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Habitual exercise training acts as a physiological stimulator for constant activation of lipolytic enzymes in rat primary white adipocytes

Junetsu Ogasawara [a, *], Tetsuya Izawa [b], Takuya Sakurai [a], Ken Shirato [a], Yoshinaga Ishibashi [a], Yoshinobu Ohira [b], Hitoshi Ishida [c], Hideki Ohno [a], Takako Kizaki [a]

[a] Department of Molecular Predictive Medicine and Sport Science, Kyorin University, School of Medicine, Mitaka, Tokyo 181-8611, Japan
[b] Graduate School of Health and Sports Sciences, Doshisha University, Kyotanabe, Kyoto 610-0394, Japan
[c] Department of Third Internal Medicine, Kyorin University, School of Medicine, Mitaka, Tokyo 181-8611, Japan

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ABSTRACT

It is widely accepted that lipolysis in adipocytes are regulated through the enzymatic activation of both hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) via their phosphorylation events. Accumulated evidence shows that habitual exercise training (HE) enhances the lipolytic response in primary white adipocytes with changes in the subcellular localization of lipolytic molecules. However, no study has focused on the effect that HE exerts on the phosphorylation of both HSL and ATGL in primary white adipocytes. It has been shown that the translocation of HSL from the cytosol to lipid droplet surfaces requires its phosphorylation at Ser-563. In primary white adipocytes obtained from HE rats, the level of HSL and ATGL proteins was higher than that in primary white adipocytes obtained from sedentary control (SC) rats. In HE rats, the level of phosphorylated ATGL and HSL was also significantly elevated compared with that in SC rats. These differences were confirmed by Phos-tag SDS-PAGE, a technique used to measure the amount of total phosphorylated proteins. Our results suggest that HE can consistently increase the activity of both lipases, thereby enhancing the lipolysis in white fat cells. Thus, HE helps in the prevention and treatment of obesity-related diseases by enhancing the lipolytic capacity.

1. Introduction

Habitual exercise training (HE) helps to maintain low body weight in laboratory animals and humans. Under a regular exercise regimen, lipolytic responses in white adipocytes are upregulated through adaptive changes in a signal transduction system referred to as a lipolytic cascade [1,2]. Physical exercise stimulates lipid metabolism by modifying lipolysis-related molecules. In mammals, the prolonged HE plays a key role in the positive regulation of energy expenditure.

It has been widely accepted that hormone-sensitive lipase (HSL) is a unique rate-limiting enzyme of lipolysis in white fat cells. The translocation of HSL from the cytoplasm to the lipid droplet surface is mediated by its phosphorylation by protein kinase A (PKA) [2,3]. However, this concept is modified by the identification of adipose triglyceride lipase (ATGL) [4]. ATGL catalyses the first step in triacylglycerol (TG) hydrolysis in the adipose tissue; this is accompanied by HSL-regulated hydrolytic degradation of TG in mammals. ATGL initially hydrolyses TG into free fatty acid (FFA), and HSL subsequently hydrolyses diacylglycerol substrate to produce an additional FFA and a monoacylglycerol [2,3]. Moreover, accumulated evidences show that the activation of ATLG is regulated by phosphorylation by PKA at Ser-404 in humans [5] or Ser-406 in rodents [6]. It has been shown that HE upregulates the action of ATGL in addition to HSL in human skeletal muscle [7] with an increase in its phosphorylation [8]. However, at present, no study has focused on the effect that HE exerts on the phosphorylation of ATGL as well as HSL in primary white adipocytes, although white adipocytes has a central role in both ATGL and HSL. The changes observed in the phosphorylation of these two enzymes by HE would help us better understand HE-induced adaptive changes in lipolytic molecules in white adipocytes because our previous study demonstrated that HE provokes behavioural changes in lipolytic molecules, thereby enhancing the lipolysis in adipocytes obtained from HE [9].

* Corresponding author.
E-mail address: junetsu@ks.kyorin-u.ac.jp (J. Ogasawara).

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In the present study, we demonstrate that HE brings about a constant increase in the levels of both phosphorylated HSL and ATGL with upregulation of these proteins in primary rat adipocytes. Our results strongly support the opinion that fitness training is imperative for prevention and treatment of obesity-related metabolic syndromes.

2. Materials and methods

2.1. Animal care and exercise training program

Four-week-old male Wistar rats (SLC, Shizuoka, Japan) were housed in groups of 2 or 3 per cage in a temperature-controlled room at 23 °C with a 12:12-h light–dark cycle. Food and water were available *ad libitum*. The animals were randomly divided into two groups: SC group (n = 5) and HE group (n = 5). The HE rats were exercised on a treadmill set at a 5-degree incline, 5 days per week for 10 weeks, according to the previously reported protocol [9–11]. The initial training intensity was 15 m/min for 20 min; thereafter, the running speed and duration were progressively increased until, after 6 weeks, the rats ran continuously at 30 m/min for 90 min. The SC rats were not subjected to the treadmill exercise. The HE rats were euthanized 36 h after the last exercise session. The rats were anesthetised with an intraperitoneal injection of sodium pentobarbital (5 mg/100 g body weight; Abbott Labs, Abbott Park, IL). Adipose tissue was rapidly removed and adipocytes were isolated using the methods described below. The Animal Care Committee of the Kyorin University School of Medicine approved the animal protocol following National Institute of Health guidelines (NIH, USA).

