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# EFFECT OF SELENIUM ON HYPOTHYROIDISM INDUCED BY METHIMAZOLE (MMI) IN LACTATING RATS AND THEIR PUPS

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The present study was undertaken to assess the effect of selenium (Se) on hypothyroidism induced by methimazole (MMI) in lactating rats and their pups. Rats were randomly divided into four groups of six each: group I served as a negative control which received standard diet; group II received orally MMI (250 mg $L^{-1}$); group III received both MMI (250 mg $L^{-1}$, orally) and Se (0.5 mg/kg of diet); group IV served as a positive control and received Se (0.5 mg $Na_2$ $SeO_3$ /kg of diet). Treatments were started from the *14th* day of pregnancy until postnatal day 14. In the MMI-exposed group, the body weight of 14-day-old pups diminished compared to controls; besides, a hypertrophy of the thyroid glands was observed. Co-administration of Se through the diet restored these parameters to near normal values. In the MMI-treated group, thyroid iodine contents and plasma thyroid hormone levels significantly decreased, while plasma TSH levels increased in pups and their mothers. These biochemical modifications corresponded histologically to closed follicles, increased vascularity and a reduction in colloid volume. Co-treatment with Se ameliorated these parameters. We concluded that the supplementation of Se in diet had beneficial effects on hypothyroidism during a critical period of life.

*Keywords:* Selenium – Methimazole – $FT_4$ – $FT_3$ – TSH – adult rats – nursing pups

## INTRODUCTION

Thyroid function is affected by various physiological conditions, for instance, food deprivation [17], iodine deficiency [42], pollutants [11, 25] and antithyroid drugs [10, 20, 37]. These compounds are able to interfere, directly or indirectly, with the synthesis of thyroid hormones which fundamentally determine the development and growth of many organs [9].

Thyroid impairment can occur after treatment with Methimazole (MMI), a biologically active compound widely used to manage hyperthyroidism associated with Grave's disease in humans [24]. MMI is absorbed by the gastrointestinal tract and concentrated in the thyroid gland [1]. It induces hypothyroidism by inhibiting the production of thyroid hormones [2], but does not interfere with peripheral tissue deiodinase activity [40].

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The administration of methimazole is known to reduce the risk of Graves' hyperthyroidism in pregnant and lactating mothers [26]. But MMI transmitted through placenta or milk [27, 28] has been shown to expose the fetuses/neonates to a risk of hypothyroidism [16]. Selenium (Se) as well as other nutritional supplements such as iodine and zinc are recommended for the treatment of hypothyroidism, rather than the administration of thyroxine [39]. Se is generally required for appropriate thyroid hormone synthesis, activation and metabolism, thus affecting thyroid function [23]. The human thyroid gland, among several organs, has the highest selenium content per gram of tissue [23].

Inorganic Se compounds, used throughout the experiment, are generally utilised as a nutritional source [31]. These species are more preferably transformed into selenoprotein than selenomethionine. Selenite is effectively incorporated into placenta during pregnancy and transferred to pups during lactation [4].

In recent years, low Se status has been associated with a thyroid dysfunction in humans [41], mice [29] and rats [13]. These cited reports consider Se as an essential trace element which may be used as a dietary supplement against thyroid impairments.

To our knowledge, there are no reports about the effects of dietary Se addition on thyroid impairments induced by MMI during late pregnancy and early postnatal periods. In the present study, we assessed the effects of selenium on MMI-induced hypothyroidism in lactating rats and their pups.

## MATERIALS AND METHODS

Adult Wistar rats, weighing about 180 g, purchased from the Central Pharmacy (SIPHAT, Tunisia), were housed at 22 ± 3 °C with a 12 hour light/dark cycle and a minimum relative humidity of 40%. They were maintained on commercial diet (SICO, Sfax, Tunisia) and tap water *ad libitum.* The standard diet contained 0.720 µg of iodine/g/diet. Iodine diet content was determined, after acid mineralization, using the catalytic method of Sandell and Kolthoff [34]. Standard diet also contained 0.17 mg of sodium selenite. Selenium diet content was determined, after mineralization, by Electrothermic Atomic Absorption Spectrometry technique (ET-AAS). Measurements were performed on a Perkin-Elmer 5100/Zeeman Atomic Absorption Spectrometer with a 196-nm wavelength.

After one week of acclimatization to the laboratory conditions and diet adaptation, virgin female rats were caged overnight with males and the presence of spermatozoa in the vaginal smear was taken as an indicator of day zero of pregnancy. The general guidelines on the use of living animals in scientific investigations (Council of European Communities [14]) and the guidelines for the care and use of laboratory animals controlled by the Tunisian Research Ministry were followed.

