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Triiodothyronine Deiodinating Activity in Brown Adipose Tissue after Short Cold Stimulation Test in Trained and Untrained Rats

A. SULLO[1], G. BRIZZI[1], N. MAFFULLI[2]

[1]Second University of Naples, Faculty of Medicine and Surgery, Department of Experimental Medicine, Human Physiology Section, Napoli, Italy, [2]Department of Trauma and Orthopaedic Surgery, Keele University School of Medicine, North Staffordshire Hospital, Thornburrow Drive, Hartshill, Stoke on Trent, Staffordshire, United Kingdom

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Summary

Interscapular brown adipose tissue (IBAT) activity is controlled by sympathetic nervous system, and factors that influence thermogenesis appear to be centrally connected to the sympathetic outflow to IBAT. Cold exposure produces a rise in BAT temperature, which is associated with an increased thyroid activity, elevated serum levels of 3,5,3'-triiodothyronine (T3), and an increased rate of T3 production. This study evaluated the effect of swimming training on 5'-triiodothyronine deiodinase (5'-D) activity in IBAT under normal environmental conditions and after short (30 min) cold exposure (TST stimulation test). 5'-D activity is lower in trained rats at basal condition, and TST increases 5'-D in IBAT of both untrained and trained rats. However, this increase is lower in trained rats. Training reduces the deiodinating activity in normal environmental conditions as well as after short cold exposure. Probably, other compensatory mechanisms of heat production are active in trained rodents.

Key words

Deiodinase activity • Thyroid hormone • Training • Brown adipose tissue • Thermogenesis

Introduction

Brown adipose tissue (BAT) is responsible for the production of most of the increase in metabolic heat production during various experimental manipulations in rodents (Fydia *et al.*1981, Girardier 1983). Interscapular BAT (IBAT) is supplied by a mixed nerve, which provides five separate branches to the individual lobes (Foster *et al.* 1982 a, b). IBAT activity is controlled by the sympathetic nervous system (Landsberg and Young 1983), and factors that influence thermogenesis appear to act centrally to modify the sympathetic outflow to IBAT (Rothwell and Stock 1984). Thyroid hormone activity closely regulates BAT thermogenesis. Cold exposure and a cafeteria diet produce a rise in BAT temperature, which is associated with increased thyroid activity, elevated serum level of 3,5,3'-triiodothyronine (T3), and increased rate of T3 production.

There is a close synergistic relationship between the sympathetic nervous system and thyroid hormone metabolism in BAT. Indeed, the type II 5'-triiodothyronine deiodinase (5'-D) found in brown fat

is under sympathetic nervous system control (Silva *et al.* 1983). This enzyme is different from 5'-D found in the kidney and liver, and is similar to 5'-D in the brain and heart (Becket and Arthur 1994). 5'-D activity increases in IBAT, but not in the liver and kidneys, after an increase of thermogenesis by injection of neostigmine into the hippocampus (Monda *et al.* 1996). This suggests that the higher serum level of T3 is due to T4 to T3 conversion in BAT, and confirms that T3 produced in BAT stimulates the thermic response of this tissue (Glick *et al.* 1985). Furthermore, the activation of type II 5'-D is probably dependent specifically on sympathetic nerves in BAT, not circulating catecholamines, although administration of pharmacological doses of exogenous adrenergic agents may increase enzyme activity (Kates *et al.* 1990).

Cold exposure (4 °C) increases BAT 5'-D activity maximally after 4 h, but this effect is evident already after 30 min (Brizzi *et al.* 1998). This confirms the key role of BAT in rapid cold adaptive mechanism to produce supplementary energy (Brizzi *et al.* 1998).

The effect of exercise training on BAT-dependent thermogenesis is controversial. For example, trained animals demonstrated a lower BAT thermogenic activity (Larue-Achagiotis *et al.* 1995), but running training had no marked effect on the thermogenic activity of BAT in rats (Segawa *et al.* 1998).

