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Effects of magnesium on cytomorphology and enzyme activities in thyroid of rats

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Till date knowledge regarding the effects of high dietary magnesium on thyroid gland is incomprehensive though certain epidemiological studies reported development of thyroid gland dysfunctions in people with chronic exposure to hard water (especially with high magnesium) despite sufficient iodine consumption. The present study is to explore the effects of chronic high dietary magnesium exposure on thyroid morphology and functional status. Male adult albino Wistar strain rats were treated with graded doses of magnesium sulphate ($MgSO_4$; 0.5, 1.0 and 1.5 g %) for 60 days and changes in different thyroid parameters were investigated. Significantly stimulated thyroid peroxidase and Na^+–K^+-ATPase and altered idothyronine 5′- deiodinase type I activities, enhanced serum thyroxine (T4) (both total and free), total triiodothyronine (T3) and thyroid stimulating hormone with decreased free T3 levels and T3/T4 ratio (T3:T4) along with enlargement of thyroid with associated histopathological changes were observed in the treated groups. The results clearly confirm that chronic high dietary magnesium exposure causes potential thyroid disruption as reported in earlier epidemiological studies.

Keywords: Hypothalamo-pituitary-thyroid axis, Magnesium, Thyroid enzymes, Thyroid hormones

Magnesium is the 11th most abundant mineral in body and the second most predominant component of hard water[1]. Being an indispensable element, it is involved in nearly all vital physiological processes essential for survival of living organisms[2]. Human interactions with magnesium have tremendously increased over the years through environmental, dietary and industrial sources, medicinal supplements as well as regular consumption of hard water by a vast population worldwide. Despite its indispensability to existence, excessive magnesium exposure elicits tremendous adverse effects in body which is reflected in the development of several disorders including hypermagnesemia[3].

Epidemiological studies conducted in certain coastal areas of Sundarban Delta[4,5] in Gangetic West Bengal, India and others studies indicated a probable relationship between water hardness (especially with high magnesium content) and persistence of endemic goiter inspite of sufficient iodine intake. Therefore a possible role of hard water in thyroid disruption has been suspected. Further the information in this regard on human as well as experimental (rat) models is still inconclusive.

The present study has been undertaken to examine the morphological, cytological and functional status of thyroid gland in experimental animals under chronic exposure of graded doses of magnesium by evaluating the histopathological changes, activities of thyroid hormone synthesizing enzymes and thyroid hormone profiles.

Materials and Methods

Maintenance and treatment of animals —About 3 months old (90±5 days) i.e. adult, 32 male albino rats (*Rattus norvegicus)* of Wistar strain weighing 140±10 g, used were maintained following the guidelines and protocols of Indian National Science Academy (INSA) and the proposal was approved by the Institutional Animal Ethics Committee (PHY/CU/IAEC/16/2008 dated 15.05.2008). They were fed on standardized i.e., normal 20% protein diet [6-8].

The animals were divided into following 4 groups of 8 each. The first group was fed with normal diet and considered as control while the second, third and fourth groups were fed the respective doses of 0.5, 1.0 and 1.5 g $MgSO_4$/100 g diet/day treatment[5,9] for 60 days. All animals were subjected to cervical

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dislocation, 24 hours after the last treatment following protocols and ethical procedures.

Reagents—Reagents were purchased from: Sigma [ouabin octahydrate, thyroxine (T4), propylthiouracil (PTU), chloramine T and poly ethylene glycol (PEG) and bromophenol blue]; Loba Cheme [potassium chloride (KCl), sodium dodecyl sulfate (SDS) or sodium lauryl sulfate, sodium meta arsenite and ascorbic acid]; Alfa Aesar (Dowex 50WX8); Pharmacia Fine Chemicals AB, Uppsala, Sweden (Sephadex G50); SRL, India [EDTA and sodium hydroxide (NaOH) pellets from BDH; imidazole, Tris base, trisodium citrate dihydrate, DTE, folin ciocalteus phenol and bovine serum albumin (BSA)] and MERCK, Germany (other reagents).

