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The effect of exercise intensity on plasma and tissue acyl ghrelin concentrations in fasted rats

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ABSTRACT

Objective: This study was conducted to investigate the effect of exercise training and feeding status on plasma and tissue acyl ghrelin concentrations.
Materials and methods: Thirty-two, eight-week-old male Wistar rats (185 ± 5 g) were randomly assigned to one of four groups: high intensity (HI: 34 m/min ~80–85% VO_2max), moderate intensity (MI: 28 m/min ~70–75% VO_2max), low intensity (LI: 20 m/min ~50–55% VO_2max), and sedentary control (SED) groups. All experimental groups performed a 12 week exercise program consisting of treadmill running on a 0° slope for 1 h/day, 5 days/week at their respective training intensity. Twenty four hours following the last training session the animals completed a 12 h fast. Rats were then killed, blood was collected and plasma separated; the fundus and soleus muscle were excised and frozen in liquid nitrogen for later analysis. Fasting levels of circulating acyl ghrelin and acyl ghrelin content in the soleus muscle and fundus, as well as glycogen in the soleus muscle were measured. Data were analyzed using one-way ANOVA.
Results: Results demonstrated that 12 weeks of exercise training combined with a 12 h fast significantly increased plasma as well as soleus muscle concentrations of acyl ghrelin in the HI and MI groups ($p<0.05$) and reduced acyl ghrelin concentrations in the fundus ($p<0.05$).
Conclusion: The results of the study indicate that chronic treadmill exercise training enhances fasting plasma acyl ghrelin in an intensity-dependent manner which is accompanied by a significant increase in soleus muscle and reduction in fundus acyl ghrelin levels.

1. Introduction

Acyl ghrelin is a 28-amino acid peptide that was isolated from the rat stomach in 1999 [1], and is a ligand for the growth hormone secretogogue receptor, GHS-R1a [2]. Acyl ghrelin [1] is also recognized as a novel player in the gut–brain regulation of energy balance [3]. The stomach is a major source of circulating ghrelin in humans [4] and in addition to being a growth hormone (GH) secretagoue, it stimulates an increase in blood glucose [5–7]. Ghrelin is present in two major forms including acyl ghrelin and des-acyl ghrelin, which lacks the O-n-octanoylation at serine 3, a critical modification for its effect on appetite [8]. Acyl ghrelin has a potent effect on eating behavior, causing an increase in hunger [2,4] and plays a key role in the central regulation of feeding and energy balance [3]. It has been demonstrated that both short and long-term injection of central and peripheral acyl ghrelin produces hyperphagia in rats and stimulates hunger in normal-weight humans [9–11]. Interestingly, total plasma ghrelin levels are significantly changed during acute and chronic alteration of nutritional status with low levels in simple obesity but higher levels after weight loss [12,13]. Moreover, ghrelin is up-regulated by fasting, insulin-induced hypoglycemia, and leptin administration [14,15].

The effect of physical exercise on total plasma ghrelin has been investigated in humans [16–19], horses [20,21], and rats [22,23]. To date, most investigations have focused on plasma total ghrelin responses to acute exercise (short-term, or long-term, resistance or aerobic exercise), but some investigations have determined effects of chronic exercise training on circulating total ghrelin concentrations. Previous studies from the investigators' lab and others have reported no change [e.g. 24,25]or reductions [26,27] in total ghrelin in response to exercise in healthy adults.

Foster-Schubert et al. [19] studied the effects of aerobic exercise for 12 months on 173 sedentary, overweight, postmenopausal women. They reported that total plasma ghrelin increased by 18% in exercisers who lost more than 3 kg of body weight. However, Morpurgo et al. [28] did not find any effect on fasting and non-fasting total ghrelin concentrations after 3 weeks of exercise training that

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reduced body weight in obese men and women. Ebal et al. [23] investigated the effects of 5 weeks of a force-resistance exercise on plasma ghrelin concentrations in rats and reported training-induced reductions in ghrelin concentrations that corresponded with 6.4% reduction in body weight. Recently our lab has reported that 6 weeks of exercise training at a low to moderate exercise intensity resulted in reduced total ghrelin levels in rat plasma and soleus muscle [29]. Animals were in a 4 h postprandial state when tissues were collected.

Regarding the fundus of the stomach, acyl ghrelin release from this tissue increases feeding behavior by stimulating NPY/AGRP-containing neurons in the arcuate nucleus of the hypothalamus [30]. Moreover, there are some data that suggest under certain conditions, exercise may suppress circulating ghrelin levels which could decrease feeding behavior [31,32]. However, acyl ghrelin, the form of ghrelin that stimulates appetite, has been investigated under acute, but not chronic exercise conditions. Since greater volume of training has a greater effect on tissues and body weight, one of the aims of the present study was to determine whether low, moderate, and high intensity/volume training affected plasma acyl ghrelin concentrations differently in a fasted state.

