The Effect of Magnesium Depletion on Thyroid Function in Rats[1]

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ABSTRACT The effects of dietary magnesium (Mg) depletion on thyroid function were studied in young male rats. The rats were fed a semipurified diet containing either 12 ppm Mg (deficient rats) or 662 ppm Mg (control rats) for 14 to 28 days. Results showed that the Mg-deficient rats had decreased body weight gain, lowered concentrations of plasma thyroxine (T_4) and Mg, but increased weight of the thyroid gland when expressed in proportion to the body weight (milligrams/100 g). There was no difference in the accumulation (uptake) of ^{131}I, 24 hours after $Na^{131}I$ injection, between the Mg-deficient and Mg-supplemented rats. The protein-bound ^{131}I ($PB^{131}I$) level and the ratio of $PB^{131}I$ to total ^{131}I in plasma was significantly reduced in Mg-deficient rats. Serum thyroid-stimulating hormone (TSH) levels after thyrotropin-releasing hormone injection (TRH, 50 ng/100 g body weight) increased fivefold at 30 minutes, but declined to near the basal level at 2 hours in both groups. No consistent difference in TSH response was observed between the two treatments. Serum T_4 response to TRH challenge was significantly reduced in Mg-deficient as compared to Mg-adequate rats at all time intervals. The reduction of T_4 level could be due to an impaired T_4 synthesis or release in Mg-deficient rats. J. Nutr. *114*: 1510–1517, 1984.

INDEXING KEY WORDS magnesium • thyroid function • thyroxine • triiodothyronine

Magnesium ion (Mg^{2+}) has been shown to activate thyroid adenyl cyclase in tissue homogenates (1, 2) and in isolated enzyme preparations (3). This divalent cation in vitro also stimulates cyclic 3'5' nucleotide phosphodiesterase (4). Since the action of thyrotropin on the thyroid gland is mediated by the formation of cyclic AMP (adenosine 3'5'-cyclic monophosphate) (5, 6), the availability of magnesium could affect the response of the gland to the pituitary hormone.

The first evidence showing that magnesium may serve to regulate the functioning of the thyroid gland itself was indicated by Kleiber et al. (7), who observed thyroid enlargement in magnesium-deficient rats. A similar observation was obtained by Corradino and Parker (8) indicating that magnesium-deficient rats had a statistically significant increase in thyroid gland size when expressed as milligrams of thyroid per 100 g

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of body weight. However, these investigators were unable to detect any effect of magnesium on the in vitro incorporation of ^{131}I into thyroxine (T_4) by thyroid tissues (8). Later, a series of studies concerning the effect of magnesium on thyroid activity in rats was reported by Heaton and associates (9–11). Their results demonstrated a significant positive correlation between magnesium concentrations in serum and the ratio of protein-bound iodine (PBI):total serum iodine, (9) as well as stimulation of thyroidal ^{131}I uptake by dietary magnesium in vivo (10). However, the addition of magnesium in vitro showed no direct effect on the iodide uptake by the thyroid (11).

Previously, we reported a significant increase of plasma phenylalanine and possibly tyrosine in magnesium-deficient rats (12). Since these two amino acids are the precursors of thyroid hormones, it is possible that magnesium depletion may affect thyroid activity. The present report concerns the effect of feeding a magnesium-deficient diet on: *1*) the concentrations of plasma T_4 and triiodothyronine (T_3), *2*) ^{131}I uptake by the thyroid after injection of a tracer dose of ^{131}I iodide, *3*) the response of serum T_4 concentration after thyrotropin-releasing hormone (TRH) injection and *4*) the response of serum thyroid-stimulating hormone (TSH) concentration after injection of TRH.

