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Exercise-induced increase in IL-6 level enhances GLUT4 expression and insulin sensitivity in mouse skeletal muscle

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ABSTRACT

A single bout of exercise is known to increase the insulin sensitivity of skeletal muscle; however, the underlying mechanism of this phenomenon is not fully understood. Because a single bout of exercise induces a transient increase in blood interleukin-6 (IL-6) level, we hypothesized that the enhancement of insulin sensitivity after a single bout of exercise in skeletal muscle is mediated at least in part through IL-6-dependent mechanisms. To test this hypothesis, C57BL6J mice were intravenously injected with normal IgG or an IL-6 neutralizing antibody before exercise. Twenty-four hours after a single bout of exercise, the plantaris muscle was harvested to measure insulin sensitivity and glucose transporter (GLUT)-4 expression levels by *ex-vivo* insulin-stimulated 2-deoxyglucose (2-DG) uptake and Western blotting, respectively. Compared with sedentary mice, mice that performed exercise showed enhanced IL-6 concentration, insulin-stimulated 2-DG uptake, and GLUT-4 expression in the plantaris muscle. The enhanced insulin sensitivity and GLUT4 expression were canceled by injection of the IL-6 neutralizing antibody before exercise. In addition, IL-6 injection increased GLUT4 expression, both in the plantaris muscle and the soleus muscle in C57BL6J mice. Furthermore, a short period of incubation with IL-6 increased GLUT4 expression in differentiated C2C12 myotubes. In summary, these results suggested that IL-6 increased GLUT4 expression in muscle and that this phenomenon may play a role in the post-exercise enhancement of insulin sensitivity in skeletal muscle.

1. Introduction

Interleukin-6 (IL-6) acts as both an inflammatory and an anti-inflammatory cytokine, depending on its cellular context, and plays various roles in glucose metabolism. Indeed, IL-6 knockout mice were reported to develop mature-onset obesity and insulin resistance [1,2]. Furthermore, the acute administration of IL-6 enhanced insulin-independent and insulin-dependent glucose uptake in human and mouse skeletal muscle [3,4]. These results suggested that IL-6 is a positive regulator of glucose metabolism in skeletal muscle. On the other hand, several studies have reported the opposite effect of IL-6 on glucose metabolism. For example, plasma IL-6 concentrations were increased in obese subjects and were negatively correlated with insulin sensitivity in humans [5]. In addition, mice treated with chronic IL-6 administration for 5 days showed hepatic insulin resistance [6] and IL-6-induced insulin resistance in myocytes, adipocytes, and hepatocytes [7–9]. In a rodent model, the blockade of IL-6 using an IL-6 receptor neutralizing antibody improved high fat diet-induced insulin resistance in skeletal muscle [10]. This inconsistency of the effects of IL-6 on glucose metabolism might be, at least in part, explained by the reaction time of IL-6. An acute, spike-shaped increase in IL-6 level is suggested to enhance insulin sensitivity. On the other hand, a chronic increase in IL-6 level is suggested to induce insulin resistance [4].

A single bout of exercise is known to increase the insulin sensitivity of skeletal muscle [11,12]; however, the underlying

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mechanism of this phenomenon has not been fully elucidated. It has been reported that a single bout of exercise transiently increases plasma IL-6 levels in both humans and rodents [13–16]. More detailed analyses showed that IL-6 starts to increase during exercise and returns to a basal level within several hours after the cessation of exercise [17]. Because, as mentioned above, a spike-shaped, short period of increase in IL-6 level might be associated with an increase in insulin sensitivity, enhanced insulin sensitivity after a single bout of exercise may be due to a transient increase in IL-6 level after exercise. However, it remains unknown as to whether IL-6 is required for enhanced muscle insulin sensitivity after a single bout exercise.

Based on this background information, in this study, we elucidated the role of IL-6 on enhanced insulin sensitivity caused by a single bout of exercise.

2. Materials & methods

Animals. Male C57BL6J mice were housed in stainless steel wire cages in a temperature-controlled room under a 12 h light–dark cycle. The animals were provided with standard chow and autoclaved tap water *ad libitum*. All animal experiments in this study were approved by the Animal Experimental Committee of Juntendo University.

Exercise protocol and tissue collection. Two days before the experiment, 8-week-old mice were accustomed to treadmill running at 5–20 m/min for 10 min. For the experiment, mice were subjected to a single bout of treadmill running at 20 m/min with a 10° uphill inclination for 90 min. Twenty-four hours after the exercise, the plantaris muscle was harvested from each mouse, frozen in liquid nitrogen, and stored at −80 °C until analysis or subjected to *ex vivo* incubation, as described below. For the measurement of IL-6 levels in serum, and phosho-Signal Tranducer and Activator of Transcription 3 (STAT3) and total STAT3 detection in muscle, mice were subjected to a single bout of exercise with the same protocol as above. Blood samples (from the inferior vena cava) and the plantaris muscle were collected before exercise, immediately after exercise, and at 1 h, 3 h, and 24 h after exercise (n = 4 for each).

