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THE EFFECT OF EXERCISE TRAINING INTENSITY ON THYROID ACTIVITY AT REST

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The influence of exercise training intensity on thyroid activity at rest was studied in male Wistar rats, weighing 114g ± 24 (mean ± SD) at the beginning of the experiment. Animals were assigned to the following groups: untrained controls and rats trained on a treadmill at the speed of 20m/min over a 5-week period with different intensities: 2 × 60 min weekly, 4 × 60 min, 6 × 20 min, 6 × 40 min and 6 × 60 min weekly. Thyroid peroxidase (TPO) and hepatic iodothyronine 5'-monodeiodinase (5'DI) activities as well as plasma thyroxine (T₄), 3,3',5'-triiodothyronine (T₃) and 3,3',5'-triiodothyronine (rT₃) concentrations were determined. Training intensity was found to influence parameters under investigation. TPO activity was decreased in groups trained 240 min (4 × 60 min and 6 × 40 min) and 360 min (6 × 60 min) weekly in comparison to control, untrained group. Furthermore, a drop in T₄ plasma concentration in all trained groups and a decrease in T₃ plasma concentration in groups exercising for 120 min (2 × 60 min and 6 × 20min) weekly, as compared to control, untrained rats, was found. Hepatic 5'DI activity and rT₃ plasma concentration were not affected by training. Thus, exercise training in rats seems to elicit the fall in TPO activity and T₄ plasma concentration at rest but without changing hepatic 5'DI activity and rT₃ plasma concentrations. A decline in T₃ plasma concentration, observed in rats trained with the lowest exercise intensities, could be regarded as transitional effect in adaptation to chronic exercise.

Key words: exercise, iodothyronine 5'-monodeiodinase, thyroid, thyroid peroxidase, thyroxine, triiodothyronine

INTRODUCTION

Thyroid hormone metabolism is influenced by alterations in energy supply and expenditure. In states of energy restriction caused by fasting or undernutrition it has been found to undergo specific changes leading to a decrease in both production and clearance rates of triiodothyronine (T₃) and thyroxine (T₄) (1—4). Although exercise training represents a condition that can produce an energy deficit, it has been shown to enhance thyroid hormone metabolism both in humans with sufficient energy intake (5—7) and ad libitum
fed animals (8—12). However, little is known about the effect of chronic exercise on thyroid hormone metabolism at rest, since kinetic studies dealing with the impact of enhanced physical activity on thyroid hormone metabolism usually did not allow to assess independently the effects of acute and chronic exercise because they did not separate them distinctly. Particularly noteworthy seems to be an increased thyroid hormone turnover found in exercise trained male rats (9, 10) shown not to increase food intake to compensate for increased energy demand attributable to enhanced physical activity (13) and to reduce their body weight gain (10, 14, 15). Moreover, exercising male Wistar rats were found to display energy economizing adaptations such as reduction in total energy expenditure (15), suppression of cold induced thermogenesis (16, 17) and brown adipose tissue activity (18). On the other hand, the influence of training on thyroid hormone plasma levels in rats at rest is not quite clear. Although training has been shown not to affect total and free T₃ and reverse T₃ (rT₃) plasma concentrations at rest (10, 14, 19), data concerning T₄ plasma concentration are not consistent. Total T₄ plasma concentration was reported to be diminished (19) or remained unchanged (14), free fraction of plasma T₄ was demonstrated to be increased (9) or decreased (10) in trained rats compared to their control, sedentary counterparts.

This study was performed to evaluate the impact of exercise training intensity on thyroid activity at rest in male rats. The activities of two enzymes involved in thyroid hormone metabolism — thyroid peroxidase (TPO) and hepatic iodothyronine 5'-monodeiodinase (5'DI) were measured together with T₄, T₃ and rT₃ plasma concentrations. Thyroid peroxidase plays a key role in thyroid hormone biosynthesis because of its ability to catalyze oxidation of iodide, the iodination of tyrosyl residues of the thyroglobulin and their coupling (20). Hepatic 5'DI catalyzes monodeiodination of both outer and inner ring of T₄, yielding T₃ and rT₃. It acts also on rT₃, providing 3,3'diiodothyronine (21).

MATERIALS AND METHODS

Animals

Male Wistar rats (n = 56) 7 week old weighing 114g ± 24 (mean ± SD) at the beginning of the experiment were housed in standard environmental conditions individually in wire mesh cages and fed ad libitum with standard rodent food.

