

Treadmill exercise promotes interleukin 15 expression in skeletal muscle and interleukin 15 receptor alpha expression in adipose tissue of high-fat diet rats

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Abstract Interleukin 15 (IL-15) has recently been proposed as a myokine involved in regulating lipid metabolism. We investigated the effect of exercise training on IL-15 content in skeletal muscle and expression of IL-15 receptor (IL-15R) in adipose tissue of obese rats. After 12 weeks of a high-fat diet, obese rats underwent treadmill running at 26 m/min (60 min each, 5 days/week for 8 weeks). High-fat diet induced obesity, with increased body weight, body fat, and lipid profile. The level of IL-15 immunoreactivity (IL-15-ir) in plasma and gastrocnemius muscle was lower in obese than control rats, and the mRNA level of IL-15 in gastrocnemius muscle was markedly decreased. The mRNA and protein levels of IL-15R in adipose tissue were markedly lower in obese rats. Compared with sedentary obese rats, treadmill running showed decreased body weight and elevated mRNA expression of IL-15 in muscle and elevated IL-15-ir level in plasma and muscle. The mRNA and protein level of IL-15R were increased in adipose tissue in treadmill running obese rats. Our results showed that exercise training improve obesity and reversed the downregulation of the IL-15 in muscle and IL-15R in adipose tissue induced by high-fat diet.

Keywords Interleukin-15 · Treadmill running · Obesity · Skeletal muscle

Introduction

Interleukin-15 (IL-15), a recently discovered cytokine that belongs to the four α -helix bundle family of cytokines, is widely expressed among tissue and cell types. In addition to lymphoid tissues, IL-15 mRNA is also expressed in cardiac muscle, lung, liver, kidney, brain, pancreas, placenta, and particularly highly expression in skeletal muscle [1]. IL-15 receptor (IL-15R) expresses in various tissue, and is characterized as trimeric in structure, composed of IL-2 receptor β (IL-2R β), the common gamma chain (γ c), and a specific IL-15R α chain [2, 3]. IL-15 is a pleiotropic cytokine that involved in the regulation of energy homeostasis [4–6]. IL-15^{-/-} mice experienced significant weight gain without altering appetite [7]. Systemically administered IL-15 reduces fat deposition in normal and obese rodents by inhibiting lipogenesis in liver and adipose tissue [8–11]. In vitro studies demonstrated that IL-15 can directly inhibit preadipocyte differentiation and lipogenesis, thus stimulating lipolysis [12–15]. Furthermore, Nielsen AR et al. reported that plasma IL-15 and muscle IL-15 mRNA were negatively correlated with obesity parameters in obese human [16]. These findings suggested that IL-15 could be a novel endogenous anti-obesity factor and that IL-15 could be markedly depressed in obesity.

A lot of population-based studies showed that regular physical activity plays an important, perhaps even a dominant role, in obesity [17–20]; however, the specific mechanism of the effects of exercise on weight loss have not been well elucidated. Recently, skeletal muscle has

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been identified as a secretory organ, and cytokines or peptides that are expressed and released by muscle cell have been termed as myokines, which could be novel mediators of the multiple health benefits of exercise [21]. In obesity, myokines, such as IL-6, tumor necrosis factor- α (TNF- α), myostatin, and brain-derived neurotrophic factor, were disturbed in skeletal muscle [22]. Contracting skeletal muscle during exercise can regulate the expression and secretion of myokines, such as IL-6 and myostatin, which involve in the weight loss in response to exercise [22]. Several lines of evidence demonstrated that IL-15 is highly expressed in skeletal muscle and functioned as a myokine [23], by muscle-to-fat axes [23], reducing adipose tissue weight [8]. However, the effect of exercise training on IL-15 expression in skeletal muscle and IL-15R α expression in adipose tissue has not been adequately investigated in obese rats. We hypothesized that exercise training can restore the depression of skeletal muscle IL-15 expression and adipose tissue IL-15R α expression in obesity. In this study, we wanted to observe the alteration in IL-15 expression in skeletal muscle, and IL-15R expression in adipose tissue during treadmill running in obese rats induced by a high-fat diet.