MATERIALS AND METHODS

Animals and diet. Four main experiments were performed on weanling and young male rats of matched age and body weight from the Sprague-Dawley strain (Charles River Breeding Laboratories, Wilmington, MA). In each experiment, these rats were randomly divided into two groups. The first group, magnesium-deficient rats, were fed the semipurified magnesium-deficient diet (powdered) (table 1) containing 12 ppm Mg. The second group, magnesium-supplemented control rats, was pair-fed an identical diet except that magnesium concentration was increased to 662 ppm. All the rats were housed individually in suspended stainless-steel cages. Room temperature at 24°C and relative humidity at 65% were held constant, and a 12-hour light/dark cycle was maintained. Deionized water was provided ad libitum. At varying intervals the rats were anesthetized with ether, and blood was drawn by cardiac puncture with and without heparin. Serum or plasma was obtained by centrifugation and stored at −25°C for the determinations of magnesium, calcium, T_4 and T_3. After the rats were killed, the thyroid, liver, kidney and right femur were immediately excised. They were cleaned and rinsed with ice-cold saline, weighed and stored at −50°C until analyzed.

TABLE 1

Composition of magnesium-deficient diet

Ingredient	Amount
	g/kg
Casein, vitamin free	200.0
DL-Methionine	3.0
Dextrose monohydrate	667.8
Corn oil	70.0
Vitamin mix[1,2]	10.0
Mineral mix[2,3]	49.2

[1]Vitamin mix provided the following (in milligrams per kilogram diet except as noted): vitamin A palmitate, 39.64; ergocalciferol, 4.405; vitamin E acetate, 242; *p*-aminobenzoic acid, 110; ascorbic acid (coated 97.5%), 0.9912 g; biotin, 441 μg; vitamin B-12 (0.1% trituration in mannitol), 29.7 μg; calcium pantothenate, 66.079; choline dihydrogen citrate, 3.496 g; folic acid, 1.98; inositol, 110; menadione (vitamin K_3), 49.56; niacin, 99; pyridoxine · HCl, 22.0; riboflavin, 22.0; thiamin · HCl, 22.0; and cornstarch, 4.666 g. [2]Purchased from Teklad, Madison, WI. [3]Mineral mix provided the following compounds in grams per kilogram diet: sodium bicarbonate, $NaHCO_3$, 12.6; potassium bicarbonate, $KHCO_3$, 15.1; sodium iodide, NaI, 0.03; calcium phosphate, dibasic, $CaHPO_4$, 14.3; calcium chloride, $CaCl_2$, 5.7; ferric citrate (10.7% Fe), 1.24; cupric sulfate, $CuSO_4 \cdot 5H_2O$, 0.013; zinc sulfate, $ZnSO_4 \cdot 7H_2O$, 0.024; manganese sulfate, $MnSO_4 \cdot H_2O$, 0.174.

Magnesium and calcium analysis. Plasma or serum was diluted with 1% oxine solution containing 2000 ppm lanthanum as $LaCl_3$. Aliquots of the solution were used to determine magnesium and calcium concentrations by atomic absorption spectroscopy by using the method of Yunice and Lindeman (13). Tissues were cleaned, weighed and dried at 125°C to constant weight, then ashed in a muffle furnace at 550°C for 12 hours. The white ash was dissolved in 0.1 ml

of concentrated nitric acid and diluted to 5.0 ml with the same diluting fluid used for plasma. Magnesium concentrations of all tissues were determined by atomic absorption spectroscopy (13). Total and free plasma T_4 and total T_3 were determined by radioimmunoassay.[2]

The T_3 uptake measures the circulating level of unbound (unsaturated) thyroxine-binding globulin (TBG). The free thyroxine index (FTI) was calculated by the multiplication of the total T_4 value by the T_3 uptake value.

Thyroid uptake of ^{131}I. A dose of 10 μCi of sodium ^{131}I (New England Nuclear, Boston, MA) per 100 g body weight was injected intramuscularly into each rat. Twenty-four hours later, blood was drawn from the heart through a heparinized syringe, and plasma was obtained by centrifugation. The thyroid glands were immediately removed, cleaned, weighed and then transferred into Pyrex counting tubes with 1 ml of 5 *N* NaOH. Upon completion of digestion, usually within 2 hours at room temperature, the radioactivity in the thyroid gland preparations was determined in a well-type scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, IL).

Measurement of labeled PBI in plasma. An aliquot of plasma was mixed with an equal amount of 30% trichloroacetic acid. The precipitate obtained by centrifugation was successively washed three times with 5% trichloroacetic acid. The radioactivities and total and trichloroacetic acid precipitates were counted by the same procedure as was used for the measurement of thyroid radioactivity. Corrections were made for background and isotopic decay.