Experimental design

After one week of standardization rats were divided into 6 groups: sedentary controls (n = 11) and groups trained on a treadmill at the speed of 20m/min during 5 weeks: 2 × 60 min weekly (n = 6); 4 × 60 min (n = 9); 6 × 20 min (n = 10); 6 × 40 min (n = 9); 6 × 60 min (n = 11) weekly.
Rats were sacrificed at the age of 13 weeks after 5 weeks of training about 24 hrs after the last exercise bout. They were anesthetized with diethyl ether and blood was collected by cardiac puncture. Thyroid and liver were weighed and immediately frozen in liquid nitrogen. Then plasma was stored at −20°C and tissues at −80°C until determinations.

**Analytical procedures**

Thyroid peroxidase activity was determined in thyroid microsomal fraction based on Alexander (22), Neary et al. (23) and Hosoya et al. (24) methods. Briefly: thyroids were thawed, washed in physiological saline and homogenized on ice in 1.1% KCl in motor driven Potter Elvehjem homogenizer during 1 min and centrifuged (15 min × 15000g). Then the pellet was discarded and supernatant was centrifuged (1hr × 105000g). Pellet was suspended in 200 μl 20 mM phosphate buffer pH 7.4 with 10 μl 0.1% Triton × 100.

TPO activity was measured by iodide oxidation assay (24). The reaction mixture contained 50 mM phosphate buffer pH 7.4, 0.135 mM H₂O₂, 13.3 mM KJ and 50—80 μg of protein in 1.5 ml. The reaction was started by the addition of 10 μl of H₂O₂ and followed spectrophotometrically at 350nm at room temperature. The amount of enzyme which gave a change of 0.001 absorbance per sec was taken as 1 unit (U). Protein was determined by the method of Lowry et al. (25) with bovine serum albumin as a standard.

Liver microsomal fraction was prepared and 5'DI activity was estimated according to procedure described by Nauman et al. (26). The livers were thawed, washed with saline and homogenized on ice in 3 vol. 20 mM buffer Tris — HCl pH 7.2 with 3 mM EDTA, 10 mM DTT and 0.25 M sucrose using a motor driven teflon pestle/glass body homogenizer. The homogenate was filtered through gauze and centrifuged for 10min at 10000g. Then the pellet was discarded and supernatant was centrifuged for 60 min at 105000g. The microsomal pellet was then suspended in the same buffer without sucrose. The microsomal fraction was stored at −80°C until further analysis.

5'DI activity was measured during 30 min incubation at 37°C. Incubation medium (400 μl) contained 50 mM Tris — HCl buffer pH 7.2, 3 mM EDTA, 1.3 nM T₄, 5 mM DTT and about 100 μg of microsomal protein. The reaction was stopped by placing test tubes on ice and adding of 800 μl of ice-cold 99% ethanol. After extraction and centrifugation (1500g, 20 min) T₃ concentration in ethanol extracts was determined immediately by radioimmunoassay. Enzyme activity was expressed as the amount of T₃ per min and mg of protein. Protein concentration was determined by the method of Bradford (27).

Thyroid hormone plasma concentrations were determined by radioimmunoassay commercial kits for T₄ (cat. no MI 88) and T₃ (cat. no MI 86) produced by POLATOM, for rT₃ (cat. no 10834) by Serono Diagnostics.

**Statistical analysis**

Statistical analysis was performed by one — way analysis of variance (ANOVA) and Student t-test.

**RESULTS**

All results and statistically significant differences between groups according to Student t-test are summarized in Table 1.
Table 1. Effect of exercise training intensity on body weight gain and resting values of thyroid activity parameters.