Materials and methods

Animals and reagents

All animal care and experimental protocols complied with the Animal Management Rules (document No. 55, 2001) of the Ministry of Health of the People's Republic of China and the guide for the Care and Use of the Laboratory Animals of Peking University Health Science Center. The 5-week-old male Sprague–Dawley (SD) rats were provided by the Animal Department, Peking University Health Science Center. The animals were housed in pairs, on a 12:12 h light–dark cycle in a temperature-controlled room at 22 ± 2 °C.

Cholesterol and aprotinin were from Sigma (St. Louis, MO, USA). IL-15 radioimmunoassay kits were from Beijing Sino-UK Institute of Biological Technology. Assay kits for total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were from Biosino Bio-technology and Science (Beijing). Antibody against IL-15R α (sc-9172), β -actin (sc-47778), and all secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Nitrocellulose membrane was from Hybond-C (Amersham Life Science, England), and the enhanced chemiluminescence kit was from Beijing Applygen Technologies. Other chemicals and reagents were of analytical grade.

Preparation of animal model

We randomly assigned 5-week-old male SD rats to 4 groups ($n = 8$ each) for treatment: (1) control, normal diet (normal diet: 14,610 kJ/kg, energy content in %: carbohydrate: 66.50; fat: 10.21; protein: 23.29); (2) control + treadmill running, normal diet and treadmill running progressively increased to reach 60 min at 26 m/min (5 days/week for 8 weeks) [24], and all training sessions took place during the afternoon (3:00–6:00 p.m.); (3) high-fat diet (HFD; 19,315 kJ/kg), 200 g fat/kg (170 g lard + 30 g corn oil to provide essential fatty acids) and 1 % cholesterol by weight plus normal drinking water [25] (The HFD was formulated to provide 40 % of total energy from fat by replacing carbohydrate energy with lard and corn oil and had the same amount of vitamins and minerals per kilojoule as the normal diet); and (4) HFD + treadmill running, training beginning 12 weeks after introducing the HFD.

At the end of the dietary and exercise protocol, rats fasted overnight but had free access to water and were anesthetized with use of urethane (1 g/kg, intraperitoneally). Blood was drawn from the abdominal aorta into syringes pretreated with EDTA (15 %), then centrifuged (3,000 rpm for 8 min, 4 °C), and plasma was kept for further analysis. Soleus and gastrocnemius muscle and visceral and subcutaneous fat were excised and weighed. All tissue samples were frozen in liquid nitrogen. Subcutaneous adipose tissue was removed from the inguinal region and visceral adipose tissue from the perirenal and epididymal fat depots. Plasma TC, TG, HDL-C, and LDL-C were measured by colorimetric enzymatic assays.

Radioimmunoassay of IL-15 level in muscle

Excised soleus and white gastrocnemius muscle were immediately acidified with 1.0 mol/L acetic acid and then heated at 100 °C for 10 min to inactivate proteases. The plasma samples were pre-treated with aprotinin (500 KIU/mL). Plasma or the supernatant extracted from skeletal muscle subjected to radioimmunoassay. The range of the standard curve was 1.02–16.3 pg/mL, in which a double-logarithmic scale showed a linear relationship with $r^2 = 0.999$. All samples were within the range of the standard curve. The detection limit was calculated to be 2.14 pg/mL. The intra- and interassay coefficients of variation were validated within our work and were 6.1 and 10.2 %, respectively. There is no cross-reactivity found with rat IL-2, IL-6, or tumor necrosis factor α (TNF- α).

Real-time PCR analysis

Total RNA from soleus and white gastrocnemius muscle and epididymal adipose tissue was isolated and reverse

transcribed by use of a reverse-transcription system (Promega, Madison, WI, USA). In total, 20 μ L of the reaction mixture underwent real-time PCR and then evaluated by SYBR Green I fluorescence. The rat primers were for IL-15: forward, 5'-CTT CTT AAC TGA GGC TGG-3' and reverse, 5'-GCA ACT GGG ATG AAA GTC-3'; IL-15R α : forward, 5'-GAA AGC AGG CAC ATC CAC-3' and reverse, 3'-GAG GCT CTC TGG TTG TGA G-5'; and β -actin: forward, 5'-GAG ACC TTC AAC ACC CCA GC C-3' and reverse, 5'-TCG GGG CAT CGG AAC CGC TCA-3'. All amplification reactions involved use of the M \times 3000 Multiplex Quantitative PCR System (Stratagene, La Jolla, CA, USA). After being denatured at 95 $^{\circ}$ C for 7 min, the solution underwent PCR for IL-15 at 95 $^{\circ}$ C for 30 s, 60 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 40 s for 43 cycles and for IL-15R at 95 $^{\circ}$ C for 30 s, 54 $^{\circ}$ C for 30 s, and 72 $^{\circ}$ C for 40 s for 45 cycles.