Brief cold exposure (30 min) is used in experimental animals to rapidly increase TRH production for enhancing thyroid function by stimulating TSH production (TSH-stimulation test = TST). This test up-regulates the hypothalamo-pituitary-thyroid axis. Under these conditions, it is possible to evaluate better the influence of cold or other factors on this regulation system. The TST is performed by transferring rapidly the experimental animals from an adaptation room at 30 °C to a cold room at 4 °C for 30 min (Cannon and Nedergaard 1983, Jones *et al.* 1996). Animals trained to exercise showed no decrease in enhanced non-shivering thermogenesis, but there is a decrease in BAT weight compared with sedentary rats, and serum T3 levels were higher in cold-adapted animals than in de-adapted rats with or without an exercise load (Moriya 1986). Exercise training in rats seems to elicit a fall in thyroid peroxidase activity and T4 plasma concentration at rest, with no changes in liver 5'-D activity and 3,3'5'-triiodothyronine (Rosolowska-Huszcz 1998).

In this study, we tested the hypothesis that training can modify T3 production in IBAT through changes in 5'-D activity in both basal conditions and after short (30 min) cold exposure (TST stimulation test).

Methods

All procedures were carried out according to the policy statement of the American College of Medicine after approval of the Ethics Committee of the Second University of Naples, Faculty of Medicine and Surgery.

Animals

We used male Sprague-Dawley rats (n = 40), weighing 280-320 g. These were housed in pairs at controlled temperature (22±1 °C) and humidity (70 %), with a 12:12 h light-dark cycle from 07.00 to 19.00 h. Laboratory standard food (Mil, Morini, Italy) and water were available at all times.

Apparatus for oxygen consumption measurement

Resting O_2 consumption was determined by indirect calorimetry. The closed circuit apparatus used was an adaptation of the calorimeter by Benedict and MacLeod (1928). Air was continuously circulated through a drying column ($CaSO_4$ Drierite), a respiratory chamber 2.5 l, and CO_2 trap (soda lime) by a peristaltic pump at a rate of 2 l/min. The oxygen reservoir was a cylindrical metal bell, which fitted in a concentric cylinder filled with water, forming an air-tight seal. The cylinder, graduated to 5 ml, was connected to the respiratory chamber. The temperature in the respiratory chamber was maintained constant by circulating water, and was monitored by an internal thermometer. O_2 consumption was determined for 60 min after 20-min equilibration.

The volume of O_2 consumed was corrected for temperature and pressure and was expressed as milliliter of O_2/min/kg b.w. $^{0.75}$ (Kleiber 1975).

Exercise

Out of the 40 male Sprague-Dawley rats included in the study, 20 rats were assigned randomly to the training group.

The physical activity consisted of daily swimming for 21 consecutive days. The animals swam six times for 5 min with a resting interval of 3 min, between 13:00 and 13:45 h. The exercise was carried out in fast flowing water at 25 °C. The flow was so fast that the animals were forced to swim vigorously, and not simply float (Monda *et al.* 1993).

Resting O_2 consumption, food intake, and body weight were measured in each animals before the training protocol, and three weeks after the beginning of training. The O_2 consumption in all animals was measured at the

same times (9.00-10.00 h). The trained rats were tested the day after the last exercise session. Before the experiment, no training was administered for three days. The rats were divided in four groups, each of 10 animals, treated as follows:

Untrained rats treated with TST (UT): after 30 min in the adaptation room (30 °C) they were transferred to a cold room (4 °C) for 30 min.

Swimming trained rats treated with TST (TT): after 30 min in the adaptation room (30 °C) they were transferred to a cold room (4 °C) for 30 min.

Untrained controls (UC): the rats were kept in the adaptation room (30 °C).

Swimming trained controls (TC): the rats were kept in the adaptation room (30 °C).

Procedure

Then the animals were anesthetized with urethan (1.2 g/kg/ body wt ip) and decapitated. Blood and IBAT were rapidly excised keeping the carcasses on ice, frozen in liquid nitrogen, and stored at –80 °C until the 5'-D enzyme assay was performed. Serum concentration of T3 and T4 were determined by radioimmunoassay (Amersham). The 5'-D activity was studied in vitro by a modified method of Leonard *et al.* (1983). The enzymatic 5'-D activity was expressed by pg of T3 for each mg of protein content in the homogenates for 60 minutes of reaction time (pg T3/mg protein/h).

Statistical analysis

All values are presented as mean ± standard error, performed on the difference between the basal values and values obtained after 3 weeks of training for food intake, resting O_2 and body weight. Statistics were evaluated using one-way analysis of variance; Newman-Keul was used as *a post-hoc* test.

In the second part of the experiment (TST), a two-way analysis of variance was used to examine the data for statistical significance. The two experimental factors included training and TST. Where significance was found, the Newman-Keul *post hoc* multiple comparison test was applied to determine differences between specific means.

