

CALCIUM ADMINISTRATION DECREASES THYROID FUNCTIONING IN MIDDLE-AGED FEMALE RATS. B. Šošić-Jurjević, B. Filipović, M. Manojlović Stojanoski, and M. Sekulić. Siniša Stanković Institute for Biological Research, 11000 Belgrade, Serbia

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Most women take calcium (Ca) supplementation for the prevention or treatment of osteoporosis at or beyond the onset of the menopause (Singh *et al.*, 2000). At the same time, this group is faced with an increased risk of thyroid disorders such as secondary and tertiary hypothyroidism (Suchartwatachai *et al.*, 2002; Schindler, 2003). According to different studies, thyroid functioning decreases with advancing age, affecting the whole hypothalamo-pituitary-thyroid axis in both humans and rats (Robusch *et al.*, 1987; Raymond *et al.*, 1992). Rodents are considered to be useful models for thyroid studies, even though significant differences between rodent and human thyroid physiology have been reported (Poirier *et al.*, 1999).

Implemented Ca treatment can affect thyroid functioning by various mechanisms that do not exclude each other. It can act indirectly, by influencing the regulatory pituitary TSH cells (Gillet *et al.*, 1990; Bergenzelz *et al.*, 1994), as well as by altering the level of peripheral conversion of T₄ to T₃ (Etling *et al.*, 1986). Calcium can also influence activity of thyroid follicular cells directly, by modulating the TSH stimulatory effect (Geberscek *et al.*, 1998; Vanvooren *et al.*, 2000).

We previously demonstrated that chronic Ca administration to middle-aged female rats affects the immunohistochemical, histological, and morphometrical features of pituitary thyrotrophic cells and thyroid tissue (Sekulić *et al.*, 1998; Šošić-Jurjević *et al.*, 2005). As a continuation of these investigations, in the present study we examined the effects of Ca administration on serum levels of thyrotropin (TSH), total thyroxine (T₄), and triiodothyronine (T₃) hormones in the same middle-aged rat model.

Female Wistar rats (14-month-old) were maintained under standard laboratory conditions (22 ± 2 °C, 12-h light/dark periods) with free access to food and water. A group of animals (Ca; n = 6) received intramuscularly 28.55 mg/kg b.w. Ca-glubionate (Novartis, Nyon, Switzerland) once a day for two weeks every day except on Sundays. Age-matched controls (C, n = 6) were treated with an equivalent volume of physiological saline. Experimental protocols were approved by the Local Animal Care Committee and conform to the recommendations given in the "Guide for the Care and Use of Laboratory Animals" (1996, National Academy Press, Washington D.C.). All animals were sacrificed 24 h after the last injection. Sera were separated from trunk blood after decapitation and stored at -70°C. To determine

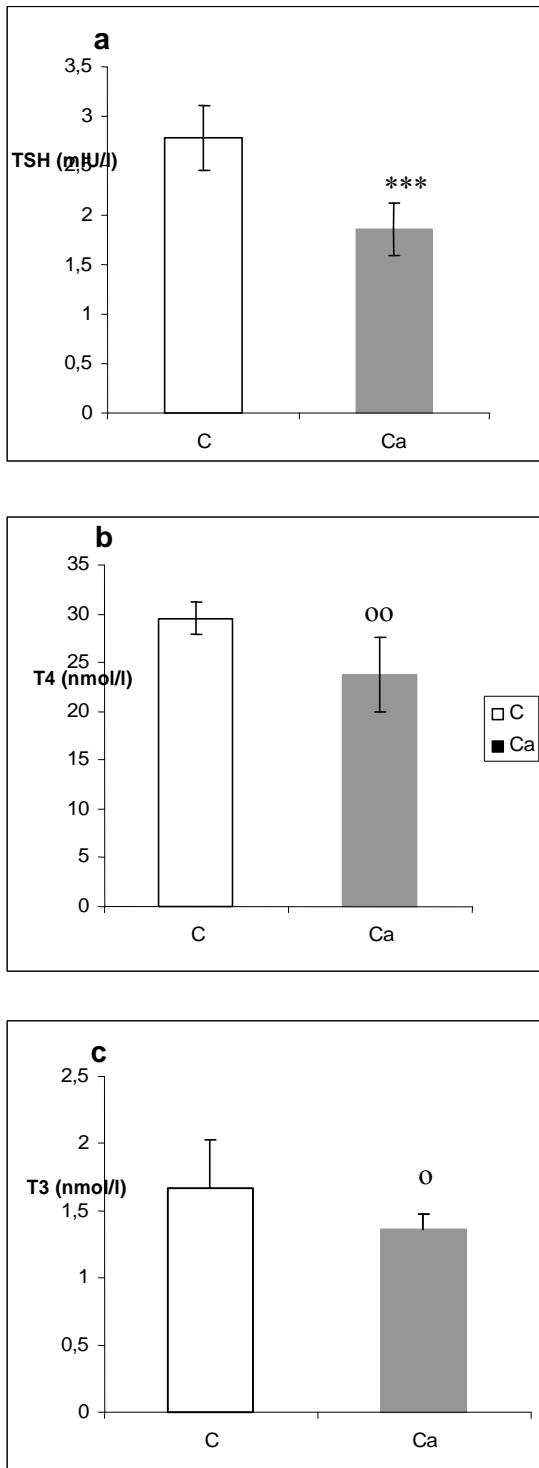
the serum concentration of TSH, total T₄, and T₃, ELISA assays were employed using a commercial human test kit (HUMAN, Wiesbaden, Germany). All serum samples were measured within the same assay in duplicate. The intra assay coefficient of variation was 5.0-13.8% for TSH, 9.8-8.7% for T₄, and 13.2-7.7% for T₃ (low and high sample levels). All results were expressed as the mean ± SD. Student's *t*-test was used for comparative evaluation. The minimum level of significance was set at p < 0.05.