Western blot analysis of adipose tissue

Epididymal adipose tissue was homogenized in lysis buffer [0.1 mol/L NaCl, 0.01 mol/L Tris-HCl (pH 7.5), 1 mmol/L EDTA, and 500 KIU/mL aprotinin]. Equal amounts of protein samples were loaded on 10 or 12 % SDS-PAGE and then transferred to a nitrocellulose membrane, for blocking with 5 % nonfat dried milk for 1 h, and then incubated with the primary antibodies anti-IL-15R α (1:600) or anti- β -actin (1:2,000) overnight at 4 $^{\circ}$ C, then secondary antibody (anti-goat or anti-rabbit IgG conjugated to horseradish peroxidase) for 1 h [26]. Protein expression was analyzed by use of NIH image software (Bethesda, Maryland, USA) and normalized to β -actin expression. All experiments were repeated at least three times.

Statistical analysis

Data are shown as mean \pm SEM. A repeated measures ANOVA model was used to examine the effects of diet, exercise, time, and their interaction on body weight during 12–20 weeks. Significance of dietary fat content and exercise was determined by two-way ANOVA and Bonferroni post-tests. The differences between groups were considered significant when $P < 0.05$.

Results

Treadmill running exercise attenuates obesity induced by a HFD

Treadmill running significantly decreased body weight ($P < 0.01$) (Fig. 1) and the ratios of visceral fat to body

