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Influence of Exercise Training and Age on Uncoupling Protein mRNA Expression in Brown Adipose Tissue

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SCARPACE, P. J., S. YENICE AND N. TÜMER. *Influence of exercise training and age on uncoupling protein mRNA expression in brown adipose tissue.* PHARMACOL BIOCHEM BEHAV **49**(4) 1057–1059, 1994.—The ability to regulate body temperature diminishes with age. Exercise training is known to increase cardiovascular performance, and there is some evidence of a cross-adaptation between exercise and cold tolerance in young rats. The present study was designed to examine the effects of physical training by treadmill running on the capacity for brown adipose tissue (BAT) thermogenesis in young and old rats. To this end, we assessed BAT uncoupling protein (UCP) mRNA expression in sedentary and exercise-trained 5- and 25-mo-old F-344 rats. The amount of UCP mRNA, whether expressed as per unit RNA or per BAT, did not change with either age or training. These data indicate that there is no cross-adaptation by exercise on adaptive thermogenesis in BAT in either young or old rats.

F-344 rats Thermogenesis Treadmill running

THE capacity for thermoregulation diminshes with age in both humans and rodents (1,16). In the rat, nonshivering thermogenesis in brown adipose tissue (BAT) is an important mediator for increasing body temperature after cold exposure. BAT thermogenesis is stimulated by catecholamine activation of adenylyl cyclase through sympathetically innervated β-adrenergic receptors (4). This process accelerates lipolysis, and the liberated fatty acids serve as substrates for mitochondrial oxidation and provide the signal to activate the mitochondrial uncoupling protein (UCP). This protein acts as an H^+/OH^- translocator that, when activated, facilitates high rates of substrate oxidation and an increase in heat production without the phosphorylation of adenosine 5′-diphosphate (7). Sympathetic stimulation leads to an immediate increase in thermogenesis by the activation of mitochondrial UCP (4). In addition, prolonged sympathetic stimulation induces the synthesis of UCP and further increases the capacity for thermogenesis (13).

Although it is somewhat controversial, there is some evidence of a cross-adaptation between exercise training and cold tolerance (2,3,5,6,12). Exercise training is known to decrease plasma catecholamines in young rats (9). The mechanism most likely involves a decrease in the biosynthesis of catecholamines as a result of the reduced expression and activity of tyrosine hydroxylase (19). Senescent rats, however, do not show these adaptive changes (19). Because sympathetic stimulation is the major regulator of thermogenesis in BAT, and because training decreases catecholamine biosynthesis, increased BAT thermogenesis would seem to be an unlikely mechanism for the cross-adaptation of training and cold tolerance, at least in young rats. However, in senescent rats, the efficiency of β-adrenergic signal transduction is decreased (18), and the effects of training on catecholamine biosynthesis are blunted (19). Moreover, senescent rats have a decreased cold tolerance and a decreased capacity for BAT thermogenesis (10,15). Thus, exercise training may preferentially increase adaptive thermogenesis in senescent rats, even when cross-adaptation is absent in young rats.

For these reasons, we designed the present study to examine the effects of physical training by treadmill running on the capacity for BAT thermogenesis in young and old rats. To this end, we assessed BAT UCP mRNA expression in sedentary and exercise trained 5- and 25-mo-old F-344 rats.

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METHOD

Animals

Female F-344 NNia rats, 3 and 23 mo of age, were obtained from Harlan Industries (Indianapolis, IN) under contract with the National Institute on Aging. Upon arrival, rats were examined and housed individually in 7 in × 10 in stainless steel cages. No serologic or bacteriologic tests were performed. All animals were maintained on Purina Rat Chow ad lib, with a 12-h light-dark cycle. At the time of sacrifice, rats were either 5 or 25 mo of age.