The mean serum concentrations of TSH, T₄, and T₃ in the control and Ca-treated groups are summarized in Fig. 1 (a-c). Chronic Ca administration to middle-aged female rats brought about a decrease of all of the examined serum parameters in comparison with the vehicle-treated control values: (i) the serum level of TSH decreased by 30.6%, p < 0.005 (Fig. 1a); (ii) the serum level of T₄ decreased by 19%, p < 0.025 (Fig. 1b); and (iii) the serum level of T₃ decreased by 22%, p < 0.05 (Fig. 1c).

These results clearly demonstrate that thyroid functioning decrease after chronic administration of Ca to middle-aged female animals.

The treatment promoted a striking reduction of serum TSH concentration. This is consistent with our previous findings that Ca supplementation brings about a decrease of the examined stereological parameters for pituitary TSH cells – the relative cellular volume and the relative percentage of thyrotropes within the pituitary unit volume in middle-aged female rats (Sekulić *et al.*, 1998). Gillet *et al.*, (1990) also found that acute and chronic hypercalcemia leads to a decrease of TSH secretion in males. It follows that, Ca treatment can affect thyroid functioning indirectly, by reducing level of its major stimulatory factor, pituitary TSH. In contrast to our findings, Klíšnik *et al.* (1990) reported increased thyroid functioning to be accompanied by a decreased TSH concentration in adult rats. This discrepancy could be due to the different ages of the examined experimental animals.

Under our experimental conditions, a significant decrease of serum T₄ and T₃ was also detected. These results can be attributed to the above mentioned decrease of TSH, since the given hormone acts as a major positive factor in the regulation of thyroid functioning. Our previous morphometric research on thyroid gland structure showed that Ca administration significantly decreases volumetric density of the thyroid follicular cell epithelium, as well as its height and index of activation rate



(Šošić-Jurjević *et al.*, 2002). In vitro studies with FTRL-5 cells demonstrated that Ca does not affect the morphology of these cells, but acts directly when administered together with TSH by reducing the thyrotrophin stimulatory effect (Gabersek *et al.*, 1998). Vanvooren *et al.* (2000) found that isoform VI of adenylyl cyclase, the enzyme crucial for TSH-induced activation of thyroid follicular cells, is negatively modulated by Ca in human and dog thyroids.

The present study also showed that Ca supplementation decreases the serum concentration of total T_3 to a greater extent than the concentration of T_4 . This is consistent with the findings of Etling *et al.* (1986), who showed that dietary calcium inhibits the conversion of T_4 to T_3 in the serum, as well as in the liver and kidneys of rats.

It can be concluded that Ca administration exerts an adverse effect on thyroid functioning in middle-aged female rats, indirectly and/or directly. However, the mechanisms underlying the observed effect are still unclear.

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References - Bergenfelz A., Ahren B. (1994) *Horm. Res.* **42**, 273-277. - Etling N., Fouque F., Garabedian M. (1986) *Reprod. Nutr. Dev.* **26** (3), 841-847. - Gillet C., Corvilain J., Matte-Hiriart J., Willems D., Bergmann P. (1990) *J. Clin. Endocrinol. Metab.* **71** (2), 516-519. - Gabersek S., Stiblar-Martuncic D., Kališnik M. (1998) *Folia Biol. (Praha)*, **44** (2), 49-52. - Kališnik M., Zorc-Plešković R., Pajer Z., Pavlin K. (1990) *Amer. J. Anat.* **189**, 201-206. - Poirier L.A., Doerge D.R., Gaylor D.W., Miller M.A., Lorentzen R.J., Casciano D.A., Kadlubar F.F., Schwetz B.A. (1999) *Regul Toxicol Pharmacol.* **30** (3), 217-222. - Raymond F., Denereaz N., Lemarchand-Beraud T. (1992) *Acta. Endocrinol. (Copenh.)* **126**, 55-63. - Robuschi G, Safran M., Braverman L.E., Gnudi A., Roti E. (1987) *Endocrin. Rev.* **8**, 142-153. - Schindler A.E. (2003) *Gynecol Endocrin* **17** (1), 79-85. - Sekulić M., Lovren M., Milošević V. (1998) *Acta. Histochem.* **100**, 185-191. - Singh N., Singh P.N., Hershman J.M. (2000) *J. Amer. Med. Assoc.* **283** (21), 2822-2825. - Šošić-Jurjević B., Filipović B., Nestorović N., Lovren M., Sekulić M. (2002) *Yug. Med. Biochem.* **21**, 261-267. - Suchartwatnachai C., Theppisai U., Jirapinyo M. (2002) *Int. J. Gynaecol. Obstet.* **77** (1), 39-40. - Vanvooren V., Allgeier A., Cosson E., Van Sande J., Defer N., Pirlot M., Hanoune J., Dumont J.E. (2000) *Mol. Cell. Endocrinol.* **170** (1-2), 185-196.

Fig. 1. Serum level of TSH (Fig. 1a), total T_4 (Fig. 1b), and T_3 (Fig. 1c) in control (C; n = 6) and Ca-treated (Ca; n = 6) middle-aged female rats. Results are means \pm SD. ° $p < 0.05$, °° $p < 0.025$, *** $p < 0.001$.