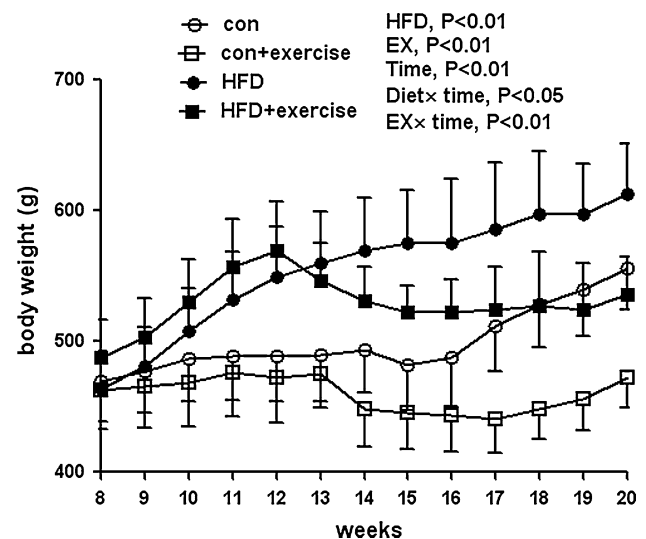


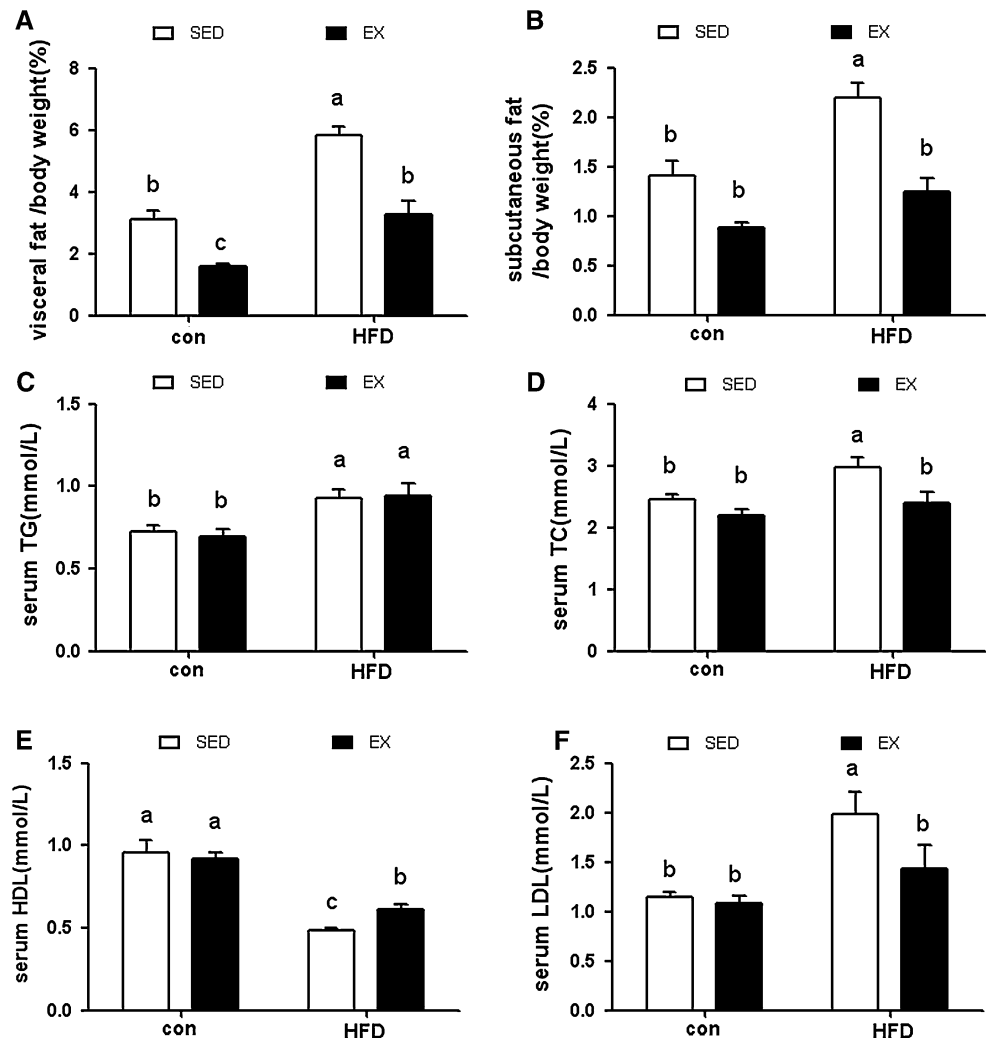
Fig. 1 Treadmill running attenuates high-fat-diet (HFD)-induced body weight. Data are mean \pm SEM, $n = 8$. A repeated measures ANOVA model was used to examine the effects of diet, exercise, time, and their interaction on body weight during 12–20 weeks. EX, exercise; SED, sedentary

weight of normal diet rats compared to the control rats ($P < 0.01$) (Fig. 2a, b). No significant difference was observed in subcutaneous fat to body weight and lipid profile between control and exercise alone groups ($P > 0.05$) (Fig. 2c–f). Body weight was significantly heavier for rats fed a HFD than control rats ($P < 0.01$) (Fig. 1). Twelve weeks after introducing the HFD, rats underwent 8 weeks of treadmill running, which significantly decreased the body weight of the HFD rats by 13 % ($P < 0.01$) (Fig. 1) and ratios of visceral and subcutaneous fat to body weight, by 40 and 44 % (all $P < 0.01$), respectively (Fig. 2a, b), compared to rats fed a HFD alone. Plasma TC, TG, and LDL-C concentrations were greater by 21 % ($P < 0.05$), 29 % ($P < 0.01$), and 73 % ($P < 0.01$), respectively, and HDL-C concentration was lower by 50 % ($P < 0.01$) in rats fed a HFD alone than control rats (Fig. 2c–f). Exercise lowered the plasma TC and LDL-C level, by 20 and 28 %, respectively, and increased HDL-C level by 28 % (all $P < 0.05$) but had no effect on plasma TG concentration ($P > 0.05$; Fig. 2c–f). Overall, exercise training prevented the effects of the HFD on body weight, body total fat, and lipid profile.

Treadmill running increased IL-15 immunoreactivity (IL-15-ir) and IL-15 mRNA level

Compared with the control group, treadmill running alone increased IL-15-ir and IL-15 mRNA level in soleus and gastrocnemius muscle by 27 % ($P < 0.01$), 26 % ($P < 0.05$) and 59 %, 65 % (all $P < 0.01$), respectively, with no significant changes in plasma IL-15-ir (Fig. 3a–e). HFD decreased IL-15-ir in plasma and gastrocnemius