Training Protocol

Rats were exercised by treadmill running in a metabolic treadmill (Model PT; Omni Tech Electronics, Columbus, OH) with electric shock avoidance. The rats were randomly selected for entry into either the control or experimental group. Rats were exercised with the lights off during their normal nocturnal cycle at approximately 70% of their peak oxygen consumption 5 days/week over 9 weeks. Peak oxygen consumption was determined at treadmill speeds of 30 m/min (young) and 20 m/min (old) for periods of 4 min. The grade was increased at the end of each period until the rat could no longer maintain that work rate. Because the peak oxygen consumption was less in the senescent rats, the final running speed was less in these animals. Each training session began with a 5-min warm-up at 15 m/min (0% grade). On day 1 (week 1) of training, the animals began exercising at 25 m/min (young) or 18 m/min (old), with a 10-min duration at 0% grade. The duration of exercise was increased by 2 min/day until the animals reached 60 min of exercise. The exercise time period remained at 60 min for the duration of the study. During weeks 1-3 the grade was gradually increased from 0 to 5%. Beginning at week 4 the speed was increased to 30 m/min (young) and 20 m/min (old), and the grade reset to 0%. During weeks 4-9 the grade was gradually increased to 12.5%. At the end of the ninth week the rats were exercising for 60 min at a 12.0% grade at speeds of 30 m/min (young) and 20 m/min (old). Animals were continuously monitored during exercise to verify that running was maintained. Nonexercised animals were placed into the treadmill for equal lengths of time with the apparatus in the off position. The ambient temperature was 26°C. We previously determined that thermoneutrality for both ages of these rats is 26°C (16).

UCP mRNA

Total cellular RNA was determined by extraction from 100 mg of minced and sonicated IBAT tissue, as described previously (16). Briefly, RNA was extracted with 1 ml of RNAzol B (Biotecx, Friendswood, TX) and 100 μl of chloroform per BAT. The extracted RNA was twice precipitated with an equal volume of isopropranolol, washed with 70% ethanol, suspended in a 10-mM EDTA solution, and heated to 65°C for 10 min. After centrifugation, the supernatant was harvested. For measurement of UCP mRNA levels, several concentrations of serially diluted RNA samples were immobilized on nylon membranes (Gene Screen; Dupont NEN, Boston, MA) and baked at 80°C for 2 h. The baked membranes were prehybridized using 25 mM potassium phosphate, 750 mM NaCl, 75 mM Na citrate, 5× Denhardt's solution, 50 μg/ml denatured salmon sperm DNA, and 50% formamide. After incubation for 14-16 h at 42°C, then membranes were hybridized with a ^{32}P random prime-labeled cDNA probe in the prehybridization buffer with the addition of 10% dextran sulfate (8). The cDNA clone for UCP was kindly provided by Dr. Leslie Kozak (Jackson Laboratory, Bar Harbor, Maine) (8). After hybridization for 14-16 h at 42°C, the membranes were washed and exposed to X-ray film (Kodak X-AR, Rochester, NY) for 96 h at −70°C using intensifying screens. Optical density per μg total cellular RNA was calculated by comparison with internal laboratory standards of BAT UCP mRNA present on each nylon membrane.

Northern analysis indicated that this probe hybridizes to two mRNA species, a major band corresponding to 1.5 kb and a minor band corresponding to 1.9 kb (16). The probe did not hybridize to any mRNA species from the cerebral cortex (16).

Statistical Analysis

Comparisons among ages and with training were determined by two-way analysis of variance (ANOVA) and Scheffé's *S* posthoc test. Analyses were performed using super ANOVA (Abacus Concepts, Berkeley, CA).

RESULTS

All rats in the exercise groups ran with minimal electrical stimulation. In the exercise groups, two young rats of 13, and five old rats of 13, did not complete the studies because of injuries (two young, two old) or death unrelated to running (three old). In the control groups, two old rats of 10 died. All 10 young control rats completed the study. Samples from one young control and one young trained rat were lost as a result of RNA degradation.

