The effect of acute exercise session on thyroid hormone economy in rats

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Abstract

The hypothalamic–pituitary–thyroid axis is affected by acute exercise, but the mechanisms underlying thyroid function changes after exercise remain to be defined. The aim of this study was to elucidate the effects of a session of acute exercise on the treadmill at 75% of maximum oxygen consumption on thyroid function of rats. Male Wistar rats were divided into five groups: control (without exercise), and killed immediately after (0 min) or 30, 60, and 120 min after the end of the exercise session. A significant increase in serum tri-iodothyronine (T3) occurred immediately after the exercise, with a gradual decrease thereafter, so that 120 min after the end of the exercise, serum T3 was significantly lower than that in controls. Total thyroxine (T4) increased progressively reaching values significantly higher than that in the control group at 120 min. T3/T4 ratio was significantly decreased 60 and 120 min after the exercise, indicating impaired T4-to-T3 conversion. Liver type 1 deiodinase activity (D1) significantly decreased at 60 and 120 min, while pituitary D1 increased progressively from 30 to 120 min after the exercise, and thyroid D1 was increased only immediately after the end of the exercise. Brown adipose tissue (BAT) type 2 deiodinase activity (D2) was significantly lower at 30 min, but pituitary D2 remained unchanged. No change in serum thyrotropin was detected, while serum corticosterone was significantly higher 30 min after the exercise. Our results demonstrate that decreased liver D1 and BAT D2 might be involved in the decreased T4-to-T3 conversion detected after an exercise session on the treadmill.


Introduction

Thyroid hormones act on multiple metabolic processes, influencing the concentration and activity of numerous enzymes, the metabolism of substrates, vitamins and minerals, and the response of target tissues to several hormones (Larsen et al. 1992). These hormones have critical roles in cell differentiation, growth, and regulation of oxygen consumption and thermogenesis (Yen 2001). Significant changes in energy metabolism occur during the exercise and are maintained for some hours thereafter, revealing challenges for energetic homeostasis. Exercise corresponds to a physical stress followed by some endocrine modifications that occur in order to counterbalance its effects on thermogenesis and substrate metabolism (Mastorakos & Pavlatou 2005). The influence of exercise on thyroid function is controversial and seems to depend on the intensity and duration of the training protocol (Rone et al. 1992).

The main thyroid gland hormonal secretion is thyroxine (T4), which is then deiodinated to generate tri-iodothyronine (T3), the most active thyroid hormone (Maia et al. 1995, Yen 2001). Types I, II, and III iodothyronine deiodinasies (D1, D2, and D3 respectively) regulate T3 production and clearance via removal of specific iodine atoms from the precursor molecule T4 or T3 itself. D1 and D2 catalyze the 5'-deiodination of T4 and are considered activating enzymes due to subsequent T3 production. The role of D3 is to inactivate T4 and T3 through their 5'-deiodination (Visser 1994, Bianco et al. 2002, Bianco & Kim 2006). Thus, deiodinases can acutely regulate serum T4 and T3 levels and also their tissue availability through their activation or inactivation.

Acute and chronic exercises elicit different effects in rats: serum levels of thyroid hormones increase after acute exercise, while the same is not observed in the majority of studies with chronic exercise protocols (Mastorakos & Pavlatou 2005). Wirth et al. (1981) observed an increase in total T3, total T4, and T3/rT3 ratio in rats immediately after the exercise for 20 min in treadmill, when compared with sedentary rats. Another study, using strenuous acute swimming exercise showed increased serum TSH levels, decreased T3 and T4 together with a blunted thyroid response to TSH stimulation immediately after the exercise protocol and a decrease in brown adipose tissue (BAT) D2 activity (Sullo et al. 2003). In humans, an acute aerobic exercise performed at 70% of...
maximum heart rate induced significant increases in total T₃ and T₄, free T₃ and T₄, and thyrotrophin (TSH); at 90% of maximum heart rate, the levels of total T₄, free T₄, and TSH were also increased, but serum T₃ and free T₃ decreased significantly (Ciloglu et al. 2005). Increased serum thyroid hormones immediately after the exercise might reflect catecholamine stimulation of hormonal secretion since a concerted action of the adrenergic system and the thyroid axis might correspond to an important physiological response to increased metabolic demands. On the other hand, high thermogenesis that takes place during the exercise could trigger mechanisms involved in the decrease in serum thyroid hormone levels, in order to prevent significant increases in body temperature.