Fig. 2 Treadmill running improves serum lipid profile in high-fat diet (HFD) rats. **a–b** Ratio of visceral fat to body weight and subcutaneous fat to body weight. **c–f** Serum levels of cholesterol (TC), TG, HDL-C, and LDL-C. Data are mean \pm SEM, $n = 8$. Different letters indicate groups differed significantly ($P < 0.05$) by two-way ANOVA. EX, exercise; SED, sedentary



muscle significantly, by 15 % ($P < 0.01$) and 26 % ($P < 0.05$), respectively, and reduced IL-15 mRNA level in gastrocnemius muscle by 40 % ($P < 0.05$), with no difference in soleus muscle (Fig. 3a–e). Compared with HFD treatment alone, treadmill running markedly elevated IL-15-ir in plasma and soleus and gastrocnemius muscle by 16 % ($P < 0.05$), 26 % ($P < 0.01$), and 47 % ($P < 0.01$), respectively, and increased IL-15 mRNA level in soleus and gastrocnemius muscle by 56 % ($P < 0.05$) and 138 % ($P < 0.01$), respectively (Fig. 3a–e).

Treadmill running increased adipose tissue IL-15R α mRNA and protein expression

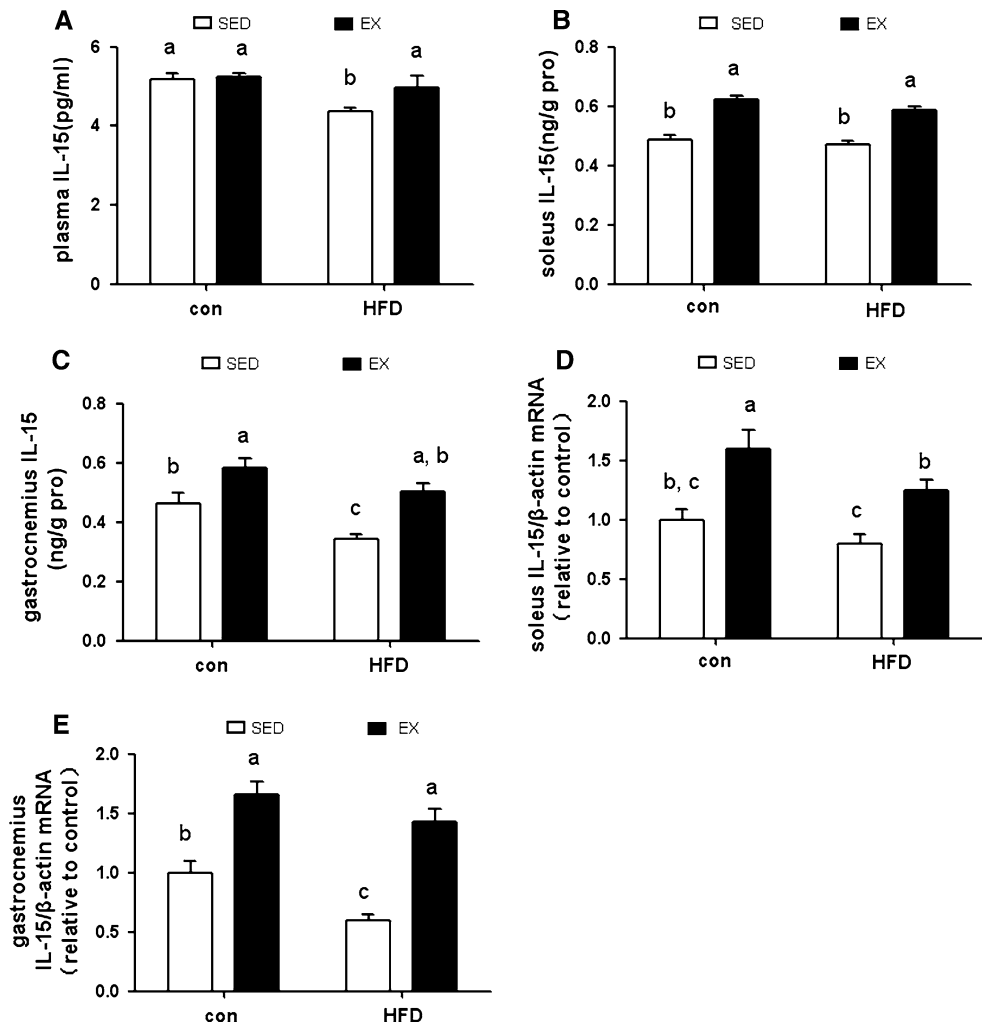
Transcripts for IL-15R α have wide tissue distribution, and adipose tissue contains an abundance of IL-15R α mRNA [9]. Compared with control rats, normal rats running the