TRH injection. After 3 weeks of dietary treatment, the rats were fasted for 16 hours and anesthetized with ether. TRH (Calbiochem, LaJolla, CA) was injected into the tail vein at a concentration of 50 ng/100 g body weight in 0.1 ml of saline. At 0, 0.5, 1.0, 2.0 and 4.0 hours after TRH injection, six rats from each dietary treatment were killed by cardiac puncture. Total serum T_4 and thyrotropin (TSH) were determined by radioimmunoassays[3] (14). The statistical evaluation of experimental observations were determined by the Student's *t*-test.

RESULTS

Growth response to magnesium depletion. Rats fed a magnesium-deficient diet in all experiments exhibited hyperirritability, peripheral vasodilation and skin lesions typical of magnesium deficiency. Similar results have been observed in earlier studies (12, 15). In addition, growth retardation (tables 2, 3 and 4) was seen in magnesium-deficient rats as compared to their respective pair-fed controls. This reduction in body weight appeared to be reversible when deficient rats were repleted with magnesium-supplemented diet (see table 7).

Magnesium and calcium concentrations. There were no differences in magnesium concentrations in the thyroid glands of magnesium-deficient and magnesium-supplemented rats (table 2). In contrast, the calcium concentrations in the thyroid of magnesium-deficient rats were significantly elevated (table 2). Similar results were observed in a magnesium repletion study (see table 7). This supports previous reports that magnesium deficiency induces a disturbance of calcium metabolism (16). Table 3 lists the results of magnesium concentrations in the plasma, femur, liver and kidney. Magnesium levels in the plasma and femur decreased significantly in rats fed a magnesium-deficient diet. No difference in magnesium concentrations of the liver and kidney were observed between magnesium-deficient and magnesium-supplemented rats. These later findings confirm the reports of McNeill, Herbein and Ritchey (17) and Sarner and Snodgrass (18).

Thyroid enlargement. The gross thyroid weight did not show any difference between the two groups. However, thyroid size in milligrams per 100 g of body weight was consistently increased in magnesium-deficient rats (tables 2, 4). An increase of several organ weights such as liver, kidney, spleen and testis in magnesium-deficient rats has been previously reported (12).

[2] Rat plasma T_4 and T_3 determinations were performed with immunoreagents and by the procedure distributed by Micromedic System Incorporated, Concept 4, Horsham, PA (1975).

[3] Rat TSH determinations were performed with the immunoreagents and by the procedure distributed by the National Institute of Arthritis, Metabolism and Digestive Diseases (1981).

TABLE 2

Effects of magnesium deficiency on thyroid weight, thyroid:body weight ratio and thyroid magnesium and calcium concentrations[1]

Measure	Mg supplemented (pair-fed)	Mg deficient	Level of significance
			P
Final body wt,[2] *g*	308 ± 22	269 ± 13	<0.01
Thyroid wt, *mg*	13.8 ± 0.9	14.6 ± 1.6	NS[3]
Thyroid:body wt ratio, *mg/100 g*	4.49 ± 0.25	5.44 ± 0.41	<0.01
Magnesium, *mg/g (wet wt)*	0.57 ± 0.07	0.65 ± 0.16	NS
Calcium, *mg/g (wet wt)*	3.44 ± 0.63	6.53 ± 1.81	<0.01

[1]Values are means ± SD; n = 6 rats per group. [2]Fifty-day-old male rats, initially weighing 179 ± 8 g (mean ± SD) for Mg-supplemented and 181 ± 8 g for Mg-deficient rats were fed experimental diet for 28 days. [3]NS, not significant.

Plasma T_4, T_3 levels, T_3 uptake and free thyroxine index (FTI) value. The values of plasma total and free T_4 and FTI were significantly reduced in magnesium-deficient rats as compared to magnesium-supplemented rats (table 3). No differences were observed in plasma T_3 level or the percentage of T_3 uptake.