<table>
<thead>
<tr>
<th>groups</th>
<th>C (n = 11)</th>
<th>2 x 60 (n = 6)</th>
<th>4 x 60 (n = 9)</th>
<th>6 x 20 (n = 10)</th>
<th>6 x 40 (n = 9)</th>
<th>6 x 60 (n = 11)</th>
<th>statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW gain (%)</td>
<td>168.9a (17.2)</td>
<td>81.5c (9.5)</td>
<td>133.8b (13.9)</td>
<td>134.7b (14.0)</td>
<td>126.5b (27.5)</td>
<td>130.9b (10.5)</td>
<td>a vs b and b vs c - p &lt; 0.01; a vs c - p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>45.7a (2.2)</td>
<td>41.9a (4.8)</td>
<td>31.8b (2.3)</td>
<td>48.4a (6.7)</td>
<td>21.4c (2.6)</td>
<td>23.7c (2.3)</td>
<td>a vs b and b vs c - p &lt; 0.05; a vs c - p &lt; 0.001</td>
</tr>
<tr>
<td>TPO (U/sec/ mg)</td>
<td>97.7a (4.4)</td>
<td>74.4b (7.8)</td>
<td>67.3b (8.2)</td>
<td>62.9b (8.1)</td>
<td>32.2c (2.7)</td>
<td>31.8c (2.3)</td>
<td>a vs b and b vs c - p &lt; 0.01; a vs c - p &lt; 0.005</td>
</tr>
<tr>
<td>T3 (ng/ml)</td>
<td>0.99a (0.05)</td>
<td>0.63b (0.08)</td>
<td>0.83a (0.04)</td>
<td>0.71b (0.04)</td>
<td>1.00a (0.08)</td>
<td>0.89a (0.08)</td>
<td>a vs b - p &lt; 0.05</td>
</tr>
<tr>
<td>rT3 (ng/ml)</td>
<td>0.131 (0.007)</td>
<td>0.132 (0.007)</td>
<td>0.120 (0.008)</td>
<td>0.109 (0.013)</td>
<td>0.114 (0.007)</td>
<td>0.117 (0.008)</td>
<td>NS</td>
</tr>
<tr>
<td>5'DI (pmol T3/ min/mg)</td>
<td>4.40 (0.13)</td>
<td>4.38 (0.13)</td>
<td>4.57 (0.23)</td>
<td>4.62 (0.22)</td>
<td>4.63 (0.23)</td>
<td>4.71 (0.16)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means with SE indicated in parantheses. Statistically significant differences are denoted by different letters, NS — lack of significance. Groups: C — control, untrained rats; 2 x 60. 4 x 60, 6 x 20, 6 x 40, 6 x 60 — rats trained, first number — exercise frequency during the week; second — daily exercise duration in minutes; n — number of rats. BW — body weight, TPO — thyroid peroxidase activity, T4, T3, rT3 — thyroxine, triiodothyronine and reverse triiodothyronine plasma concentration (respectively), 5'DI — iodothyronine 5'-monodeiodinase activity in liver.

Body weight gain in all trained groups was significantly reduced compared to the control rats. Group trained twice weekly for 60 min gained significantly less than the other trained groups. ANOVA: effect of daily exercise duration: p < 0.02; effect of frequency during the week: p < 0.0001.

Thyroid peroxidase activity was influenced by daily exercise duration (ANOVA: p < 0.0001), but not by its frequency during the week (ANOVA: p < 0.1). In rats trained 6 x 60, 6 x 40 and 4 x 60 min weekly TPO activity was significantly reduced compared to sedentary controls and rats trained 6 x 20 and 2 x 60 min. In groups trained 6 x 40 and 6 x 60 min it was significantly lower than in group trained 4 x 60 min.

Plasma T4 concentration in all trained groups was lower than in sedentary animals. ANOVA revealed significant influence of daily exercise duration (p < 0.0002) and frequency (p = 0). In groups trained 6 x 20, 2 x 60 and
$4 \times 60$ min values were statistically not different, but significantly higher than in groups trained $6 \times 60$ and $6 \times 40$ min.

Plasma $T_3$ concentration was diminished in groups trained $2 \times 60$ and $6 \times 20$ min in relation to sedentary rats and the other trained groups. ANOVA indicated significant influence of daily exercise duration ($p < 0.0002$).

Plasma $rT_3$ concentration and 5' DI activity were neither influenced by exercise frequency nor by duration. In all trained groups the values did not differ statistically significantly from those of control group.

**DISCUSSION**

This study has demonstrated that chronic enhancement of physical activity in rats can elicit a diminish in TPO activity and $T_4$ plasma concentration in resting state without changing plasma $T_3$ and $rT_3$ concentrations and hepatic 5'DI activity.

The decrease in body weight gain, which occurred in all trained rats compared to sedentary controls, could be attributed to inadequate energy intake in relation to increased energy demand which has been stated in chronically exercising male Wistar rats (13, 15). Suprisingly, body weight gain did not differ between groups trained with various exercise intensities except the group exercised twice weekly. It could be supposed, that the lower body weight gain in group trained with the least frequency could be the result of an emotional stress.

Thyroxeine plasma concentration and TPO activity were reduced depending on the weekly training dose. However, $T_4$ level was decreased in all trained rats while TPO activity was diminished in rats trained at least 240 min. weekly. With the 240 min. of training performed weekly both $T_4$ plasma level and TPO activity were more reduced when exercise was applied with shorter daily duration but more frequently.