These previous studies show contradictory results possibly due to the different protocols of exercise intensity used, but could also reflect differences due to the time after the exercise when the animals or humans have been evaluated. Thus, a time-course study might better define the changes that occur in the pituitary–thyroid hormone axis in exercised animals.

Hence, the aim of the present study was to elucidate the effects of a session of acute exercise on thyroid function just after the exercise protocol and during the recuperation period (30, 60, and 120 min). Since thyroid gland economy was adapted to run 2–3 days at 17 cm/s during 5 min. After the adaptation period, each rat was submitted to a maximum exercise protocol that consisted of 20 min treadmill running at 75% of maximum oxygen consumption velocity (VVO₂) and a constant slope of 10%. The animals completed at least 75% of the stage. Mean room temperature was maintained at 22 °C and a stainless steel grid at the end of the treadmill provided an electrical stimulus to keep the rats running. All tests and exercise protocol were carried out with a motor-driven treadmill chamber (Panlab – LSI Letica model, Sao Paulo, Brazil) and VO₂ was measured by an oxygen analyzer (Sable Systems FC-1B O₂, Las Vegas, NV, USA) at a constant flow rate of 500 ml/min. VO₂ consumption was obtained by the following formula: \( \text{VO}_{2\text{max}} = \left( \% \text{O}_2 \text{ max} - \% \text{O}_2 \text{ basal} / 100 \right) \times 500 \text{ ml/min} / \text{weight (kg)} \) (Werneck-de-castro et al. 2006).

After the experimental period, the animals were killed by decapitation and the blood was collected for hormone concentration analyses. Serum was obtained after centrifugation of the blood at 1500 g for 15 min and stored at −20 °C. Rat tissues were dissected out, weighed, and stored in liquid nitrogen until processing for enzymatic measurements.

**RIA for total T₃ and T₄, TSH, and corticosterone**

The measurement of serum TSH levels was carried out using a specific RIA for rat TSH obtained from the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK, Bethesda, MD, USA), and expressed in terms of reference preparation 2.

Serum total T₃ and T₄ concentrations were measured using commercial kits (T₃, DLS-3100 Active; T₄, DLS-3200 Active; TX, USA), based on the presence of specific antibodies adhered to the internal surface of propylene tubes. Rat hormone-free serum was used in the standard curves for total T₃ and T₄ and TSH. All the procedures were carried out following the recommendations of the kit.

The levels of serum corticosterone were measured by specific RIA for rats and mice (MP Biomedicals, LLC, Solon, OH, USA).

**Thyroperoxidase (TPO) iodide oxidation activity**

For extraction of TPO, each rat thyroid gland was homogenized in 50 mM Tris–HCl buffer (pH 7.2) and centrifuged at 200 000 g (4 °C for 35 min. The pellet was suspended in digitonin (1%, w/v; Sigma) and stored for 24 h at 4 °C. After this incubation period, centrifugation was repeated in the same conditions, and the supernatant containing solubilized TPO was stored at −20 °C (Moura et al. 1989, Carvalho et al. 1994).

TPO iodide oxidation assay (Nakashima & Taurog 1978, Pommier 1978, Moura et al. 1989, Carvalho et al. 1994) was performed as follows: 1·0 ml of 50 mM sodium phosphate buffer (pH 7·4) containing 24 mM KI and 11 mM glucose was placed in a 2 ml cuvette; the volume of the solubilized TPO added was 10 to 100 μL, and the final volume was adjusted to 2·0 ml with 50 mM sodium phosphate buffer (pH 7·4). The assay was started by the addition of 10 μl of 0·1% (w/v) glucose oxidase (Boehringer Grade I). The increase in absorbance at 355 nm (ΔA₃₅₅ nm/min, triiodide formation)
was followed for 2 min on a Hitachi spectrophotometer (U-3000). The A353 nm/min was determined from the linear portion of the reaction curve and corrected for a blank determined in the absence of TPO. One unit of iodide oxidation activity is defined as A353 nm/min = 1.0. The activity was related to protein concentration in the enzyme preparation. Protein concentration was measured by the method of Bradford (1976).