Uptake of ^{131}I by thyroid glands and plasma $PB^{131}I$. The effect of magnesium deficiency on ^{131}I uptake by the thyroid gland is shown in table 4. The radioactivities found in the thyroids and total plasma of magnesium-deficient rats were not significantly different from the values obtained in their corresponding controls. However, plasma $PB^{131}I$ values and the ratio of plasma $PB^{131}I$ to total plasma ^{131}I (table 4) were substantially decreased to magnesium-deficient rats. The reduction in ratio was due mainly to the increased radioactivity in total plasma and the decreased radioactivity in PB-^{131}I.

Serum T_4 response. Table 5 shows that the serum T_4 value of magnesium-deficient rats before TRH treatment was less than 50% of that of the supplemented animals. After TRH administration, the response of serum T_4 by magnesium-supplemented rats was

TABLE 3

Effects of magnesium deficiency on magnesium concentration in plasma, liver, kidney, and femur and plasma thyroxine (T_4) and triiodothyronine (T_3) levels, and values of free thyroxine index (FTI)[1]

Measure	Mg supplemented (pair-fed)	Mg deficient	Level of significance
			P
Final body wt,[2] *g*	270 ± 12	230 ± 9	<0.01
Magnesium concn			
Plasma, *mEq/L*	1.35 ± 0.25	0.36 ± 0.11	<0.01
Liver, *mg/g dry wt*	0.685 ± 0.105	0.713 ± 0.091	NS[3]
Kidney, *mg/g dry wt*	0.819 ± 0.138	0.789 ± 0.027	NS
Femur, *mg/g dry wt*	3.889 ± 1.347	1.528 ± 0.567	<0.05
Plasma T_4			
Total, *μg/dl*	4.72 ± 0.51	3.64 ± 0.52	<0.02
Free, *μg/dl*	1.50 ± 0.24	1.10 ± 0.32	<0.05
Plasma T_3, *ng/dl*	82.2 ± 9.35	78.2 ± 7.85	NS
T_3 uptake, %	57.2 ± 1.28	56.8 ± 1.45	NS
FTI	2.70 ± 0.25	2.07 ± 0.32	<0.01

[1]Values are means ± SD; n = 8 rats/group. [2]Forty-two-day-old male rats initially weighing 150 ± 7 g (mean ± SD) for Mg-supplemented rats and 147 ± 5 g for Mg-deficient rats, were fed experimental diets for 21 days. [3]NS, not significant.

TABLE 4

Effects of magnesium deficiency on thyroidal uptake of ^{131}I, the activity of total plasma ^{131}I, protein-bound (PB) ^{131}I and the ratio PB^{131}I:total plasma ^{131}I in 24 hours[1]

Measure	Mg supplemented (pair-fed)	Mg deficient	Level of significance
			P
Final body wt,[2] *g*	160 ± 12	124 ± 18	<0.05
Thyroid:body wt ratio, *mg/100 g*	9.0 ± 0.74	11.41 ± 1.12	<0.05
Thyroid ^{131}I uptake, *cpm/mg*	4788 ± 1139[3]	4403 ± 1136	NS[4]
Plasma ^{131}I activity			
Total, *cpm/ml*	6849 ± 2686	8313 ± 1411	NS
PBI, *cpm/ml*	601 ± 100	421 ± 47	<0.05
PBI:total ratio	0.105 ± 0.04	0.052 ± 0.01	<0.05

[1]Values are means ± SD; *n* = 8 rats/group. [2]Five-week-old male rats, initial body weight for Mg-supplemented rats, 92 ± 9.0 g, (mean ± SD) Mg-deficient rats, 108 ± 28 g, fed the experimental diets for 2 weeks. [3]After an overnight fast, each rat received an i.m. injection of Na^{131}I in saline (10 μCi/100 g body weight) and was killed by heart puncture 24 hours later. [4]NS, not significant.

slight, but appeared to have peaked at 2 hours and returned to baseline levels at 4 hours. In magnesium-deficient rats, the response of serum T_4 to TRH challenge was somewhat greater. But the differences in T_4 values before and after TRH injection was insignificant. However, when the comparison was made between serum T_4 levels at each time interval following TRH treatment, a significant reduction was observed in magnesium-deficient rats compared with the corresponding control animals (table 5).