Twenty-four pregnant female Wistar rats were randomly divided into four groups of six each. The first group served as a negative control which received 0.17 mg of sodium selenite ($Na_2SeO_3$/kg of diet); the second group (MMI) received orally 250

mg $L^{-1}$ of methimazole $C_4H_6N_2S$ (Sigma, France); animals of the third group (MMI + Se) were treated orally with methimazole (250 mg $L^{-1}$) and selenium was added to their diet as $Na_2SeO_3$ (0.5 mg/kg) (Sigma, France); the fourth group (Se) served as a positive control which received a supplement of selenium in their diet (0.5 mg $Na_2SeO_3$/kg of diet), from the 14$^{th}$ day of pregnancy until day 14 after delivery. The methimazole dose and the treatment beginning were chosen according to Schwartz et al. [35] since it induced the classical picture of primary hypothyroidism without lethal effects. The supplement dose of selenium (0.5 mg/kg of diet) used in our experiments and in other findings [21] gave high protection against hypothyroidism. Lower selenium doses gave less protection while higher doses were not much more effective [22]. Pregnant female rats were allowed to deliver spontaneously three weeks after coitus. Within 24 hours after birth, the litters were reduced to 8 pups for each mother (four males and four females if possible) to ensure standardized nutrition and maternal care [18].

On postnatal day 14, the animals were anesthetized with chloralhydrate administered intraperitoneally. Forty-eight pups and six dams in each group were weighed. Blood samples were collected from the brachial artery (pups) and by aortic puncture (dams) and then they were centrifuged at $2200 \times g$ for ten minutes. Plasma samples were drawn and kept at –20 °C until analysis. Only six plasma samples were randomly chosen for the determination of free thyroxine ($FT_4$), free triiodothyronine ($FT_3$) and TSH levels by radio-immunoassay kits (Immunotech, France, refs 1363, 1579 and Biocode Hycel, ref: AH R001, respectively). Some thyroid glands of each group randomly taken from pups (n = 36) and dams (n = 5) were weighed and preserved at –20 °C until iodine determination by Sandell and Kolthoff method [34].

Other thyroid samples destined for light microscopy were dissected together with a piece of trachea, fixed in Bouin's solution, embedded in paraffin and serially sectioned at 5 μm and stained with hematoxylin-eosin [19].

The data were analyzed using the statistical package program Stat View 5 Soft Ware for Windows (SAS Institute, Berkley, CA). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test as a post-hoc test for comparison between groups [treated groups (MMI, MMI + Se, Se) vs (negative controls)] and [MMI + Se] vs [MMI, Se]. Student unpaired *t*-test was also used when comparison between two groups was required. All values were expressed as mean ± S.D. Differences were considered significant if $p < 0.05$.

## RESULTS

Food and water consumption was reduced by 50 and 20%, respectively, in MMI-treated mothers (Table 1). When selenium was supplemented to the diet, a partial recovery occurred only in food intake (+63%) in (MMI + Se)-treated mothers when compared to MMI-treated group (Table 1). In Se-treated group, food intake, ingested Se and iodine quantities increased by 23%.

*Table 1*
Daily food and water consumption, MMI, selenium and iodine quantities ingested by lactating rats

| Parameters and treatments | Food consumption (g/day/rat) | Water consumption (mL/day/rat) | Ingested MMI quantities (mg/day/rat) | Ingested Se quantities (µg/day/rat) | Ingested iodine (µg/day/rat) |
|---|---|---|---|---|---|
| Negative control | 30.702± 1.670 | 40.208±1.587 | – | 5.233±0.531 | 21.163±1.270 |
| MMI | 15.236±0.613*** | 32.270±0.892*** | 8.506±0.176 | 2.590±0.500 | 10.800±0.410*** |
| MMI+Se | 41.545±2.805***+++†† | 30.312±1.701*** | 7.578±0.425+++ | 20.772±1.402*** | 29.912±2.020***+++ |
| Positive control (Se) | 48.888±2.609*** | 40±2.222*** | – | 24.444±1.304*** | 35.200±1.878*** |

Values are mean± SD for six rats in each group.
MMI or Se group vs negative control group: ***$p < 0.001$,
MMI group vs (MMI + Se) group: +++$p < 0.001$.
Se group vs (MMI + Se) group: ††$p < 0.01$.

*Table 2*
Effects of methimazole (MMI), selenium (Se) and their combination on body and thyroid weights, thyroid iodine content obtained from 14-day-old rats and their mothers

| Parameters and treatments | Negative control | MMI | MMI+Se | Positive control (Se) |
|---|---|---|---|---|
| Body weight (g) | | | | |
| Offspring (n = 48) | 22.100±0.248 | 12.710±0.150*** | 19.172±1.147*** | 24.000±0.490 |
| Mothers (n = 6) | 170.981±6.217 | 168.500±5.885 | 168.500±5.885†† | 203.667±9.500** |
| Thyroid weight (mg) | | | | |
| Offspring (n = 36) | 2.152±0.085 | 4.900±0.160*** | 2.446±0.374+++†† | 1.950±0.122* |
| Mothers (n = 5) | 10.833±0.698 | 46.375±1.634*** | 24.75±0.500***+++††† | 9.500±0.577 |
| Thyroid iodine contents (µg I/thyroid) | | | | |
| Offspring (n = 36) | 1.677±0.046 | 0.093±0.002*** | 0.780±0.092***+++††† | 2.413±0.045*** |
| Mothers (n = 5) | 11.124±0.088 | 0.048±0.009*** | 4.489±0.78***+++††† | 15.029±0.036*** |

Values are mean ± SD.
MMI or Se group vs negative control group: *p < 0.05, **p < 0.01, ***p < 0.001.
MMI group vs (MMI + Se) group: +++p < 0.001.
Se group vs (MMI + Se) group: ††p < 0.01, †††p < 0.001.