**D1 assay**

One pituitary gland, one thyroid gland, or 25 mg of liver were homogenized in 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 10 mM dithiothreitol (pH 6.9). Homogenates (150 μg of protein for pituitary samples and 30 μg of protein for liver and thyroid) were incubated in triplicate for 1 h at 37 °C with 1 μM [125I] rT3 (Perkin–Elmer Life and Analytical Sciences, Boston, MA, USA), and 10 mM dithiothreitol (USB/Invitrogen) in 100 mM potassium phosphate buffer containing 1 mM EDTA (pH 6.9) in a reaction volume of 300 μl, as described previously (Berry et al. 1991, Fortunato et al. 2006). Blank incubations were carried out in the absence of protein. The reaction was stopped at 4 °C in an ice bath with the addition of 100 μl fetal bovine serum (Cultilab, Campinas, Brazil) and 200 μl trichloroacetic acid (50%, v/v) followed by vigorous agitation. The samples were centrifuged at 8 000 g for 3 min and the supernatant was collected for the measurement of 125I liberated during the deiodination reaction. A specific D1 inhibitor (1 mM propylthiouracil (PTU)) was added to completely block deiodinase activity; thus, in our assay conditions, only D1 activity was measured.

D1 activity was related to the protein concentration in the homogenates. Protein concentration was measured by the method of Bradford (1976), after incubation of homogenates with NaOH (2.5 M).

**D2 assay**

One pituitary gland or 25 mg BAT were homogenized in 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 10 mM dithiothreitol (pH 6.9). Homogenates (50 μg protein for pituitary and 150 μg for BAT samples) were incubated in triplicate, 2 h for pituitary and 3 h for BAT, at 37 °C with 1 nM [125I] T4 (Perkin–Elmer Life and Analytical Sciences, Boston, MA, USA), and 10 mM dithiothreitol (USB/Invitrogen) in 100 mM potassium phosphate buffer containing 1 mM EDTA (pH 6.9) in a reaction volume of 300 μl, as described previously (Berry et al. 1991, Fortunato et al. 2006). Blank incubations were carried out in the absence of protein. The reaction was stopped at 4 °C in an ice bath with the addition of 100 μl fetal bovine serum (Cultilab) and 200 μl trichloroacetic acid (50%, v/v) followed by vigorous agitation. The samples were centrifuged at 8 000 g for 3 min and the supernatant was collected for the measurement of 125I liberated during the deiodination reaction. D2 activity was related to the protein concentration in the homogenates. Protein concentration was measured by the method of Bradford (1976), after incubation of homogenates with NaOH (2.5 M).

**Short-term radioiodide uptake: sodium–iodide symporter (NIS) activity**

We have previously demonstrated that the measurement of radioiodide uptake 15 min after 125I-NaI administration (short-term iodide uptake) reflects iodide transport through the NIS without the influence of in vivo thyroid iodine organification activity (Ferreira et al. 2005). Thus, in order to evaluate the in vivo NIS function using thyroid radioiodine uptake measurements without the influence of the TPO iodine organification reaction, the animals received Na125I (250 000 c.p.m., i.p., Amersham) 15 min before decapitation. The thyroids were removed and weighed. The radioactivity of the thyroid glands was measured using a γ-counter (LKB, Mount Waverley, Brazil) and expressed as percentage of total 125I injected per mg of thyroid.