Thyroid weight—Thyroid gland weights were recorded following the experimental protocols of Chandra *et al* [6].

Histologic, histometric/morphometric and semi-quantitative assay—Histopathologic, morphometric as well as semiquantitative assay of thyroid glands were performed following the experimental protocols of Chandra *et al* [6].

Thyroid peroxidase (TPO) assay—Thyroid peroxidase (TPO) activity was measured by a method of Alexander[10].

Thyroidal sodium potassium triphosphatase (Na^+-K^+-ATPase) assay—Thyroidal sodium-potassium adenosine triphosphatase (Na^+-K^+-ATPase) activity was measured as per Esmann *et al.* [11], with slight modification[12,13].

Thyroidal 5'-deiodinase type I (DI) Assay—Iodothyronine 5'-deiodinase type I (5'-DI) activity was measured according to the method of Ködding *et al.*[14].

Protein estimation—Proteins were estimated by the method of Lowry *et al.*[15], using bovine serum albumin (BSA) as the standard protein.

ELISA of serum total thyroxine (tT4), total triiodothyronine (tT3), free thyroxine (fT4) and free triiodothyronine (fT3)—Circulating tT4, tT3, fT4 and fT3 levels were assayed using respective ELISA kits obtained from RFCL Limited, India. The respective sensitivity of the tT4, tT3, fT4 and fT3 assays was 0.4 µg/dL, 0.04 ng/mL, 0.05 ng/dL and 0.04 pg/mL respectively.

Radioimmunoassay (RIA) of thyroid stimulating hormone (TSH)—Serum levels of thyroid stimulating hormone (TSH) were assayed by RIA using reagents supplied by Rat Pituitary Distribution and NIDDK (California, USA). NIDDK rTSH was iodinated using the Chloramine T method [16] with carrier free ^{125}I.

Statistical analysis—Data were expressed as mean ± SD. One-way analysis of variance (ANOVA) test was first carried out to test whether there is any difference between the mean values of all groups. Values of the experimental groups were compared with that of the control group by multiple comparison *t*-test if differences between groups were established. A value of $P < 0.05$ was interpreted as statistically significant [17].

Results and Discussion

Changes in enzyme activity—The results showed gradual, significant ($P < 0.05$) elevation in the thyroid peroxidase (TPO) (Fig. 1 A) and thyroidal Na^+-K^+-ATPase (Fig. 1 B) enzyme activities in a dose–dependent manner while thyroidal 5´-Deiodinase Type I (DI) (Fig. 1 C) activity showed a striking biphasic response, i.e., inhibition after administration of moderate (1.0 g %) dose but elevation after high (1.5 g %) dose of magnesium sulphate in treated groups.

TPO, the principal enzyme essential for all the major steps of thyroid hormone biosynthesis, is TSH-dependent[2]. It is thus logical to suggest that, the higher TPO activity as observed in the present study is presumably a TSH-mediated means for optimal adaptation to thyroid dysfunction[2] caused by chronic exposure to high dietary magnesium.

A striking biphasic response pattern of deiodinase activity was observed upon magnesium exposure depending on the dose, i.e., lower enzyme activity at medium (1.0 g %) dose but higher at high (1.5 g %) dose. However, further studies are required to explain this observation. The exact role of high magnesium on DI activity is yet to be established. The relationship and interaction between magnesium and selenium has been well characterized as evidenced by alterations in the level of these two elements in various physiological as well as pathological conditions. Magnesium and selenium possess strong anti-oxidant actions; besides selenium is an integral part of the deiodinase enzyme structure[18]. Therefore the alterations in the activity of DI at different doses of magnesium could be explained in this light, though it cannot be confirmed from the present study.

