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Exercise as a new physiological stimulus for brown adipose tissue activity

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Abstract *Background and aim:* Brown adipose tissue (BAT) plays a major role in body energy expenditure counteracting obesity and obesity-associated morbidities. BAT activity is sustained by the sympathetic nervous system (SNS). Since a massive activation of the SNS was described during physical activity, we investigated the effect of endurance running training on BAT of young rats to clarify the role of exercise training on the activity and recruitment state of brown cells.

Methods and results: Male, 10-week-old Sprague Dawley rats were trained on a motor treadmill (approximately 60% of VO_2max), 5 days/week, both for 1 and 6 weeks. The effect of endurance training was valuated using morphological and molecular approaches.

Running training affected on the morphology, sympathetic tone and vascularization of BAT, independently of the duration of the stimulus.

Functionally, the weak increase in the thermogenesis (no difference in UCP-1), the increased expression of *PGC-1α* and the membrane localization of MCT-1 suggest a new function of BAT. Visceral fat increased the expression of the *FOXC2*, 48 h after last training session and some clusters of UCP-1 paucilocular and multilocular adipocytes appeared.

Conclusion: Exercise seemed a weakly effective stimulus for BAT thermogenesis, but surprisingly, without the supposed metabolically hypoactive effects. The observed browning of the visceral fat, by a supposed white-to-brown transdifferentiation phenomena suggested that exercise could be a new physiological stimulus to counteract obesity by an adrenergic-regulated brown recruitment of adipocytes.

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Introduction

Metabolically active brown adipose tissue (BAT) plays an important and exclusive role in maintaining body energy homeostasis through the adaptive thermogenesis [1].

The recent demonstration of the presence of active BAT in human has renewed scientific interest because of its well documented role in preventing diet-induced obesity, insulin resistance and type 2 diabetes: genetic ablation of BAT in mice is accompanied by obesity whereas experimental conditions capable of increasing the number of brown adipocytes reduce insulin resistance and obesity [2].

Development and maintenance of the brown phenotype in adipose tissue is sustained by sympathetic nervous system (SNS) and noradrenergic post-ganglionic neurons, through β −adrenergic receptors signaling [3]. Noradrenergic fibers are strictly associated to brown but not to white adipocytes [4] and their density in the adipose organ results positively correlated with both functional activity and proportion of brown cells [5].

Weight gain and insulin resistance are associated with low SNS activity [6–8] and reduced turnover of noradrenaline in BAT [6,9], whereas the anti-obesity effect of some drugs seems to be associated with a stimulation of sympathetic mechanisms [10].

A massive activation of the SNS was observed during exercise [11,12], but the influence of exercise training on BAT activity is not clear: running training results in a burst of sympathetic activity with a transient heat production in BAT [13,14] and chronic running training was found markedly reduce the expression of UCP-1 in BAT without reduction of either total protein content and UCP-1 or guanosine 5′-diphosphate binding in the mitochondria [15]. On the contrary, Scarpace demonstrates no changes in total RNA content and UCP-1 gene expression [16].

The absence of the BAT thermogenesis during chronic exercise and the decrease of BAT weight [15,16] and DNA content [17] suggested that BAT is hypoactive during exercise [1].

To clarify the role of exercise training on the activity and recruitment state of BAT we investigated the effect of endurance training (by running) on BAT using morphological and molecular approaches.

Methods

Animals and exercise program

Male Sprague Dawley rats (Charles-River, Milan, Italy), 10 weeks old, with initial body weights of 340–370 g were used. Animals were housed singly in a temperature-controlled room at 22 °C with a 12/12 h light–dark cycle. Food and water were available *ad libitum*.

The exercised rats were subjected to exercise training on a motor treadmill gradually progressing toward 18 m/min for 60 min, 5 days/week, for 1 (acute exercise) ($n = 20$) and 6 weeks (chronic exercise) ($n = 8$). This working intensity was approximately 60% of VO_2max, which was estimated based on published data [18,19]. The week before the experiment, the rats were submitted to a short period of exercise (10 min) on a treadmill to enable them to become accustomed to the experimental procedure.

After the final exercise training, the animals were sacrificed immediately (E) or 48 h after the last exercise session (E-recovery). A sedentary group (C) was used as control.

For cold acclimatization experiments, 10-week-old rats ($n = 6$) were transferred to 4 °C for 1 week.

Animal care and handling were in accordance with Italian Institutional Guidelines.

At the designated time, all the animals were deeply anesthetized with an intraperitoneal injection of sodium thiopental (45 mg/kg body weight) (Pentothal Sodium, Abbott, Italy) and interscapular-BAT (iBAT), retroperitoneal White Adipose Tissue (rpWAT) and epididymal-WAT (eWAT) were immediately dissected out. For RNA isolation, tissues were immediately removed, immersed in RNA later solution (Qiagen) and either immediately processed or stored at −80 °C for later processing.

For histological analysis, the animals were transcardially perfused with 4% formaldehyde in 0.1 M phosphate-buffer (PB), pH 7,4. iBAT, rpWAT and eWAT were dissected and postfixed by overnight immersion in the same fixative. Samples were dehydrated in ethanol and paraffin-embedded. For transmission electron microscopy, iBAT tissue fragments were postfixed in 2% glutaraldehyde, 2% formaldehyde in 0.1 M PB, pH 7.4 and embedded in an Epon–Araldite mixture.

Light microscopy and immunohistochemistry

4-μm-thick sections were obtained and stained with haematoxylin-eosin to assess morphology. For immunohistochemistry, sections were incubated with polyclonal anti-UCP-1, anti-Tyrosine Hydroxylase (TH), anti-Monocarboxylate Transporter 1 (MCT-1), anti-eIF4E-BP1 (phosphoT70) and by the biotinylated forms of the lectin *Bandeiraea simplicifolia* agglutinin (BS-1). For details, see Supplementary materials.

Morphometric analysis

TH immunoreactive (i.r.) parenchymal nerve fibers (i.e., fibers closely associated to brown adipocytes) were counted in the iBAT, as previously described [4].

Quantification of isolectin-positive vessels per adipocyte was obtained by counting 20 different fields from each animal [$n = 6$ (acute exercise) and $n = 4$ (chronic exercise) for each condition]. The results were expressed as the number of capillary per adipocyte.

The quantification of UCP-1 i.r. adipocytes was performed on the right rpWAT. 4-μm-thick slides were randomly sectioned every 300 μm. UCP-1 i.r. cells were counted in all the sections and UCP-1 i.r. adipocytes density (number/mm^2) was calculated for each animal.

For details, see Supplementary materials.

Gene expression analysis

RNA was extracted using RNeasy Mini Kit (Qiagen), treated with DNase I and reverse transcribed using Omniscript RT Kit (Qiagen) and random primers (Promega) according to