**Statistical analyses**

The results are expressed as mean ± S.E.M. Data from total T3, total T4, corticosterone, T3/T4 ratio, and deiodinase activities were analyzed by one-way ANOVA test followed by Dunnett’s multiple comparison tests. The results of serum TSH, NIS, and TPO were analyzed by Kruskal–Wallis test followed by Dunnett’s multiple comparison tests. Statistical analyses were done using the software GraphPad Prism (version 4, GraphPad Software, Inc., San Diego, CA, USA). P ≤ 0.05 was considered statistically significant.

**Results**

**Serum total T3, T4, TSH, and corticosterone concentrations**

Immediately after the exercise, a significant increase in serum total T3 was detected (P < 0.05 versus control); however, at 30 min after the exercise, serum T3 returned to the basal levels and continued to decrease thereafter, reaching values that were statistically lower than controls at 120 min (P < 0.05) (Fig. 1A). Serum T4 was significantly higher at 120 min after the end of the exercise in comparison with the control and 30 min groups (P < 0.01) (Fig. 1B). The T3/T4 ratio was significantly lower at 60 min in comparison with the control group (P < 0.05) and further decreased at 120 min, differing from all the other groups (120 min versus C = P < 0.001; 0 = P < 0.01; 30 = P < 0.01; 60 min = P < 0.05) (Fig. 1C). These data indicate a decrease in the T4 to T3 conversion during the period of adaptation to exercise. Serum TSH did not change significantly after this acute exercise protocol (Fig. 1D). Serum levels of corticosterone were significantly higher at 30 min after the exercise session when compared with the control (P < 0.05) and normalized thereafter (Fig. 2).
TPO activity

TPO is a key enzyme in the biosynthesis of thyroid hormones, and an alteration on its activity could account for changes in T4 and T3 biosynthesis. Corroborating with previous findings that discard acute changes in TPO activity, the protocol of an intense acute exercise did not modify the activity of this enzyme (CZ3.53 ±G0.71, n=9; 0 min CZ3.55 ±G0.30, n=8; 30 min CZ3.56 ±G0.17, n=8; 60 min CZ4.30 ±G0.38, n=10; 120 min CZ3.65 ±G0.38 U/mg protein, n=9).

Short-term radioiodide uptake: NIS function

Although the NIS function might be acutely modulated by several factors, no changes in the short-term radioiodide uptake were seen among the groups (CZ0.036 ±G0.002, n=11; 0 min CZ0.036 ±G0.003, n=12; 30 min CZ0.036 ±G0.005, n=8; 60 min CZ0.041 ±G0.009, n=9; 120 min CZ0.043 ±G0.006%I125 per mg thyroid, n=11). Since thyroid iodide uptake did not change significantly, our data indicate no functional changes in thyroid hormone biosynthesis pathways after the acute exercise protocol. T3 increase detected immediately after the exercise might be secondary to thyroid secretion stimulation by catecholamine and/or to increased peripheral production of T3.

D1 enzymatic activity

Thyroid D1 activity was significantly increased at the end of the exercise when compared with the control group (Fig. 3C), but normalized thereafter, which could explain,
at least in part, the rapid increase in serum T₃ observed. Immediately after the end of the exercise session, hepatic D1 activity was normal and then progressively decreased, reaching significantly lower values in comparison with the control group, at 60 and 120 min (P<0.01) (Fig. 3A), a mechanism probably involved in the progressive serum T₃ decrease after the end of the exercise. Conversely, pituitary D1 activity significantly increased at 30, 60, and 120 min in comparison with the control (P<0.01) and 0 min groups (P<0.001); at 60 and 120 min, pituitary D1 was also significantly increased when compared with 30 min (Fig. 3B).

**Discussion**

Exercise is a stressful situation that challenges body homeostasis, so that the organism has to reestablish a new dynamic equilibrium in order to minimize cell damage. One of the systems affected is the hypothalamic–pituitary–thyroid axis (Mastorakos & Pavlatou 2005). While increased metabolic demands occur during exercise, higher thermogenesis is counterbalanced by mechanisms that are activated in order to dissipate heat-like vasodilatation. Both metabolism of substrates and thermogenesis are tightly regulated by thyroid hormones, and we show herein some of the mechanisms involved in thyroid function alterations that occur immediately after the exercise and for some hours thereafter. The intensity, duration, and type of exercise may elicit different patterns of response, and thus the results obtained are contradictory. The utilization of experimental animals to clarify these questions is useful to discard variables that are commonly found in human subject studies, apart from the possibility of studying the mechanisms involved in the hormonal changes observed.