Serum TSH response. Before TRH injection the amount of circulating serum TSH of magnesium-deficient rats was approximately the same as magnesium-supplemented rats (table 6). At 30 minutes the increase of serum TSH was substantial and appeared to have peaked in both groups. This increase was then markedly reduced to 60% (of the peak value) in magnesium-supplemented rats and to 40% in magnesium-deficient rats at 60 minutes while animals injected with TRH reached comparable values at 30 minutes postinjection. The difference between two treatments was statistically significant. In both groups the values of serum TSH at 120 minutes were about the same as the values before injection. By 240 minutes after TRH injection, serum TSH values were less than basal values in both magnesium-supplemented and magnesium-deficient rats.

Magnesium repletion. The results summarized in table 7 demonstrate that the repletion for 1 week with magnesium-supplemented diet to magnesium-deficient rats restored the values of plasma T_4 to normal. Thus, the impairment of T_4 metabolism appeared to be readily reversible.

DISCUSSION

The results of these experiments show that the activity of the thyroid gland can be influenced by dietary magnesium deficiency.

TABLE 5

Effect of magnesium deficiency on the response of serum total thyroxine (T_4) after TRH injection[1]

Time	Serum total T_4: Mg supplemented (pair-fed)	Serum total T_4: Mg deficient	*P*-value
	μg/dl		
Before TRH injection	6.68 ± 0.70	3.13 ± 1.13	<0.001
Hr after TRH injection			
0.5	5.71 ± 0.43	4.86 ± 0.75	<0.050
1.0	6.38 ± 1.08	4.75 ± 0.70	<0.025
2.0	7.35 ± 0.85	5.60 ± 1.28	<0.025
4.0	6.46 ± 0.55	5.16 ± 0.64	<0.010

[1]Values are means ± SD for *n* = 6 rats/group. TRH, thyrotropin-releasing hormone, or TRF, thyrotropin-releasing factor, modified (2-L-3-methylhistidine TRF, *p*-Glu-L-3-Me-His-L-Pro-NH_2, Calbiochem-Behring Corp., La Jolla, CA, Lot 210059), 50 ng in 0.05 ml saline for each 100 g body weight, i.v.

TABLE 6

Effect of magnesium deficiency on the response of serum thyroid-stimulating hormone (TSH) after thyrotropin-releasing hormone (TRH) injection[1]

Hr after TRH injection	TSH response: Mg supplemented (pair-fed)	TSH response: Mg deficient	*P*-value
	ng/ml		
0	2,278 ± 939	2,127 ± 904	NS[2]
0.5	10,649 ± 2,716	10,079 ± 2,650	NS
1.0	5,880 ± 1,181	3,921 ± 1,057	<0.02
2.0	3,200 ± 1,185	2,528 ± 967	NS
4.0	1,512 ± 404	1,461 ± 492	NS

[1]Values are means ± SD; $n = 6$. [2]NS, not significant.

The increased size of thyroid glands, the reduction of plasma T_4 concentration, the decreased ratio between PB^{131}I and total plasma ^{131}I and the decreased FTI are indications of thyroidal dysfunction in magnesium-deficient rats. The lack of difference in T_3 uptake pointed out that the reduced plasma T_4 was apparently not due to the circulating level of unbound TBG. Whether these defects are related to the reported functional impairment in the liver (17), pancreas (18, 19), kidney (20, 21) and heart (22) of magnesium-deficient rat is not clear. Despite drastically reduced plasma magnesium concentration, thyroidal magnesium content in magnesium-deficient rats was not significantly altered indicating that magnesium is probably indirectly involved in thyroid function. Heaton and Lucas (11) have demonstrated that the rate of thyroid hormone synthesis from radioactive iodide in vitro and the concentration of cyclic AMP within the thyroid glands were unaffected by magnesium status. However, the influence of magnesium deficiency on biosynthesis of thyroxine in vivo was not reported.