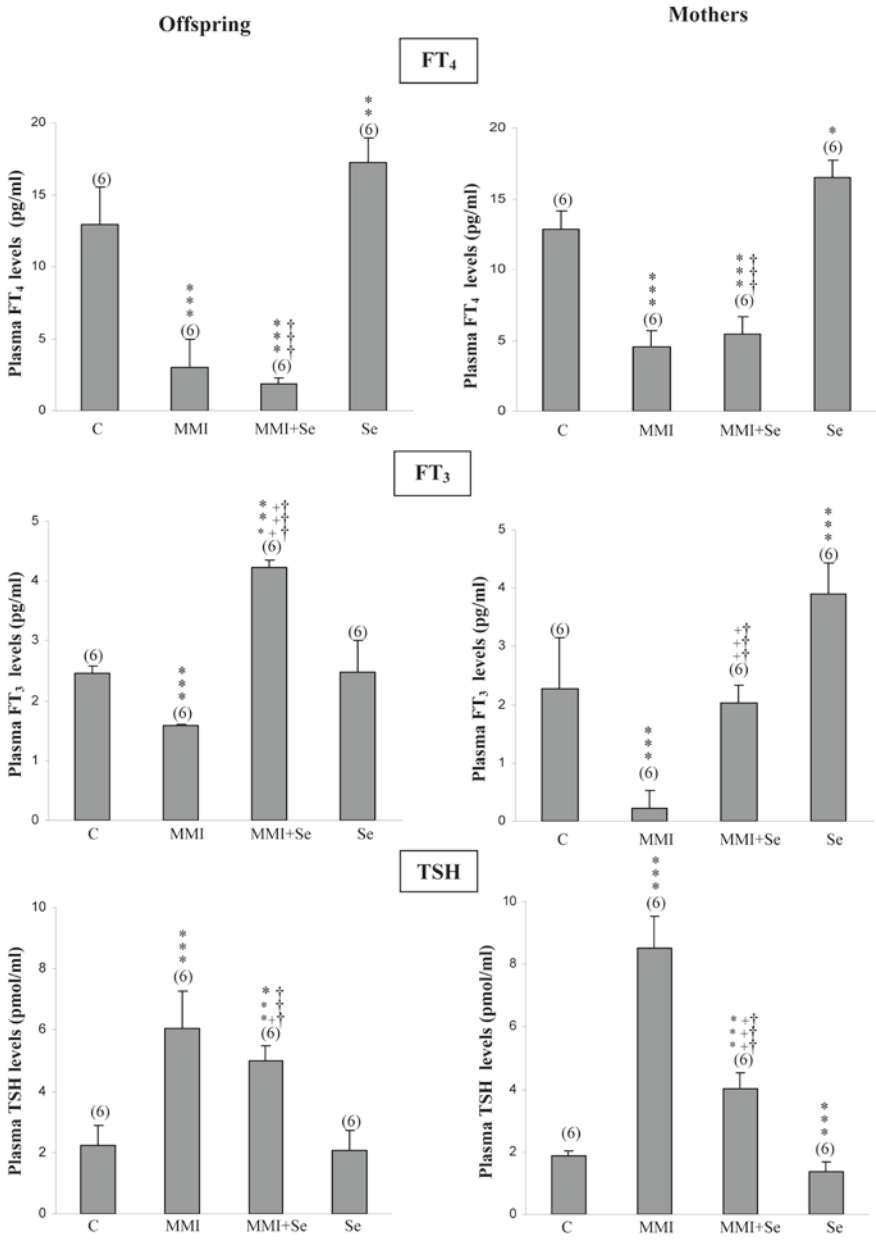


*Fig. 1.* Effects of methimazole (MMI), selenium (Se) and their combination on plasma thyroxine ($FT_4$), triiodothyronine ($FT_3$) and TSH levels obtained from14-day-old rats and their mothers. Values are mean ± SD for six rats in each group. MMI or Se group vs negative control group: *$p<0.05$, **$p<0.01$, ***$p<0.001$. MMI group vs (MMI + Se) group: +$p<0.05$; +++$p<0.001$. Se group vs (MMI + Se) group: †††$p<0.001$

Compared to control, 14-day-old rats (n = 48), whose mothers received 250 mg of MMI in their drinking water, showed a decrease of 42% in their body weights (Table 2). When selenium was supplemented to the diet of MMI-treated dams, a partial recovery occurred in the body weight (+29%) of pups. In their mothers (n = 6), no significant changes were obtained. Body weights of rats, which received 0.5 mg Se/kg as supplement in their diet, were not changed when compared to negative control, while, in their mothers an increase of 16% in body weight was obtained.