The body weight and intrascapular BAT weight of the young rats, as expected, were considerably less than those of the senescent rats (Table 1). Training had no measurable effect on body weight in rats of either age. In contrast, BAT weight declined with training in rats of both ages (Table 1). After 9 weeks of training, the peak oxygen consumption (while running) significantly increased by 12 and 10%, respectively, for young and old rats, compared with pretraining levels (data not shown). This indicated that a similar level of training occurred in both ages of rats.

To determine the effects of training on the capacity of thermogenesis in BAT, the amount of UCP mRNA was determined. The total amount of RNA recovered was greater in the senescent rats but not significantly changed with training in rats of either age (Table 2). The amount of UPC mRNA per unit of recovered RNA was unchanged with age or training (Table 2). When the total amount of UCP mRNA per BAT

TABLE 1

EFFECT OF TRAINING AND AGE ON BODY AND BAT PARAMETERS

	Young		Senescent	
	Control	Trained	Control	Trained
Body Weight (g)*	199 ± 4	191 ± 3	270 ± 4	262 ± 5
BAT Weight (mg)†	295 ± 15	238 ± 9‡	450 ± 25	363 ± 20‡
RNA/BAT (μg)*	280 ± 24	192 ± 16	379 ± 30	324 ± 18

Data represent the mean ± SE of nine young control, 10 young trained, eight old control and eight old trained rats.

*$p < 0.001$ (body weight) and $p < 0.03$ (RNA/BAT) for difference with age. No significant difference with training.

†$p < 0.001$ for difference with age and with training.

‡$p < 0.02$ for difference with training compared with age-matched control by Scheffé's *S* posthoc analysis.

TABLE 2

EFFECT OF TRAINING AND AGE ON BAT UCP mRNA

	Young		Senescent	
UCP mRNA	Control	Trained	Control	Trained
Arbitrary U/μg total RNA	97 ± 14	119 ± 11	100 ± 10	89 ± 6
Arbitrary U/BAT	412 ± 64	500 ± 64	526 ± 65	417 ± 32

Data represent the mean ± SE of nine young control, 10 young trained, eight old control, and eight old trained rats. There were no significant differences with age or training.

was considered, there still was no change with either age or training (Table 2).

DISCUSSION

The ability to regulate body temperature in response to a variety of environmental challenges diminishes with age (1,14). One of several factors contributing to this may be a reduced capacity for thermogenesis. Sympathetic stimulation mediated by β-adrenergic signal transduction is the primary regulator of BAT thermogenesis (4). With senescence, β-adrenergic signal transduction and β-adrenergic-stimulated mitochondrial GDP-binding in BAT are reduced, suggesting diminished thermogenic capacity in BAT with age (15,17).

The heart is another tissue in which β-adrenergic signal transduction is diminished with age (18). There is also a decrease in cardiovascular performance with age. Exercise training increases cardiovascular performance and increases β-adrenergic signal transduction in hearts from senescent rats, partially offsetting the decrease in signal transduction with age (11,18). Because physiologic function and β-adrenergic signal transduction decrease in both heart and BAT with age, and because training improves cardiovascular performance as well as the efficiency of signal transduction in aged hearts, we predicted that training should increase thermogenic capacity in BAT, at least in senescent rats. β-Adrenergic signal transduction mediates both the increase in GDP binding and the induction of UCP mRNA (4,13). Previous studies indicated that the GDP binding and blood flow to BAT was unchanged in young rats after exercise (12,20). Thus, we chose to investigate the adaptive process mediated by β-adrenergic signal transduction—that is, the induction of UCP mRNA. The results of this study confirm our previous report that the levels of UCP mRNA are unchanged with age (16), and indicate that there is no effect of training at either age on UCP mRNA expression in intrascapular BAT.

There is some evidence that exercise training increases cold tolerance in humans and rats (2,5,6). However, other evidence indicates that there is no cross-adaptation—in particular, nonshivering thermogenesis after cold exposure is not increased by prior exercise training (3,12). The results of this report support this latter notion that there is no cross-adaptation between exercise training and thermogenic capacity in BAT of either young or old rats.

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