2.2. Preparation of primary adipocytes

Adipocytes were isolated using a method developed by Rodbell [12]. Briefly, fat pads were minced with scissors and placed in plastic vials in buffer A (Krebs–Ringer bicarbonate solution buffered with 10 mM HEPES, pH 7.4, containing 5.5 mM glucose and 2% (w/v) fatty acid-free bovine serum albumin) with 200 nM adenosine and collagenase type 1 (3 mg/ml, Worthington Biochemical, Lakewood, NJ). Collagenase digestion was performed at 37 °C in a water-bath shaker. After 60 min, the contents of the vials were filtered and centrifuged at 100 × *g* for 1 min. The layer of floating cells was then washed three times with buffer A. The cells were transferred to centrifugation tube, and then the proteins were extracted.

2.3. Protein extraction

Isolated primary adipocytes were washed three times with phosphate-buffered saline (137 mM NaCl, 8.1 mM Na_2HPO_4, 2.68 mM KCl, 1.47 mM KH_2PO_4). The cells were centrifuged at 800 × *g* at room temperature for 2 min. The packed cells were homogenized in ice-cold homogenization buffer (Pierce, Rockford, IL), including both protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN) and phosphatase inhibitor cocktail (Sigma, St Louis, MO), by 20 passages through a 5/8-inch, 27-gauge needle attached to a syringe, at 4 °C. The homogenate was centrifuged at 40,000 × *g* at 4 °C for 30 min. The obtained supernatant was centrifuged again, and the obtained clear sample was used as the cell extract for immunoblotting analysis. The samples were frozen at −80 °C for later analysis.

2.4. Sample preparation for Phos-tag analysis

Phos-tag acrylamide was purchased from NARD Institute Ltd. (Amagasaki, Japan). The cell lysate was dialyzed in the Xpress Micro Dialyzer MD100 system (Funakoshi Co., Ltd., Tokyo, Japan) to remove detergent. Dialyzed samples were mixed with Laemmli's sample buffer and then placed in a heat block at 95 °C for 5 min. The samples were cooled and loaded onto a 6% Phos-tag SDS-polyacrylamide gel. After electrophoresis, the proteins were transferred onto a PVDF sequencing membrane (Millipore Corporation, Billerica, MA) according to the manufacturer's protocol. Immunoblotting analysis was performed as described below, using HSL and ATGL antibodies. The principle of Phos-tag SDS-PAGE has been described in the paper by Kinoshita et al. [13].

2.5. Immunoblotting analysis

There was no significant difference between the protein levels in the cells from the different groups (data not shown). Therefore, identical amounts of each sample were run on the same gel. The samples were mixed with Laemmli's sample buffer and then placed in a heat block at 100 °C for 3 min. The cooled samples were loaded onto a 9–12% SDS-polyacrylamide gel. After electrophoresis, the proteins were transferred onto a PVDF sequencing membrane (Millipore Corporation, Billerica, MA). The PVDF membrane was first incubated for 60 min in TBS-T (100 mM of Tris–HCl, pH 7.4, 150 mM of NaCl and 0.1% Tween 20) containing 5% skim milk. After incubation, the PVDF membrane was incubated with a specific antibody in the TBS-T at 4 °C overnight. The antibodies against the following antigens were used at a 1:1000 dilution: HSL, ATGL phosphorylated at Ser-406, β-actin (Abcam, Cambridge, UK), p-ATGL, HSL phosphorylated at Ser-563, and HSL phosphorylated at Ser-660 (Cell Signaling Technology, Inc., Danvers, MA). After washing, the membranes were incubated for 60 min with anti-rabbit or anti-goat immunoglobulin G (1:2000 dilution)-conjugated horseradish peroxidase antibody (DakoCytomation, Glostrup, Denmark). The membranes were washed, and the immunoreactive bands were detected using the ECL system (GE Healthcare, Buckinghamshire, UK) by Kodak X-ray film (Kodak, Tokyo, Japan).

2.6. Statistical analysis

Values represent the means ± S.D. The significance of differences between means was assessed using the Scheffe's test after the analysis of variance had been performed to establish that there were significant differences between the groups. $P < 0.05$ was regarded as significant.

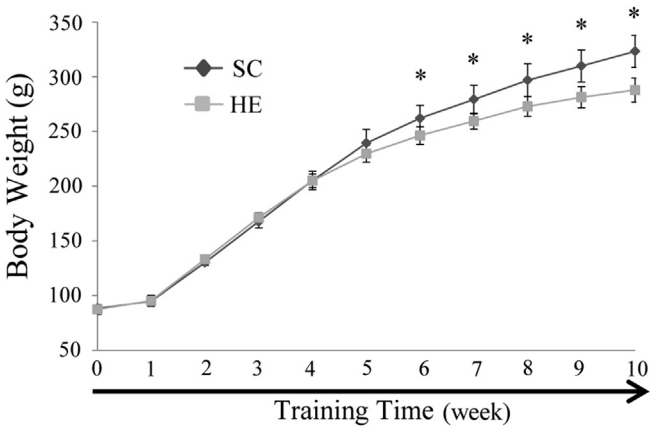

Fig. 1. The effect of habitual exercise training on the alteration in body weight. The body weights were measured by weight scale. HE-induced loss of body weights were observed from six week period of training time. *P < 0.05 vs. sedentary control.

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