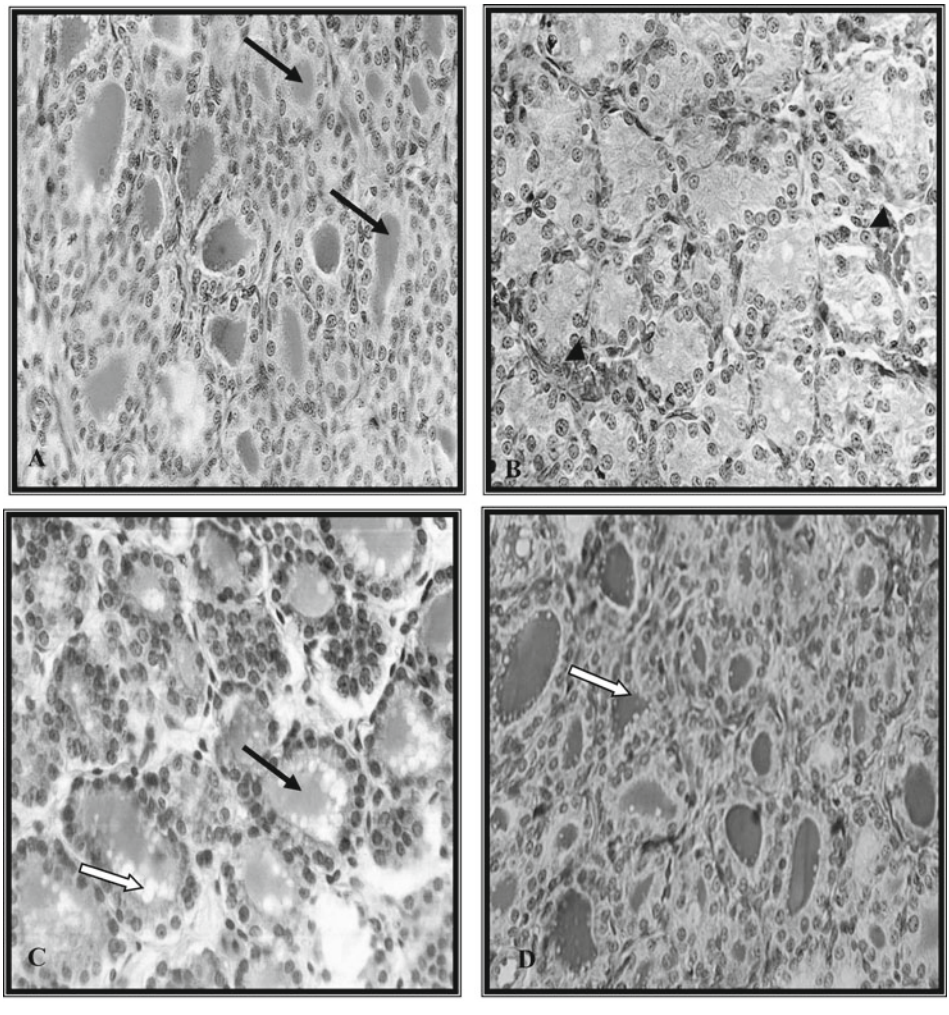


*Fig. 2.* Histological sections of thyroid glands obtained from 14-day-old rats, controls (A) and treated with MMI (B), MMI+Se (C) or selenium (D) from the 14$^{th}$ day of pregnancy until the 14$^{th}$ day after parturition. Haematoxylin-eosin, ×400. Arrows indicate thyroid follicles; arrowheads indicate blood vessels; open arrows indicate resorption vacuoles