The effect of exercise on TPO activity, according to the author's knowledge, has not been examined as yet. Some insight into the effect of exercise on thyroid gland activity could yield results of studies on thyroidal iodine uptake, although they were conducted both during rest and periods of enhanced daily activity. They revealed decrease in iodine uptake in trained rats and humans (18, 29). Rats exercising spontaneously were found to store only half as much iodine in the thyroid as did the nonexercising controls with the amount of exercise and amount of iodine being negatively correlated (28). This effect has been suggested to be connected with depression of thyroid activity or increase in thyroid hormone production and release. However, a significant difference in the rate of renewal of thyroidal iodine between sedentary and exercising rats was not stated (28). Thyroidal iodine uptake as well as TPO synthesis and activity are stimulated by TSH (30, 31)). In trained rats, both unstimulated
TSH values and TRH — stimulated TSH responses have been found to be decreased compared to ad libitum fed nonexercising rats (14). It must be also taken into consideration that insulin, known to stimulate TPO gene expression (32), has been shown to be reduced by training both in humans (33, 34) and rats (35). Effect of training on thyroid activity could be mediated by brain systems known to inhibit TRH and/or TSH secretion, such as dopaminergic and NPY systems (36, 37) and those suggested to stimulate, such as serotonergic (38). Exercise training was stated to stimulate the hypothalamic NPY (39) and dopaminergic (40) systems. On the other hand, a downregulation of brain serotonin receptors by training was suggested (41). All of these effects could lead to decline in TRH/TSH secretion and reduction in stimulation of thyroid.

Decrease in TPO activity could contribute to a fall in T₄ plasma concentration found in exercising rats. In previously reported investigations training — induced decrease in T₄ plasma concentration was associated with changes in T₄ metabolism and distribution. A decline in T₄ half life and elevation in T₄ secretion rate were reported in horses together with decrease in protein bound iodine plasma concentration (8). In rats, an increase in T₄ metabolic clearance rate, as well as decrease in T₄ plasma half life and total distribution space have been observed (9). However, a decline in T₄ plasma concentration was also stated in trained men to appear with decrease in degradation rate of T₄ (6). Increase in T₄ plasma concentration demonstrated by Kattz and Selgrad (42) in exercising rats compared to nonexercising controls was connected with increase in T₄ total body pool and total volume of distribution and decrease in its plasma clearance rate.

Decrease in T₃ plasma concentration demonstrated in the present experiment could be treated as transitional stage in adaptation to enhanced physical activity, since it was found in rats trained with the lowest intensity. Unaltered plasma T₃ concentration, found in this experiment in rats trained at least 240 min weekly compared to untrained controls, has been also reported by others in trained rats (10), mice (12) and men (6, 7). Despite plasma T₃ concentration being unaffected by training, changes in T₃ metabolism have been found in trained men (6, 7) and laboratory rodents (10, 12). In trained men elevation in T₃ degradation rate together with increase in metabolic and urinary clearance rates have been shown (6, 7). Total volume of distribution, disposal rate and total body pool were found to be positively correlated with aerobic capacity (7). In trained rats an increase in T₃ metabolic clearance rate was demonstrated (10), while in mice with chronic enhancement of physical activity an increase in T₃ production rate without changes in clearance rate was noted (12).

Lack of changes in T₃ plasma concentration together with decrease in T₄ plasma level was stated to be associated with elevation in T₄ concentration in liver and diminution in T₃ concentration in heart and kidney in trained rats
compared to non — exercising controls (19). In another study, exercising rats were found to increase both T₄ and T₃ concentration in liver and their total distribution space (10).

It should be mentioned, that only in experiment performed by Rone et al. (7) in men effects of training and acute exercise were separated since subjects did not continue exercise during turnover study. Results obtained in this study clearly demonstrate, that enhanced T₃ metabolism represents event existing in resting conditions. Contrary to this, studies reporting enhanced T₄ and T₃ metabolism in trained rats (9, 10) did not enable the distinction between periods of activity and rest.

Despite elevation in T₄ and T₃ concentration in liver and increased metabolism of T₄ in trained rats reported by others (9, 10), hepatic 5'DI activity at rest was found to be unaffected by training in the present study, which is in agreement with the previous data obtained in rats (9) and mice (12). In addition, 5'deiodinating activity was found not to be altered in kidneys and muscles in trained rats (9). Unchanged plasma rT₃ level found in exercising rats in this study according to data obtained by others in men (43, 44) and rats (14) could confirm lack of training effect on hepatic 5'deiodinating activity in the resting state, since rT₃ plasma concentration is considered to be an index of 5'DI activity in liver (45). In the study conducted on mice after four weeks of spontaneous enhanced physical activity, decrease in hepatic 5'DI activity was observed in animals submitted to feeding restriction (12). Therefore, decrease in 5'DI activity could appear above the certain stage of energy deficit and/or shortage of its specific stimulant, such as glucose or T₃ (46, 47).

A decline in TPO activity at rest could contribute to the energy conservation trends observed in trained male rats (15, 18). It could be supposed that exercise training induced adaptations in thyroid hormone metabolism found in rats known not to increase their food intake to compensate for the energy expenditure attributable to exercise could also occur in humans submitted to the energy restriction, as in the treatment of obesity.

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