those fed the C diet after feeding of Mg-deficient diet (R group). There was no significant difference on hepatic gene expression pattern between the MD28 and MD35 groups. A feeding period-depending difference existed; expressions of 734 genes changed on day 28 and those of 637 genes on day 35. The comparison suggested that 357 among these genes showed the common expression pattern, while 377 and 280 genes were changed within 28 days and 35 days, respectively. Gene expression profiles were compared among the C35, MD35 and R groups. It was resulted that profiles in the R group approximated to those in the C35 group. It is indicated that the hepatic gene expression pattern tends to return to the original state in association with enhancing the dietary Mg amount up to the normal level. Then we focused on the 357 genes that had shown a significant expression difference between the MD28 and MD 35 groups, with the result that 80 % of those gave a reverse direction of expression change when the MD diet was replaced with the C diet.

Thus, the expression of these genes returned to the original level that had initially been observed by feeding with the C diet.

Key words: magnesium deficiency, rat, nutrient metabolism, DNA microarray,

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