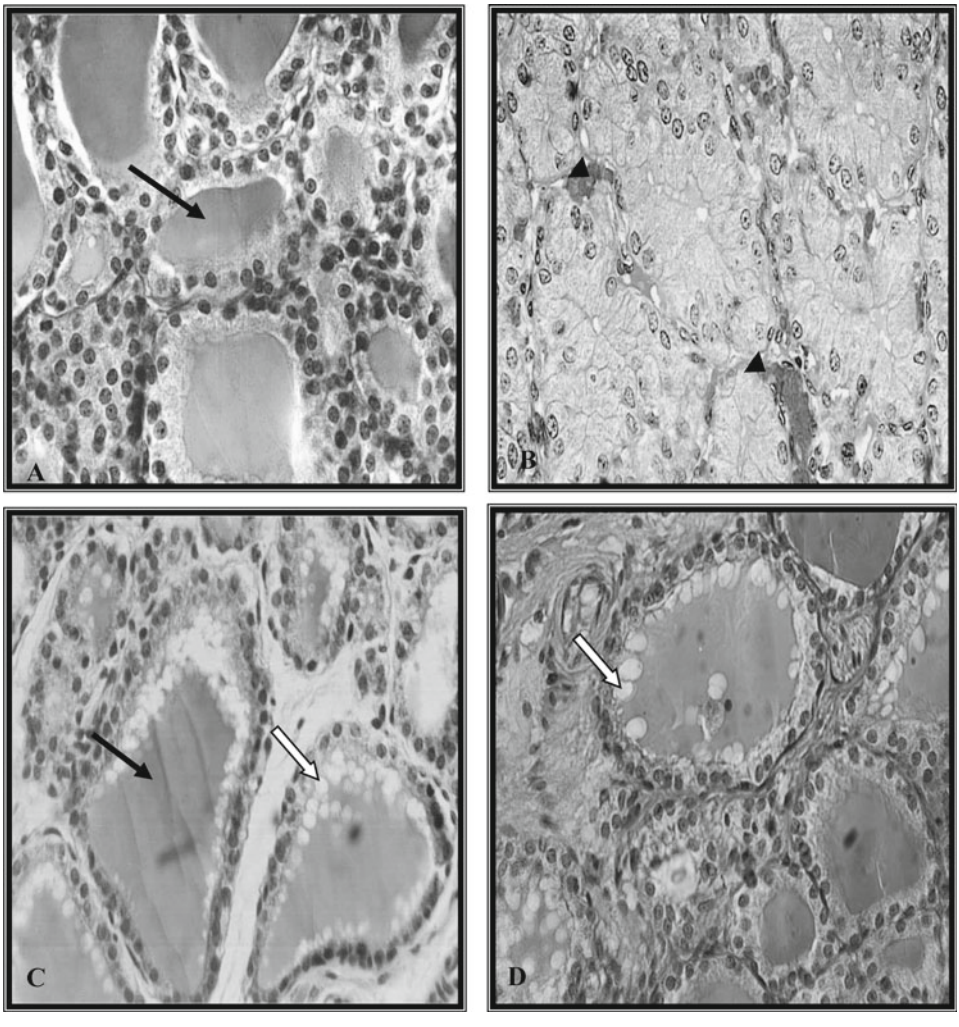


*Fig. 3.* Histological sections of thyroid glands obtained from adult rats, controls (A) and treated with MMI (B), MMI + Se (C) or selenium (D) from the 14th day of pregnancy until the 14th day after parturition. Haematoxylin-eosin, ×400. Arrows indicate thyroid follicles; arrowheads indicate blood vessels; open arrows indicate resorption vacuoles

Compared to negative and positive controls, an hypertrophy of the thyroid gland was noted: (+56%) in pups and (+77%) in MMI-treated mothers (Table 2), probably due to the increase of plasma TSH levels by 63% in pups and 78% in dams (Fig. 1). When selenium was supplemented to the diet of MMI-treated dams, thyroid weights were recovered totally in pups and partially in their mothers (–47%), compared to MMI-treated group, in spite of a partial return of plasma TSH levels (–17 and –53%) (Fig. 1).

Plasma thyroid hormone levels decreased by 37 and 80% for $FT_3$ and by 64 and 77% for $FT_4$ in MMI-treated pups and dams, respectively (Fig. 1). The reduction of plasma thyroid hormone levels was the result of the decrease in daily ingested iodine (–54%) and in thyroid iodine content by 99% in pups and 94% in their mothers (Table 2). After selenium supplementation to the diet of MMI-treated dams, a partial amelioration of $FT_4$ levels (+7%) in pups and a 38% decrease in dams were noted when compared to MMI-treated rats (Fig. 1), while plasma $FT_3$ levels returned to normal values in dams and exceeded normal values in pups (Fig. 1). In addition, an increase in ingested iodine (+68%) and in thyroid iodine content (+41%) was observed in (MMI + Se)-group when compared to MMI-treated rats (Table 1). In positive controls (Se group), thyroid iodine content and plasma $FT_4$ increased, respectively, by 29 and 22% in pups and by 25 and 25% in their mothers when compared with negative controls. Plasma $FT_3$ was not changed in pups and increased by 41% in dams.

In the present study, biochemical data confirmed the histological aspects of thyroid glands. In fact, light microscopy revealed histological changes in the thyroid glands of MMI-treated dams and pups compared to controls which were characterized by closed follicles, follicular cell hyperplasia, an increased vascularisation, as well as a decrease in colloidal volume. In fact, all vesicular cavities surrounded by cuboidal epithelial cells were empty (Figs 2B, 3B), while in control rats, most follicles were lined by flattened epithelium cells and filled with colloid (Figs 2A, 3A). In positive controls (Se group), most follicles were filled with colloid and their vesicular cavities contained empty vacuoles resulting from colloid resorption (Figs 2D, 3D). When selenium was supplemented to the MMI-treated group diet, a significant improvement in thyroid histological pictures was observed but did not reach that of controls. Vesicular follicle cavities contained empty vacuoles resulting from colloid resorption, indicating an hyperactivity of the thyroid gland (Figs 2C, 3C).