The present study showed enhanced activity of the ouabin-sensitive thyroidal Na^+-K^+-ATPase enzyme, which is involved in the rate-limiting step of thyroid hormone biosynthesis, i.e., the active iodide transport

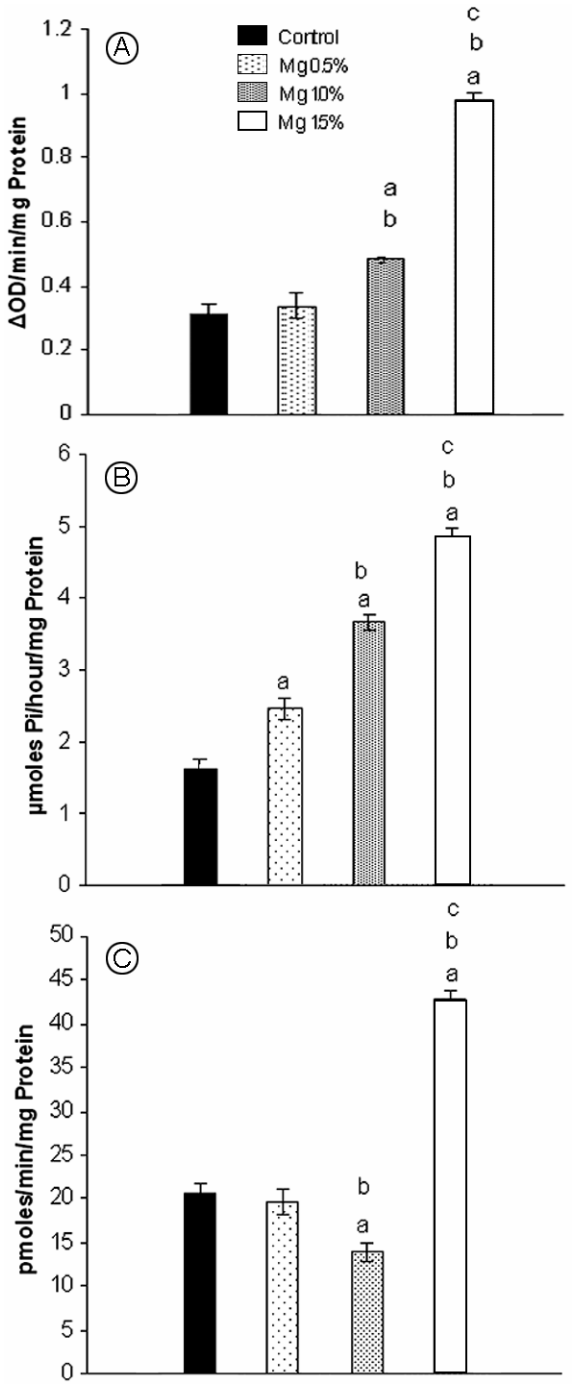

Fig. 1—Effects of magnesium at different doses on thyroid peroxidase (TPO) (a), thyroidal Na^+-K^+-ATPase (b) and 5'-Deiodinase Type I (DI) (c) activities in experimental animals for 60 days [Values are mean ± SD of three pooled samples. Each pool containing a mixture of three thyroid glands isolated from three individual rats. The assay was repeated twice. One-way analysis of variance (ANOVA) test followed by a multiple comparison *t*-test. Mean values are significantly different by ANOVA at $P < 0.05$. [a]Control versus other groups; [b]calcium 0.5% versus calcium 1.0% and calcium 1.5%; [c]calcium 1.0% versus calcium 1.5%].

in thyroid gland. High iodide uptake, followed by its higher capture by the thyroid while the renal iodide clearance remains constant, is preceded by an elevated Na^+-K^+-ATPase activity in thyroid dysfunction. Iodide trapping capacity is directly proportional to the number of transporters per cell as well as number of cells[19,20]. Previous studies have strongly suggested increased Na^+-K^+-ATPase gene expression as well as its activity in response to elevated TSH levels[2]. Therefore the elevated Na^+-K^+-ATPase activity (Fig. 1 b) as observed in the present study is a functional consequence of high TSH activity (as found in the present investigation) induced by high dietary magnesium.