Table 1. The changes in food intake, resting O_2 consumption (ml/kg b.w.$^{0.75}$) and body weight before and after 3 weeks of training.

Variables	Untrained rats		Trained rats	
	UT	UC	TT	TC
Food intake (g)	2.00±0.41	2.05±0.36	12.00±0.91*	11.90±0.82*
Resting O_2 consumption	0.70±0.41	0.35±0.38	6.65±0.91*	7.05±0.88*
Body weight (g)	30.70±2.34	31.35±1.38	32.62±1.94	31.63±0.74

Data are means ± S.E.M. *TT and TC > UT and UC ($p<0.01$), UC: untrained control rats, UT: untrained rats undergoing TST, TC: trained control rats, TT: trained rats undergoing TST.

Results

The differences between food intake, measured before the treatment and 3 weeks after the training protocol, are shown in Table 1. Analysis of variance showed statistical differences between trained (TT and TC) and untrained (UC and UC) groups [$F(3,20) = 62.46$, $p<0.01$]. The *post hoc* test showed a difference between UT and TT ($p<0.01$), UT and TC ($p<0.01$), TT and UC ($p<0.01$), and TC and UC ($p<0.01$).

The changes in resting O_2 consumption are shown in Table 1. O_2 consumption increased in UT, RT, UC, and TC, respectively. Analysis of variance showed a statistical differences between trained (TT and TC) and untrained (UC and UC) groups [$F(3,20) = 42.16$, $p<0.01$]. The *post hoc* test showed a difference between UT and TT ($p<0.01$), UT and TC ($p<0.01$), TT and UC ($p<0.01$), and TC and UC ($p<0.01$).

Body weight did not differ significantly among the groups.

Regarding the serum level of T3 of trained and untrained rats undergoing the TSH stimulation test, two-way analysis of variance showed significant effects for TST [$F(1,10) = 78.234$, $p<0.01$], for training [$F(1,10) = 77.235$, $p<0.01$], and for the interaction between training and TST [$F(8,80) = 20.132$, $p<0.01$]. The *post hoc* test

showed a difference between UT and UC ($p<0.01$), UT and TT ($p<0.01$), UT and TC ($p<0.01$), UC and TC ($p<0.01$), and TT and TC ($p<0.05$).

The serum level of T4 of trained and untrained rats undergoing the TSH stimulation test were not significantly different between the groups.

Two-way analysis of variance showed significant effects 5'-D activity in the BAT of trained and untrained rats undergoing TSH stimulation test [$F(1,10) = 77.235$, $p<0.01$], for training [$F(1,10) = 80.235$, $p<0.01$] and for the interaction between training and TST [$F(8,80) = 21.235$, $p<0.01$]. The *post hoc* test showed a difference between UT and UC ($p<0.01$), UT and TT ($p<0.01$), UT and TC ($p<0.01$), UC and TT ($p<0.01$), UC and TC ($p<0.01$), and TT and TC ($p<0.05$).

Table 2. Serum level of T3 (ng/dl) and T4 (μg/dl) as well as 5' deiodinase activity (pg T3/mg protein/h) in the IBAT.

Variables	Untrained rats		Trained rats	
	UT	UC	TT	TC
Serum T3 level	94.12±3.10*	82.24±2.34^{+}	86.32±2.47$^{\#}$	76.12±4.15
Serum T4 level	4.22±0.34	4.32±0.51	4.23±0.42	4.36±0.16
5'deiodinating activity	638.34±13.17*	422.42±11.62§	345.46±14.19$^{\#}$	304.20±12.59

Data are means ± S.E.M. *UT > UC and TT and TC ($p<0.01$); $^{+}$ UC > TC ($p<0.01$); $^{\#}$ TT > TC ($p<0.05$); § UC > TT and TC ($p<0.01$), UC: untrained control rats, UT: untrained rats undergoing TST, TC: trained control rats, TT: trained rats undergoing TST.

Discussion

In the present investigation, we used swimming as the exercise of choice because it is a stressful stimulus and causes different functional changes than other forms of exercise. In thermoneutral water, swimming produces adaptive changes somewhat similar to running and those produced by cold acclimatization (Harri and Kuusela 1986). On the other hand, the adaptations in thermoneutral water are similar to those from swimming in cold water (Harri and Kuusela 1986). This suggests that changes are due to the particular physical activity *per se* and only partially depend on the temperature.