## DISCUSSION

Our results showed, after MMI treatment, a decrease in plasma thyroid hormone levels in dams and their progeny. In fact, plasma $FT_3$ and $FT_4$ levels decreased significantly when compared to control. This might be explained, according to previous findings, either by the transplacental transfer of MMI from mothers to their fetuses [27] or by its transfer from breast milk to suckling pups [28]. In fact, MMI involved an important decrease in thyroid iodine content, as demonstrated by our results. Iodine uptake by the thyroid gland probably became insufficient, causing a decrease in the intrathyroidal iodine pool. This might be explained, as reported by Dorea [15], by the inhibition of sodium iodide coupled symport transport abolishing the prolactin effects on iodide accumulation and incorporation in the mammary gland. In fact, iodine is easily absorbed by the gastrointestinal tract and avidly taken up by the thyroid gland through the $Na^+/I^-$ Symporter (NIS), a transmembrane carrier protein. Iodine uptake mechanism is also present in the lactating mammary gland [38] and allows the element to be absorbed against a high concentration gradient. A low total

iodide pool may impose a reduction in thyroid iodine content and a critical limitation of hormonal synthesis, since iodine is necessary for thyroid hormone synthesis [8]. Furthermore, MMI inhibits the first step of the biosynthetic pathway, which is the incorporation of iodide into tyrosine residues on thyroglobulin catalyzed by thyroid peroxidase (TPO) [7]. Although the detailed mechanism of its action is still not clear, the available information reveals that this drug may block the thyroid hormone synthesis by coordinating to the metal center of TPO [33].

Iodine uptake by the thyroid gland requires ATP energy. Moreover, the incorporation of this halogene in the thyroid gland is stimulated by TSH and blocked by antithyroid drugs [12]. Our results suggest that the administration of MMI provokes thyroid hyperplasia in rats. Indeed, the thyroid gland weight is increased. The mechanism for this response is an increase in TSH levels, as seen in the present study, due to a decrease in the production of thyroid hormones. The changes in thyroid weights in our study are also similar to those described by Ampong et al. [3].

It is well known that thyroid hormones are necessary for growth. Treatment with MMI leads to a reduction in the body weight of suckling rats, which may be explained by reduced thyroid hormone levels as demonstrated by our results and confirmed by previous studies carried out on rats [5] and chicken [32] treated with MMI. Moreover, Porter [30] demonstrated that MMI inhibited the *in vivo* differentiation of somatotroph cells in chick embryo and pituitary cells in the rats fetuses.

On the other hand, a reduction in daily food consumption by MMI-treated lactating rats could explain the pups' growth perturbations. Our results are in agreement with previous findings of Aragão et al. [5]. When selenium was supplemented to the diet of MMI-treated rats, a partial recovery occurred in the body weight of pups. So the growth improvement could be explained by an increase either in daily food consumption by lactating rats or in plasma $FT_4$ and $FT_3$ levels. Moroever, the improvement in TH synthesis could explain the pups' growth amelioration. In fact, a partial return of plasma $FT_4$ levels was observed, while plasma $FT_3$ levels exceeded control values. The increase in TH synthesis in suckling pups could be explained by the transfer of selenium either through placenta or breast milk from mothers to their fetuses as reported in rats [6]. According to Smith and Picciano [36], Se requirement increases during pregnancy and lactating due to the increase in requirement for the maternal transfer of Se to the fetus and the newborn. In addition, Anan et al. [4] demonstrated that sodium selenite, ingested by dams, was transformed into selenoprotein P which is effectively incorporated into placenta during pregnancy. Se-containing protein is also transferred to pups as well as seleproteins during lactation, resulting in the reduction of those compounds in the dam's body.

The biochemical modifications cited above confirmed the histological aspects of the thyroid glands. In fact, we have observed morphological changes in follicular cells. Histological changes, seen in the thyroid gland of MMI-treated rats, were characterized by follicular cell hyperplasia suggestive of an enhanced thyroid activity, an increase in follicular number and vascularity and a decrease in colloid. Se supplementation improved the histological features of the thyroid gland evidenced by its return to normal aspects in which the phenomena of proliferation and cell death must have

been involved. However, higher TH plasma levels occurred in (MMI + Se) group, exerting a negative feed back on the pituitary which reduced TSH levels. Restoring iodide, after Se supplementation to diet, reduced the hypertrophy of follicle epithelial cells and increased the colloidal volume. Some thyroid follicles became larger than those observed in the MMI-treated group.

The correlated findings of thyroid histology and thyroid hormone imbalance show a hypothyroidism reflected by a great decrease in free plasma $T_4$ and $T_3$ levels and by its major consequence, a growth retardation induced by MMI treatment. The increased plasma TSH levels, thyroid weights and the thyroid hyperplasia are consistent with a consequent stimulation of pituitary cells.

These effects were reversed at least in part when the diet was supplemented with selenium. In fact, in our study, with the addition of selenium to diet, a partial recovery occurred in body and thyroid gland weights, thyroid iodine content and plasma TSH levels of both mothers and pups. Our data are in agreement with the findings of Golstein et al. [21] after the addition of 0.5 mg/kg of selenium to diet in perchlorate-treated rats.