Changes in hormonal profile—Serum hormonal profile also found to be modulated by magnesium treatment (Table 1). Serum total as well free thyroxine (T4) levels of the experimental rats were found to be higher than their respective control groups. Serum total triiodothyronine (T3) level showed a striking bidirectional response, i.e., reduction at moderate (1.0 g %) dose but elevation at high (1.5 g %) dose in the magnesium sulphate treated groups, resembling that of deiodinase activity. Serum free T3, however, showed a gradual retardation in dose-dependent fashion. T3/T4 ratio (T3:T4) was also markedly decreased in the magnesium treated groups. Finally, a significant dose- dependent increase in thyroid stimulating hormone (TSH) was observed in the magnesium treated experimental groups as compared to control.

Higher total T4 levels in the treated groups could have resulted from the higher TPO activity following magnesium treatment[2]. The present results depicted a resemblance between DI activity and total T3 levels.

Table 1—Effects of magnesium at different doses on serum total and free thyroxine (T4), total and free triiodothyronine (T3), TSH levels and total T3:T4 in experimental animals for 60 days [Values are mean ± SD from 8 observations each]

Parameters	Groups			
	Control	$MgSO_4$ 0.5g%	$MgSO_4$ 1.0g%	$MgSO_4$ 1.5g%
Serum Total T4[a]	5.28±0.44	5.63±0.22	5.74±0.15	7.59±0.31*
Serum Total T3[b]	1.17±0.05	1.20±0.05	0.72±0.03*	1.27±0.04*
Serum Free T4[c]	1.11±0.04	1.17±0.07	1.34±0.05*	1.45±0.05*
Serum Free T3[d]	1.44±0.02	1.34±0.03	1.22±0.04	1.09±0.03*
Serum TSH[b]	2.98±0.09	3.10±0.17	3.30±0.06*	3.54±0.05*
Total T3:T4	0.22±0.04	0.21±0.04	0.12±0.03*	0.17±0.03*

One-way analysis of variance (ANOVA) followed by multiple comparison *t*-test. *$P < 0.05$ ANOVA. [a]µg/dL, [b]ng/mL, [c]ng/dL, [d]pg/mL.

Serum T3 levels synchronized with DI response, i.e., decreased at moderate (1.0 g %) but increased at high (1.5 g %) dose of magnesium. Serum T3/T4 ratio (T3:T4) was also altered concomitantly in the Mg treated groups. Since deiodinase regulates the conversion of T4 to T3, any change therefore in the activity of DI is obviously reflected in the altered serum thyroid hormone profiles as well as total T3:T4.

Serum free T4 and T3 levels, the more precise indicator of thyroid gland functioning[21], were also modulated following magnesium treatment (Table 1). Most of the thyroid hormones remain bound to plasma proteins (mainly TBG or thyroxine binding globulin) while in circulation; therefore alterations in the affinity or binding capacity of these proteins may modulate thyroid hormone levels. The discrepancy between the total and free T3 levels at the high dose of magnesium treatment, as observed in the present study, might have resulted from the altered TBG levels[22], though it could not be ascertained from the present study.

A fall in T3/T4 ratio (T3:T4) is known to trigger the hypothalamic release of TSH[6] which is also evident in the present study.

Changes in absolute and relative thyroid gland weight—Absolute thyroid gland weights were significantly ($P < 0.05$) higher in all the treatment groups compared to control while the change in relative thyroid gland weight of the dose I was not significant ($P > 0.05$), though it was significantly ($P < 0.05$) higher in dose II and dose III $MgSO_4$ treated groups (Table 2).

Histological changes—Histological studies of thyroid glands of the control as well as the magnesium sulphate (dose I) treated groups of rats showed more or less similar, typical features with no pathological changes, while thyroid gland sections of the magnesium sulphate treated dose II and dose III groups showed prominent hypertrophy and or hyperplasia of thyroid follicular epithelial cells with the colloid taking up more eosin, especially in the high (1.5 g %) dose (Fig. 2).