The information on thyroid uptake of radioactive iodide as related to magnesium depletion is unclear and controversial. Corradino and Parker (8) reported that the thyroid gland of magnesium-deficient rats contained more ^{131}I, 24 hours after injection of K^{131}I, than those of rats fed higher dietary levels of magnesium. The injected dose was not based on body weight, and the results were expressed as percent of injected dose remaining in thyroid after 24 hours. Different findings were obtained by Heaton and Lucas (11) who observed that iodide uptake by the thyroid gland after a single dose (10 μCi ^{125}I/rat) was enhanced by magnesium loading and inhibited by magnesium deficiency when the results were expressed as a ratio between counts per gland and counts per 1.0 ml of serum. Since body size of magnesium-deficient rats was approximately 20% less than normal weight, it is more appropriate that the injected dose should be proportional to body weight. Again, due to the differences in thyroid weight between magnesium-deficient and magnesium-supplemented rats, perhaps thyroidal uptake should be calculated as radioactivity per unit weight of thyroid. The data indicate that thyroid ^{131}I uptakes in vivo of magnesium-deficient rats and their pair-fed controls were insignificantly different and allow us to suggest that magnesium depletion may not alter the

TABLE 7

Effect of magnesium repletion on plasma T_4 concentration[1]

Measure	Mg supplemented (pair-fed)	Mg repleted[2]	Mg deficient
No. of rats	6	6	7
Final body wt, *g*	268 ± 10	258 ± 9	225 ± 11*
Thyroid wt, *mg*	12.1 ± 0.1	11.5 ± 0.8	13.4 ± 0.5
Thyroid:body wt ratio, *mg/100 g*	4.45 ± 0.25	4.62 ± 0.31	5.97 ± 0.41*
Thyroid magnesium, *mg/g (wet wt)*	0.58 ± 0.07	0.59 ± 0.01	0.65 ± 0.16
Thyroid calcium, *mg/g (wet wt)*	3.52 ± 0.11	4.21 ± 0.21	5.73 ± 0.83*
Plasma T_4, *μg/dl*	4.85 ± 0.27	4.62 ± 0.32	3.45 ± 0.18*

[1]Male rats with initial body weight of 125 ± 5 g were fed the respective experimental diets for 3 weeks. Values are means ± SD. [2]Male rats received Mg-supplemented diet ad libitum for 1 week after they had been on Mg-deficient diet for 2 weeks. *Significantly different from Mg-supplemented pair-fed and Mg-repleted rats ($P < 0.05$).

iodine trapping process (table 4). Whether this is equally true in the oxidation of iodide and/or in the iodination process remains to be investigated.

Since the response of serum TSH to TRH injection revealed no clear differences among samples, it would appear that the ability of the pituitary gland to respond to TRH was not influenced by magnesium deficiency. In contrast the marked reduction in T_4 levels suggests that the thyroid gland's ability to respond to its hormone production or release may be impaired in magnesium-deficient rats. The reduction in T_4 response may have resulted from an impaired T_4 synthesis in vivo or release by thyroid gland of magnesium-deficient rats.

It is well established that hypothyroidism causes hypercholesterolemia (23–25) and hyperlipoproteinemia (26). In hypothyroidism an increase in plasma low density lipoprotein (LDL) levels also has been observed, which is attributed to a decrease of LDL degradation (27). The defect in LDL removal can be reversed by the administration of thyroxine (27). Recently Rayssiguier et al. (28) reported that magnesium deficiency in rats results in an increase in plasma triglyceride and free cholesterol levels. In addition, with magnesium deficiency, both triglyceride and cholesterol levels were significantly increased in the very low density lipoprotein (VLDL) and LDL fractions (28). These findings in conjunction with depressed T_4 levels (table 3) in magnesium-deficient rats indicate that the alteration in thyroid activity may be partially responsible for the observed hypercholesterolemia. It would be intriguing to know whether a magnesium-depleted state in humans would similarly result in thyroid dysfunction.

Further studies are needed to determine the effects of magnesium depletion on T_4 metabolism and related hypercholesterolemia. Such information becomes useful for assessment of the association between dietary magnesium deficiency, thyroid function, plasma lipid disorders and cardiovascular diseases (22, 29).

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