Recent evidence suggests that skeletal muscle is an important site of ghrelin action. Ghrelin and GHS-R have been found in skeletal muscle [29,33] and it has been shown that growth hormone secretagogues can alter both electrical and contractile activity in skeletal muscle [34]. Moreover, GHS receptors have been localized in skeletal muscle [35]. Collectively, these studies suggest that the fundus and skeletal muscle are important sites of ghrelin action. Thus, alterations of skeletal muscle and fundus acyl ghrelin after exercise training could be important and informative. Therefore, the second aim of this study was to address the question regarding what changes in tissue acyl ghrelin levels can be observed as a result of 12 week exercise training. Thus, we conducted this study to examine the effect of three different exercise training intensities on fasting plasma, soleus muscle and fundus acyl ghrelin concentrations. We chose the soleus muscle for several reasons. First, the soleus muscle is a predominantly slow twitch muscle that has served as a useful tissue to study glycogen depletion/resynthesis in previous investigations [36]. Second, most of its muscle fibers would be stressed (recruited) in our animal model experiment for all 3 exercise intensities (low, moderate and high intensities). Moreover, previous data from our revealed alterations in total ghrelin content following 6 weeks of running [29] and thus determination of the acyl form could be very important.

2. Materials and methods

2.1. Animals

All experiments involving animals were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Ethics Committee of the School of Medical Sciences, Tarbiat Modares University (TMU), Tehran.

Thirty-two, eight-week-old male Wistar rats (185 ± 5 g) were randomly assigned to one of four groups as follows: 1) sedentary control (SED), 2) low intensity training (LI: 20 m/min ~50–55% VO_2max), 3) moderate intensity training (MI: 28 m/min ~70–75% VO_2max), and 4) high intensity training (HI: 34 m/min ~80–85% VO_2max). These treadmill running speeds have been used previously to elicit the respective low, moderate and high intensity %VO_2max values for Wistar rats [37,38] and previous data from our lab suggests that training 60 min/day, 5 days/week, 25 m/min for 6 weeks affected total ghrelin levels in rat soleus muscle [29]. Thus we utilized a reasonable training time period to produce expected acyl ghrelin adaptations. Groups of four rats were housed in cages under controlled light/dark (12/12 h) and temperature (22 ± 1 °C) conditions, and were provided with food and water ad libitum. Animals were familiarized to laboratory conditions for 1 week before experiments began.

2.2. Exercise training protocol

The training groups exercised on a rodent motor-driven treadmill on a 0° slope, 60 min/day, 5 days/week for 12 weeks. During the 1st week of training the rats ran at a treadmill speed of 10 m/min for 15 min. During the 2nd and 3rd week of training the treadmill speed and exercise duration gradually increased until the animals were running for 60 min/day at the prescribed speed depending upon the group assignment (low intensity: 20 m/min, ~50–55% VO_2max; moderate intensity: 28 m/min ~70–75% VO_2max; high intensity:34 m/min ~80–85% VO_2max). The treadmill speed and exercise duration were then held constant for the remainder of the training period.

2.3. Blood collection and tissue preparation

After a 12-h fast and 36 h after the last training session to minimize the effect of acute exercise, the rats were terminally anesthetized with a mixture Ketamine™ (30–50 mg/kg body wt, ip) and Xylazine (3–5 mg/kg body wt, ip), after which their blood was harvested from the abdominal aorta. Blood was collected in tubes that contained both EDTA as well as p-hydroxymercuribenzoic acid (1 mM in the final sample volume). The PHMB was prepared in a 100 mM concentration in potassium phosphate buffer containing 1.2% NaOH (10 N) and then adding 10 μL of this solution for each mL of blood. The samples were centrifuged at 3500 rpm for 10 min at 4 °C and supernatants were transferred into separate tubes. 100 μL of 1 N HCl was added per mL of plasma and centrifuged at 3500 rpm for 5 min at 4 °C then supernatants were transferred into separate tubes and quickly stored at −80 °C. The rat soleus muscle and fundus were quickly collected and frozen by immersion in liquid nitrogen and then stored at −80 °C until analyzed. Tissue samples were homogenized in five volumes of buffer containing 0.9% NaCl, 50 mM Tris–HCl, 12 μM leupeptin, using a Potter–Elvejheim homogenizer set at 800 rpm and cooled in ice. The homogenates were centrifuged at 9000 rpm for 10 min at 4 °C. Supernatants were collected and analyzed.

2.4. Measurements

Plasma and tissue acyl ghrelin concentrations were assayed according to the manufacturers' instruction using rat ELISA kits specific for determinations of acyl ghrelin (SPI BIO, Montigny le Bretonneux, France) using a plate reader (Opsys Microplate Reader, Dynex Technologies, Franklin, MA). Soleus muscle glycogen was determined by a commercial kit (Glycogen Colorimetric kit, Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma glucose was determined by an enzymatic (GOD–PAP, Glucose Oxidase–Amino Antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran); the intra-assay coefficient of variation and sensitivity of the method were 1.3% and 1 mg/dL, respectively. Rat plasma insulin was determined by an ELISA method (Mercodia AB, Uppsala, Sweden), with an intra-assay coefficient of variation and sensitivity of 4.1% and 0.07 μg/L, respectively.

2.5. Statistical analysis

The Kolmogorov–Smirnov test was used to determine the normality of distribution, and variables were found to be normally distributed. A one-way analysis of variance (ANOVA) was performed to determine whether variable differences existed among four groups. Significant differences were identified using a least significant difference post hoc test. All data are expressed as mean ± SD and significance was set at the alpha level of $p<0.05$.