Morphometric/histometric changes—The morphometric/histometric analyses (Table 3) following chronic exposure to magnesium sulfate showed significant ($P < 0.05$) increase in the areas of both the follicular cells as well as colloid following magnesium treatment.

Semiquantitative assessment—Semiquantitative assessment of thyroid follicles (Table 3) revealed the presence of small and medium sized follicles maximally with relatively few large follicles in the control group while the proportion of large, medium sized follicles increased in the magnesium treated groups.

Excess TSH secretion is eventually manifested in thyroid histomorphological alterations. Histopathological studies of thyroid glands of the control group showed the typical features with no pathological changes. However, depending upon the treatment to graded doses of magnesium sulphate, the glands exhibited prominent hypertrophic and hyperplastic developments of follicular epithelial cells. The colloid taking up more eosin[23], especially in the high (1.5 g %) dose indicating high TSH promoted pathophysiological modifications of thyroid. The morphometric/histometric analyses following chronic exposure to magnesium sulfate also revealed certain exclusive histological findings including significant increase in the areas of both the follicular cells as well as colloid following magnesium treatment, in comparison to control. Semiquantative assessment of thyroid follicles revealed the maximal presence of small and medium sized follicles with relatively few large follicles in the control group while the large and medium sized follicles predominated in the treated groups, indicating development of magnesium mediated disruptive changes. The hypertrophic and hyperplastic changes were reflected in significant increase in both total/absolute and relative thyroid gland weight in treated groups than their respective controls as observed, which are indicative of thyroid dysfunction upon chronic high magnesium exposure.

Table 2—Effect of magnesium on body weight, absolute thyroid gland weight, relative thyroid weight and food consumption
[Values are mean ± SD from 8 observations each]

Parameters	Groups			
	Control	Mg 0.5 g%	Mg 1.0 g%	Mg 1.5 g%
Absolute thyroid weight (mg)	10.63±1.85	13.50±2.93*	20.50±1.60*	22.75±2.60*
Relative thyroid weight (mg/100g body weight)	6.66±0.81	7.33±1.04	10.58±0.59*	11.34±0.74*

One-way analysis of variance (ANOVA) followed by multiple comparison *t*-test. *$P < 0.05$ ANOVA.