In our study, the rats were submitted to an exercise session at 75% of maximal oxygen consumption, during 20 min, and the thyroid function was assessed immediately after and 30, 60, and 120 min after the end of the exercise protocol. Serum total T₃ levels were significantly higher immediately after the end of the exercise, while serum total T₄ and TSH levels were not altered. These findings are in agreement with the results found by Límanová et al. (1983), in a study with untrained young subjects, and those by Wirth et al. (1981), who showed an association of increased T₃ with an augmented norepinephrine response (Wirth et al. 1981, Límanová et al. 1983). Increased serum T₃ immediately after the end of an acute exercise session is probably caused by the adrenergic stimulus that occurs during the exercise, since exercise protocols at 60% of maximum oxygen capacity or greater are accompanied by large increases in circulating catecholamine levels, and the ability of adrenergic stimulus to increase basal thyroid hormone secretion is well established (Ahren et al. 1986, Coggan et al. 2000). However, in other studies, the levels of T₃, T₄, and TSH at the end of the exercise were unaffected or decreased by the exercise protocol. While Sullo et al. (2003) found a decrease in serum total T₃ and T₄ concentrations and an increase in TSH in rats at the end of a strenuous swim exercise. Krotkiewski et al. (1984) in a study with obese women showed no changes in these hormone levels. The divergence among these works is probably due to the different exercise protocols executed, as we know that catecholamine secretion depends on the intensity and duration of exercise.

TPO and NIS are two important proteins involved in the biosynthesis of thyroid hormones; while TPO is the enzyme that catalyzes the formation of T₃ and T₄, NIS is a symporter that transports two sodium ions along with one iodide into the follicular thyroid cell (Carvalho et al. 1994,
Eskandari et al. (1997). In our study, exercise had no effect on TPO and the NIS function. This is the first report evaluating the effect of acute exercise on these parameters. Rosolowska-Huszcz (1998) studied the effect of different intensities of 5-week exercise in rats and have shown a decrease in TPO activity at the higher intensities (Rosolowska-Huszcz 1998). The primary hormonal stimulator of TPO and NIS is TSH (Gerard et al. 1988, Uyttersprot et al. 1997, Ferreira et al. 2005). Serum TSH was not acutely modulated by the exercise protocol used herein, and the activities of TPO and NIS did not change, discarding a possible acute effect of catecholamines or other hormonal regulators on these parameters after intense acute exercise.

Krotkiewski et al. (1983) evaluated the changes in thyroid hormones at 30, 60, and 90 min after the end of the exercise in obese women and have shown a progressive decrease in serum TSH along with a progressive increase in serum total hormones at 30, 60, and 90 min after the end of the exercise. We are interested in order to clarify their physiological role after an acute exercise protocol. Eskandari et al. (2002) showed the activity of these enzymes after an acute exercise protocol.

In conclusion, we show herein that serum thyroid hormone changes that follow an acute exercise protocol seem to be mainly due to the regulation of both thyroid and liver D1 and BAT D2. The understanding of iodothyronine deiodinases regulation by corticosterone and adrenaline, as well as by other mediators induced by exercise, is of great interest in order to clarify their physiological role after an acute exercise protocol.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Programa de Núcleos de Excelência (PRONEX/CNPq), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). R S F is a recipient of a fellowship from CNPq, D L I from CAPES, and A S P from FAPERJ.

Acknowledgements

We are grateful for the technical assistance of Norma Lima de Araújo Faria, Advaldo Nunes Bezerra, and Wagner Nunes Bezerra.

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Received in final form 27 May 2008
Accepted 4 June 2008
Made available online as an Accepted Preprint 5 June 2008

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