In conclusion, this study showed that the hypothyroid state, induced by methimazole, could be prevented by selenium supplementation in diet. This trace element can be of great fundamental importance to health since it activates thyroid hormone synthesis.

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REFERENCES

1. Aboul-Enein, H. Y., Al-Badr, A. A. (1979) Methimazole. In: Forey, K. (ed.), *Analytical Profiles of Drug Substance.* Academic Press, New York 18, pp. 351–371.
2. Aletrari, M., Kanari, P., Partassides, D., Loizou, E. (1998) Study of the british pharmacopeia method on methimazole (thiamazole) content in carbimazole tablets. *J. Pharm. Biomed. Anal. 16,* 785–792.
3. Ampong, B., Honda, H., Kogo, H. (2002) Effect of hypothyroidism on β-adrenoreceptor-mediated relaxation in the rat thoracic aortae: A time-dependent study. *Vascul. Pharmacol. 38,* 149–155.
4. Anan, Y., Ogra, Y., Somekawa, L., Suzuki, K. T. (2009) Effects of chemical species of selenium on maternal transfer during pregnancy and lactation. *Life Sci. 84,* 888–893.
5. Aragão, C. N., Souza, L. L., Cabanelas, A., Oliveira, K. J., Pazos-Moura, C. C. (2007) Effect of experimental hypo- and hyperthyroidism on serum adiponectin. *Metab. Clin. Exper. 56,* 6–11.
6. Archimbaud, Y., Grillion, G., Poncy, J. L., Masse, R. (1992) $^{75}$Se transfer via placenta and milk, distribution and retention in fetal, young and adult rat. *Radiat. Prot. Dosimetry 41,* 147–151.
7. Bandyopadhyay, U., Biswas, K., Banerjee, R. K. (2002) Extrathyroidal actions of antithyroid thionamides. *Toxicol. Lett. 128,* 117–127.
8. Beaufrère, B., Bresson, J. L., Briend, A., Ghisolfi, J., Goulet, O., Navarro, J., Putet, G., Ricour, C., Rieu, D., Turck, D., Vidailhet, M. (2000) La nutrition iodée chez l'enfant. *Arch. Pediatr. 7,* 66–74.