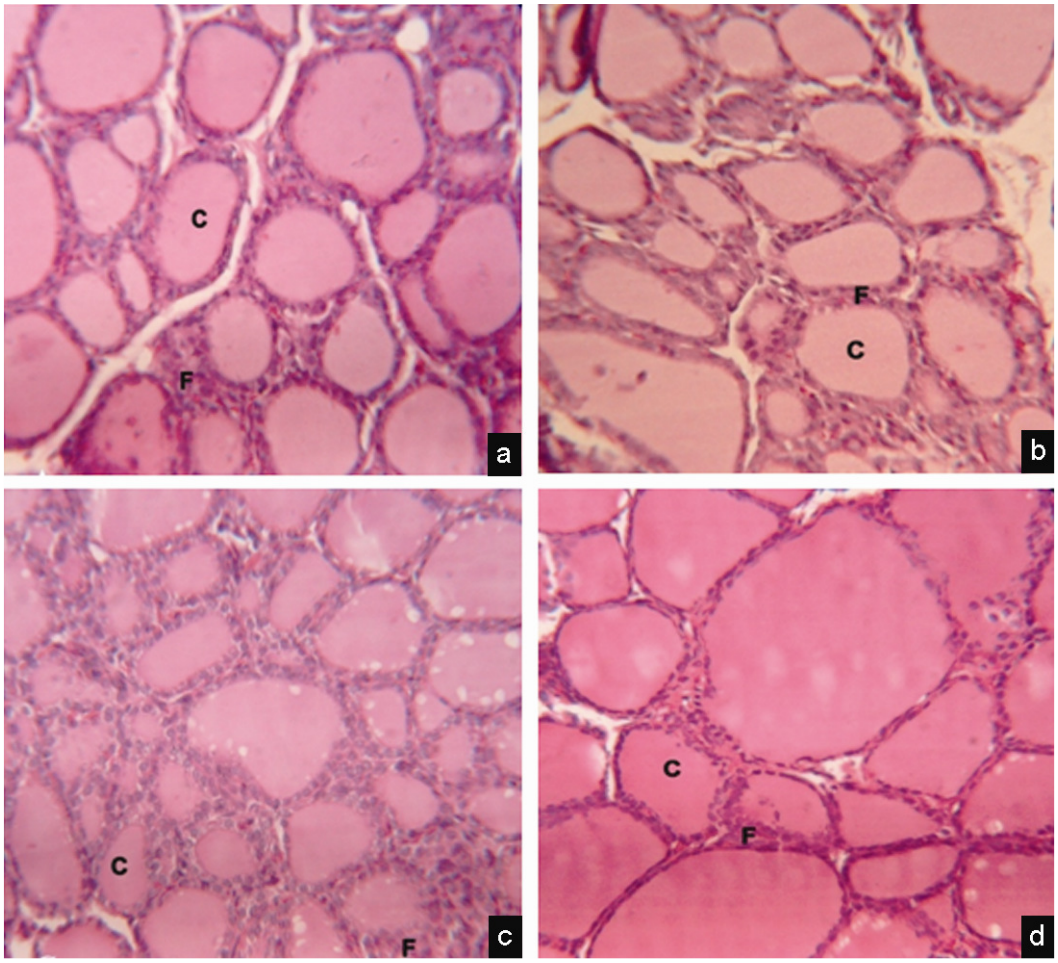

Fig 2— Effects of $MgSO_4$ at different doses on rat thyroid. Photomicrographs of paraffin-embedded H & E- stained rat thyroid sections. (a) control animals, (b) rats treated with 0.5 % $CaCl_2$ (dose I). (c) rats treated with 1.0 % $CaCl_2$ (dose II), (d) rats treated with 1.5 % $CaCl_2$ (dose III). C= Colloid, F= Follicle of thyroid. 400X, H & E.

Table 3—Morphometric/histometric and semiquantitative assessment of thyroid follicles of experimental animals subjected to different doses of magnesium for 60 days
[Values are mean ± SD from 6 observations each]

Groups	Parameters					
	Mean individual follicular area (mm^2)	Mean colloidal area in individual follicle(mm^2)	Small follicles (%)	Medium follicles (%)	Large follicles (%)	Immature follicles (%)
Control	1.45±0.05	1.19±0.02	46	23	26	5
Mg 0.5 %	1.52±0.08	1.28±0.04	46	30	20	4
Mg 1.0 %	1.75±0.06*	1.42±0.03*	38	43	24	6
Mg 1.5 %	1.91±0.05*	1.50±0.06*	30	33	33	4

One-way analysis of variance (ANOVA) followed by multiple comparison t-test. *P < 0.05 ANOVA. Small follicle = Follicular diameter of 5-10 µm; Medium follicle = Follicular diameter of 11-15 µm; Large follicle = Follicular diameter of 16-20 µm; Immature follicle = Follicular diameter 0-5 µm.

Conclusion

The present investigation concludes that, thyroid hormone synthesizing enzymes, as well as thyroid hormone profiles underwent extensive modulation upon chronic exposure to relatively high dietary magnesium that eventually resulted in the modifications of thyroid histoarchitecture as manifested in its enlargement resembling goiter. All these changes resulted for the actions of excessive magnesium at cellular and molecular levels.

The present study is thus an attempt to explain the mechanistic details of thyro-modulating actions of relatively high exposure of chronic magnesium.

Disclosure statement

The authors declare that there are no conflicts of interest.

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