Exercise causes a higher increase of resting O_2 consumption, in accordance with the results reported both in rodents (Hill *et al.* 1983, 1984) and in humans (Poehlman and Horton 1989, Mole 1990).

The food intake and body weight of our trained experimental animals are apparently in contrast with the findings of other authors, who found that exercise, such as running, leads to reduced energy intake that contributes to a delayed energy deposit as fat and protein (Richard and Arnold 1987, Richard and Rivest 1989, Rivest *et al.* 1989a). In our experiment, the type of exercise was different, and was performed in the light period, whereas in the experiments of other authors the exercise program was carried out in the dark phase of the light-dark cycle. The possible increase of CRF, which is considered to be an anorectic factor (Morley 1987, Rivest *et al.* 1989b), could not significantly influence the consummatory behavior, because the CRF increment, due to the stress of exercise, could be too distant from the start of feeding (which occurs prevalently in the dark phase).

The increase in food intake was in agreement with the findings of Harri and Kuusela (1986), which indicated that food intake was increased by swimming both at 36 °C and at 30 °C, which induced hypothermia.

The second part of our experiment showed that trained rats had lower serum levels of T3 and deiodinating activity (about 25%) than untrained rats at basal condition. These results are in agreement with Rosolowska-Huszcz (1998), who observed a decline in serum level of T3 as effect in adaptation of chronic exercise, and Larue-Achagiotis *et al.* (1995), who demonstrated a lower BAT thermogenic activity in trained animals than in sedentary rats. In contrast, Segawa *et al.* (1998) found that running training had no overt effect on the thermogenic activity of BAT in rats. Neverthelss, the authors of the latter report also found a decrease in BAT mass that could represent an absolute reduction of 5'-D activity with respect to sedentary rats with unchanged BAT mass.

Short cold treatment, produces supplementary T3 by sympathetic activation, and increases deiodinating activity in organs directly involved in adaptation to functional requirements, and particularly in the IBAT, which can produce rapidly more heat locally, thus balancing the decreasing body temperature. We found an increase in serum level of T3 and deiodinating activity which was significantly lower in trained rats than in untrained rats. Indeed, our results have shown an increase of about 50 % in untrained rats contrasting with an increase of about 20 % in trained rats. The decreased 5'-D activity in IBAT could result from exercise-induced lower sympathetic activity, which aims to reduce energy dissipation. These results confirm the findings of Leblanc *et al.* (1982) who demonstrated that exercise training (swimming 2 h/day for 10 weeks) reduced food intake and body weight gain, and failed to increase norepinephrine-induced thermogenesis in rats. In another experiment, Lambert and Jonsdottir (1998) found that hypothalamic concentrations of norepinephrine were reduced after exercise training. The presence of a positive relationship between arterial and hypothalamic norepinephrine levels suggested an association between noradrenergic neuronal activity of the hypothalamus and sympathetic nervous function.

In conclusion, we found that training by swimming can reduce T3-induced thermogenesis in IBAT through changes in 5'-D activity both under basal conditions and after short (30 min) cold exposure (TST stimulation test). This effect can be explained by a lower sympathetic activity both at rest and after a cold stress. Probably, other compensatory mechanisms of heat production are active in trained rodents. For example, exercise training with a running wheel shifts threshold temperatures for heat loss and heat production to higher levels, which may rise a core temperature level (Sugimoto *et al.* 2000). Other authors found that stimuli associated with elevated blood pressures and heart rates in sedentary humans, such as sustained handgrip to fatigue or 2 min of cold exposure were significantly attenuated in trained subjects (O'Sullivan and Bell 2001).

Although chronic exercise conditioning has been shown to alter basal thermoregulatory processes (Rowsey *et al.* 2001), the relationship between exercise and thermoregulation is not clear yet (Briese 1998). Long-term exercise causes marked morphological and functional changes that can be explained by blunting of sympathetic vasomotor activation that follows training, such as an increase in muscle mass and capillary density, a decrease in fat mass, improvement of cardiovascular and respiratory responses to both exercise-dependent and exercise-independent stimuli.

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Reprint requests

N. Maffulli, Department of Trauma and Orthopaedic Surgery, Keele University School of Medicine, North Staffordshire Hospital, Thornburrow Drive, Hartshill, Stoke on Trent, Staffordshire, ST4 7QB, England, E-mail: n.maffulli@keele.ac.uk