IL-6 neutralization. Thirty to 60 min before a single bout of treadmill running, mice were intravenously administered with 2.5 μg of IL-6 neutralizing antibody (n = 8) or control IgG (n = 8) (R&D Systems, Minneapolis, MN, USA). Mice not subjected to exercise were used as the sedentary group (n = 8).

Recombinant IL-6 injection and skeletal muscle analyses. Several doses of recombinant mouse IL-6 (BD Biosystems, San Jose, CA, USA) were intravenously injected into C57BL6J mice to reach a concentration of 50 pg/mL, 100 pg/mL, 1 ng/mL, 10 ng/mL, and 100 ng/mL (total blood volume estimated as 3.0 mL/mice, n = 4 each). Twenty-four h after the IL-6 injection, soleus and plantaris muscles were harvested from each mouse, frozen in liquid nitrogen, and stored at −80 °C until analysis.

***Ex vivo* incubation and 2-deoxyglucose uptake**. *Ex vivo* incubation and 2-deoxyglucose (DG) uptake measurements were performed as described previously [18].

ELISA for IL-6. IL-6 levels in serum were measured by sandwich ELISA using Mouse IL-6 Quantkine ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

Cell culture. C2C12 myoblasts were grown in 6-well plates until confluence. After reaching confluence, the medium was switched to DMEM containing 2% horse serum (HS) for 6–7 days to induce myoblast to myotube differentiation. Differentiated C2C12 myotubes were treated with 100 pg/mL recombinant IL-6 (BD Biosystems, San Jose, CA, USA). The durations of IL-6 treatment are described below and illustrated in Fig. 4A; 3 h of IL-6 treatment followed by 21 h of incubation in culture medium (2% HS in DMEM), 6 h of IL-6 treatment followed by 18 h of incubation in culture medium, 12 h of IL-6 treatment followed by 12 h of incubation in culture medium, 24 h of IL-6 treatment and 24 h of incubation in culture medium as a control (No treatment, NT). After a total of 24 h of treatment, cells were washed twice with phosphate buffered saline (PBS) and protein extraction was performed as described below.

Western blotting. Skeletal muscles and C2C12 myotubes were homogenized in RIPA buffer (50 mM HEPES [pH 7.5], 150 mM NaCl, 1% NP-40, 0.1% sodium deoxycholate, 1 mM EDTA, 5 mM benzamidine, 10 mM β-glycerophosphate, 1 mM Na_3VO_4, 50 mM NaF, and 1 mM phenylmethylsulfonyl fluoride) containing Halt protease inhibitor cocktail (ThermoFisher Scientific, Rockford, IL, USA). After centrifugation, protein concentrations were measured using the BCA method and lysates were adjusted to 1.0 mg/mL using Laemmli sample buffer, then 10 μg of total protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis transferred to polyvinylidene difluoride membranes (Merck Millipore, Darmstadt, Germany), blocked with Blocking One (Nacalai Tesque, Kyoto, Japan) and incubated overnight with antibodies against phospho-STAT3 (Tyr705), total STAT3, phosoho-Akt (Ser473), phospho-Akt (Thr308), total Akt, GLUT4, or β-actin (Cell Signaling Technology, Danvers, MA, USA) at 4 °C. Membranes were incubated with the appropriate secondary antibody conjugated to horseradish peroxidase and visualized using ECL plus (ThermoFisher Scientific, Rockford, IL, USA) and the LAS3000 imaging system (FUJIFILM, Tokyo, Japan). Visualized protein bands were analyzed using Image J software. All data were expressed as arbitrary units compared with the sedentary group, which was set to 1.0. All analyses were repeated in duplicate (*in vivo* studies) or triplicate (*in vitro* study).

Statistical analysis. Data were analyzed using one-way ANOVA. If ANOVA indicated a significant difference, then the Tukey honestly significant difference was performed to determine the significance of the difference between each of the mean values.

3. Results

3.1. IL-6 neutralizing antibody administration abolishes exercise-induced increase in insulin responsiveness in mouse plantaris muscle

In the present study, we used the same single bout of exercise protocol as our previous study, which enhanced insulin-stimulated glucose uptake in the plantaris muscle 24 h after exercise [18]. Using this exercise protocol, we observed an increase in circulating IL-6 levels, which returned to baseline levels at 24 h after exercise (Fig. 1A).

To test whether this increase in circulating IL-6 levels is associated with the exercise-induced increase in muscle insulin sensitivity, we administered an IL-6 neutralizing antibody or control IgG intravenously 30–60 min before a single bout of treadmill running. An increased phosphorylation level of STAT3, which is a major downstream target of the IL-6 receptor, was observed 1 h after the single bout of exercise, and the enhanced STAT3 phosphorylation was abolished by the administration of an IL-6 neutralizing antibody before the exercise (Fig. 1B). In this setting, consistent with our previous study using the same exercise protocol [18], the single bout of exercise increased insulin-stimulated 2-DG uptake in the control IgG-injected group compared with the sedentary group (Fig. 1C and D). However, administration of the IL-6 neutralizing antibody before exercise canceled this effect (Fig. 1C and D). These results suggested that IL-6 is required for the enhanced insulin sensitivity in the plantaris muscle after a single bout of exercise.

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