9. Beckett, C. J., Arthur, J. R. (1994) Hormone-nuclear receptor interactions in health and disease. The iodothyronine deiodinases and 5'-deiodination. *Baillieres Clin. Endocrinol. Metab. 8,* 285–304.
10. Ben Hamida, F., Soussia, L., Guermazi, F., Rebai, T., Zeghal, N. (2001) Effets de deux antithyroïdiens (propyltiouracile et perchlorate) sur la fonction thyroïdienne de la souris en période d'allaitement. *Ann. Endocrinol. 62,* 446–453.
11. Bouaziz, H., Soussia, L., Guermazi, F., Zeghal, N. (2005) Fluoride-induced thyroid proliferative changes and their reversal in female mice and their pups. *Fluoride 38,* 185–192.
12. Carrasco, N. (1993) Iodide transport in the thyroid gland. *Biochim. Biophys. Acta 154,* 65–82.
13. Colzani, R. M., Alex, S., Fang, S. L., Stone, S., Braverman, L. E. (1999) Effects of iodine repletion on thyroid morphology in iodine and/or selenium deficient rat term fetuses, pups and mothers. *Biochimie 81,* 485–491.
14. Council of European Communities (1986) Council instructions about the protection of living animals used in scientific investigations. *Official Journal of the European Communities* (JO 86/609/CEE) L358, pp. 1–18.
15. Dorea, J. G. (2002) Iodine nutrition and breast feeding. *J. Trace Elem. Med. Biol. 16,* 207–220.
16. Dussault, J. H., Ruel, J. (1987) Thyroid hormones and brain development. *Annu. Rev. Physiol. 49,* 321–334.
17. Fetoui, H., Bouaziz, H., Mahjoubi-Samet, A., Soussia, L., Guermazi, F., Zeghal, N. (2006) Food restriction induced thyroid changes and their reversal after refeeding in female rats and their pups. *Acta Biol. Hung. 57,* 391–402.
18. Fishbeck, K. L., Rasmussen, K. M. (1987) Effect of repeated cycles on maternal nutritional status, lactational performance and litter growth in ad libitum-fed and chronically food-restricted rats. *J. Nutr. 117,* 1967–1975.
19. Gabe, M. (1968) *Techniques histologiques.* Masson & Cie, Paris.
20. Ghorbel, H., Fetoui, H., Mahjoubi, A., Guermazi, F., Zeghal, N. (2008) Thiocyanate effects on thyroid function of weaned mice. *C. R. Biol. 331,* 262–271.
21. Golstein, J., Corvilain, B., Lamy, F., Paquer, D., Dumont, J. E. (1988) Effects of a selenium deficient diet on thyroid function of normal and perchlorate treated rats. *Acta Endocrinol. 118,* 495–502.
22. Hotz, C. S., Fitzpatrick, D. W., Trick, K. D., L'Abbé, M. R. (1997) Dietary iodine and selenium interact to affect thyroid hormone metabolism of rats. *J. Nutr. 127,* 1214–1218.
23. Köhrle, J. (1999) The trace element selenium and the thyroid gland. *Biochimie 81,* 527–533.
24. Koornstra, J. J., Kerstens, M. N., Hoving, J., Visscher, K. J., Schade, H. J., Gort, H. B. W., Leemhuis, M. P. (1999) Clinical and biochemical changes following 131I therapy for hyperthyroidism in patients not pretreated with antithyroid drugs. *Neth. J. Med. 55,* 215–221.
25. Mahjoubi-Samet, A., Fetoui, H., Soussia, L., Guermazi, F., Zeghal, N. (2005) Dimethoate effects on thyroid function in suckling rats. *Ann. Endocrinol. 66,* 96–104.
26. Mandel, S. J., Cooper, D. S. (2001) The use of antithyroid drugs in pregnancy and lactation. *J. Clin. Endocrinol. Metab. 86,* 2354–2359.
27. Marchant, B., Alexander, W. D. (1972) The thyroid accumulation, oxidation and metabolic fate of 35 S-methimazole in the rat. *Endocrinology 91,* 747–756.
28. Marchant, B., Brownlie, B. E., Hart, D. M., Horton, P. W., Alexander, W. D. (1977) The placental transfer of propylthiouracil, methimazole and carbimazole. *J. Clin. Endocrinol. Metab. 45,* 1187–1193.
29. Miyazaki, K., Watanabe, C., Mori, K., Yoshida, K., Ohtsuka, R. (2005) The effects of gestational arsenic exposure and dietary selenium deficiency on selenium and selenoenzymes in maternal and fetal tissues in mice. *Toxicology 208,* 357–365.
30. Porter, T. E. (2005) Regulation of pituitary somatotroph differentiation by hormones of peripheral endocrine glands. *Domest. Anim. Endocrinol. 29,* 52–62.
31. Rock, M. J., Kincaid, R. L., Carstens, G. E. (2001) Effects of prenatal source and level of dietary selenium on passive immunity and thermometabolism of newborn lambs. *Small Ruminant Res. 40,* 129–138.
32. Rosebrough, R. W., Russell, B. A., McMurtry, J. P. (2006) Studies on doses of methimazole (MMI) and its administration regimen on broiler metabolism. *Comp. Biochem. Phys. A 143,* 35–41.

33. Roy, G., Das, D., Mugesh, G. (2007) Bioinorganic chemistry aspects of the inhibition of thyroid hormone biosynthesis by anti-hyperthyroid drugs. *Inorganica Chimica Acta 360,* 303–316.
34. Sandell, E. B., Kolthoff, I. M. (1937) Microdetermination of iodine by a catalytic method. *Microchem. Acta 1,* 9–25.
35. Schwartz, H. L., Ross, M. E., Oppenheimer, J. H. (1997) Lack of effect of thyroid hormone on late fetal rat brain development. *Endocrinology 138,* 3119–3124.
36. Smith, A. M., Picciano, M. F. (1986) Evidence for increased selenium requirement for the rat during pregnancy and lactation. *J. Nutr. 116,* 1068–1079.
37. Soussia, L., Ben Hamida, F., Guermazi, F., Zeghal, N. (2004) Induction et réversibilité d'action du thiocyanate sur la fonction thyroïdienne chez le rat en période d'allaitement. *Ann. Endocrinol. 65,* 451–458.
38. Tazebay, U. H., Wapnir, I. L., Levy, O., Dohan, O., Zuckier, L. S., Zhao, Q. H., Deng, H. F., Amenta, P. S., Fineberg, S., Pestell, R. G., Carrasco, N. (2000) The mammary gland iodide transporter is expressed during lactation and in breast cancer. *Nat. Med. 6,* 871–878.
39. Thiel, R., Fowkes, S. W. (2007) Down syndrome and thyroid dysfunction: Should nutritional support be the first-line treatment? *Med. Hypotheses 69,* 809–815.
40. Van Doorn, J., Roelfsema, F., Van Der Heide, D. (1983) The effects of propylthiouracil and methimazole on the peripheral conversion of thyroxine to 3,5,3-triiodothyronine in athyreotic thyroxine-maintained rats. *Acta. Endocrinol. 103,* 509–520.
41. Zagrodzki, P., Szmigiel, H., Ratajczak, R., Szybinski, Z., Zachwieja, Z. (2000) The role of selenium in iodine metabolism in children with goiter. *Environ. Health Perspect. 108,* 67–71.
42. Zimmermann, M. B. (2008) Iodine requirements and the risks and benefits of correcting iodine deficiency in populations. *J. Trace Elem. Med. Biol. 22,* 81–92.