treadmill showed increased mRNA and protein levels of IL-15R α by 28 % ($P < 0.05$) and 45 % ($P < 0.01$), respectively, in adipose tissue, which was decreased in HFD rats by 58 and 56 % (all $P < 0.01$) (Fig. 4a–c), respectively. Treadmill running ameliorated the reduced mRNA and protein levels of IL-15R α in HFD rats by 148 % ($P < 0.01$) and 70 % ($P < 0.05$) (Fig. 3a–c), respectively.

Discussion

This study was undertaken to determine if exercise stimulated the skeletal muscle IL-15 expression and adipose tissue IL-15R α expression in obesity. In this study, plasma IL-15 content and gastrocnemius IL-15 mRNA expression and content were lower in HFD rats, however, no

Fig. 3 Treadmill running increased IL-15 immunoreactivity (IL-15-ir) and IL-15 mRNA level. **a–c** IL-15-ir content in plasma and soleus and gastrocnemius muscle. Data are mean \pm SEM, $n = 6$. **d–e** Real-time PCR analysis of mRNA level of IL-15 in soleus and gastrocnemius muscle. Data are mean \pm SEM, $n = 3$. Different letters indicate groups which differed significantly ($P < 0.05$) by two-way ANOVA. HFD, high-fat diet; EX, exercise; SED, sedentary



difference in soleus. Exercise training increased soleus and gastrocnemius IL-15 mRNA expression and content not only in HFD rats, but also in normal diet rats. Additionally, treadmill running restored the adipose tissue IL-15 $R\alpha$ mRNA and protein expression in HFD rats. Therefore, our data indicated that exercise improved obesity and reversed the downregulation of the IL-15 in muscle and IL-15R in adipose tissue induced by HFD.

Skeletal muscle behaving as an endocrine organ, express several myokines and has paracrine or endocrine effects, which involved in metabolism regulation. IL-15 was the most highly expressed of all cytokines measured at the mRNA level in human skeletal muscle [27]. Biologically active IL-15 has been detected in primary human myoblast and cultures supernatant [28], IL-15 has been regarded as a myokine [23]. In obese human, plasma IL-15 and vastus lateralis muscle IL-15 mRNA were found negatively related to obesity parameters [16]. In this work, we found that plasma and muscle IL-15

content in gastrocnemius decreased in HFD rats, however, no difference in soleus. Furthermore, we also found that muscle IL-15 mRNA was downregulated in obese rats and no significant difference was observed in soleus. These data indicated that IL-15 expression in rats is regulated in a muscle fiber type-dependent manner.

There are inconsistent findings from previous studies as to the effects of exercise on IL-15 expression in skeletal muscle and plasma IL-15 content [29]. However, most subjects of previous studies were normal individuals, and changes in IL-15 level in obese subjects after exercise were unclear. In the current study, exercise training markedly increased IL-15-ir content and mRNA expression in soleus and gastrocnemius muscle in chow-fed and HFD rats. Furthermore, two-way ANOVA tests demonstrated that there was no significant interaction between exercise and diet, which indicated that exercise itself can significantly affect the skeletal muscle IL-15 expression independently of weight loss. In contrast,

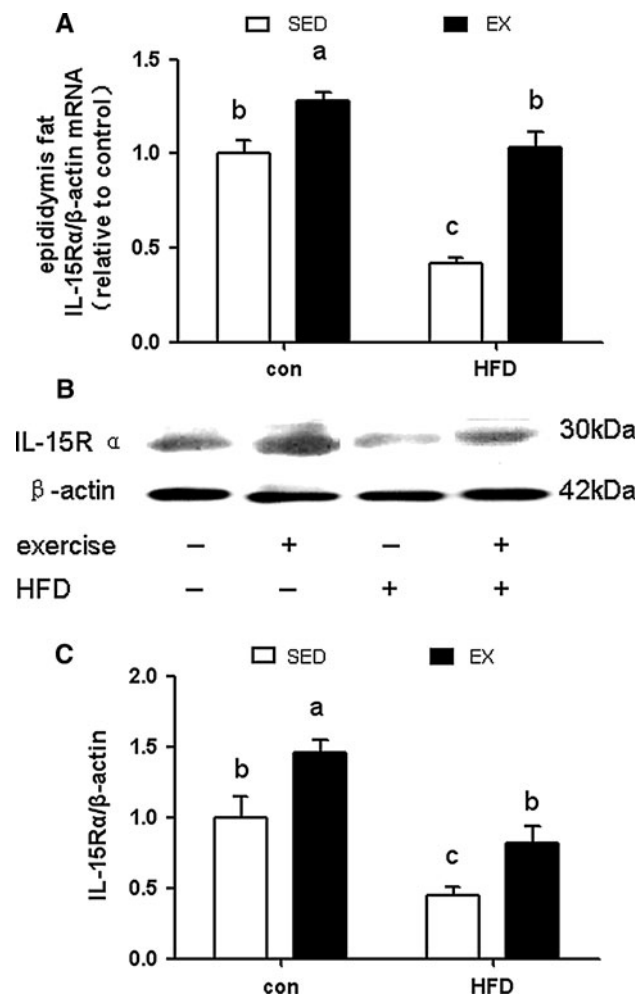


Fig. 4 Treadmill running increased IL-15 receptor α (IL-15R α) mRNA and protein expression in adipose tissue. **a** Real-time PCR analysis of mRNA level of IL-15R α . **b** Western blot analysis of protein level of IL-15R α and **c** quantification of IL-15R α . Data are mean \pm SEM, $n = 3$. Different letters indicate groups differed significantly ($P < 0.05$) by two-way ANOVA. HFD, high-fat diet; EX, exercise; SED, sedentary

Christiansen et al. reported that exercise alone had no effect on muscle IL-15 expression in obese subjects who undertook a program of exercise, very low energy diet, or the combination of diet and exercise [30]. The difference of diet regimen and exercise regimen may result in the discrepancy. Additionally, we found that treadmill running increased plasma IL-15 level in HFD rats, but no change in normal diet rats. Studies of Tamura et al. [31] and Richman et al. [32] reported that serum IL-15 content was increased immediately following acute exercise. In our work, the plasma was sampled at the next day morning of the end of exercise protocol, rather than immediately after exercise, which hinted that chronic exercise may not affect the circulating IL-15 level. Alternatively, IL-15 forms functional complexes with soluble high affinity IL-15R α in serum, which will interfere with the detection of IL-15 [33]. Therefore, the

exact role of exercise on circulating IL-15 content still awaits further research.

IL-15 receptor (IL-15R) is a heterotrimeric receptor comprising IL-15R α plus IL-2R β and IL-2R γ , and all three subunits were expressed in adipose tissue [9]. However, little or no IL-15 mRNA has been detected in undifferentiated preadipocytes or differentiated adipocytes [23]. Quinn et al. reported that IL-15 functions in a muscle-to-fat endocrine axis and has powerful actions in the regulation of fat-lean body composition [34]. However, the effect of HFD and exercise training on adipose tissue IL-15R expression is yet unknown. So we detected the IL-15R expression in adipose tissue, rather than other tissue. Whether other tissues contribute to adipose tissue reduction need to be further investigated. In our work, we found that the expression of IL-15R α in adipose tissue decreased in obese rats. IL-15R α binding with its unique high affinity ligand IL-15, functions primarily to confer high affinity binding to the receptor β and γ_c subunits, which are responsible for signal transduction [29, 35]. Therefore, the decreased protein level of IL-15R α may blunt the sensitivity of adipose tissue to IL-15. Treadmill running stimulated the IL-15R α expression in normal diet rats and restored the IL-15R α expression in HFD rats; however, the regulation of IL-15R α was still unknown. Our data showed that both exercise and diet significantly affected the adipose tissue IL-15R α expression, which indicated that exercise itself and exercise-induced adipose tissue reducing involved in the regulation of the adipose tissue IL-15R α expression.

In summary, we showed that long-term treadmill running improved obesity and reversed the downregulation of the IL-15 in muscle and IL-15R in adipose tissue induced by high-fat diet.

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Ethical standards All animal care and experimental protocols complied with the Animal Management Rules (document No. 55, 2001) of the Ministry of Health of the People's Republic of China and the guide for the Care and Use of the Laboratory Animals of Peking University Health Science Center.

Conflict of interest The authors declare that they have no conflict of interest.

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