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Treadmill training enhances rat agouti-related protein in plasma and reduces ghrelin levels in plasma and soleus muscle

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Abstract

Ghrelin and agouti-related protein (AgRP) are orexigenic peptides secreted from stomach mucosa and the arcuate nucleus of the hypothalamus, respectively. Both peptides affect feeding behavior and play a role in energy balance, glucose homeostasis, and adiposity. The purpose of the current study was to determine the effects of moderate-term (6 weeks) running regimen on resting levels of ghrelin, AgRP, adenosine triphosphate, and glycogen in soleus muscle as well as plasma concentrations of the orexigenic hormones. Eighteen adult Wistar male rats (12 weeks old, 235-255 g) were randomly assigned to training (n = 10) and control (n = 8) groups. The training group ran for 60 min/d, 5d/wk at 25 m/min and 0% grade for 6 weeks. Forty-eight hours after the last exercise session, rats were killed; and soleus muscle and plasma were collected and frozen in liquid nitrogen for later analysis. Results demonstrated that 6 weeks of treadmill exercise reduced ghrelin and increased AgRP levels in plasma. Trained rat soleus muscle had higher levels of glycogen but not adenosine triphosphate or AgRP compared with untrained controls. Data indicate that training lowers ghrelin levels in rat soleus and plasma, which is accompanied by higher plasma AgRP and soleus glycogen content.

1. Introduction

Ghrelin and agouti-related protein (AgRP) are signaling peptides that affect feeding behavior, energy homeostasis, and adiposity [1,2]. Ghrelin is primarily secreted from stomach mucosa and AgRP from hypothalamic neuropeptide Y containing neurons of the arcuate nucleus (ARC) [3,4]. Both peptides are also expressed by extrahypothalamic [3-7] and extragastric [6-8] tissues. Ghrelin and AgRP are up-regulated by fasting, insulin-induced hypoglycemia, and leptin administration [8-10]. Moreover, ghrelin is involved in the regulation of feeding behavior through activation of both neuropeptide Y and AgRP neurons [11,12].

It has been proposed that AgRP uptake by peripheral tissues (including liver, heart, lungs, and skeletal muscle) could be regulated by tissue, important for tissue signaling, and altered in response to fasting [6]. Moreover, one investigation reported that, after the expression plasmid of the AgRP gene driven by mouse β-actin, pActAgRP, was electroporated (transferred) into leg muscle of the mouse, body weight subsequently increased [13].Furthermore, daily food intake significantly increased in these mice. In addition, it has been shown that administration of ghrelin increases glucose transporter 4 expression in soleus muscle [4] and has a positive effect on insulin sensitivity. Thus, AgRP and ghrelin may be important for muscle function.

All experiments involving the 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, Tehran, Iran.

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The effects of different forms of physical exercise on plasma and brain total and acylated ghrelin in humans [14-23] and rats [24-28] have been studied by several investigators, with some conflicting findings. Both no change [19] and reductions [18,20,21,28] in plasma total ghrelin levels have been documented in response to acute exercise; however, continuous-exercise (training) studies have revealed significant increases in women who lost weight [22], no change in obese subjects [29], and reductions in rats who lost weight [26]. Data regarding AgRP and exercise are sparse, with only 1 study documenting acute increases in plasma AgRP concentrations after circuit training [16]. Thus, this study was conducted to investigate the effect of a 6-week treadmill exercise training regimen on soleus muscle and plasma ghrelin and AgRP concentrations. Because AgRP is up-regulated by fasting and insulin-induced hypoglycemia, a second aim was to determine whether changes in plasma and soleus muscle ghrelin and AgRP are accompanied by changes in soleus muscle glycogen and adenosine triphosphate (ATP) content. It was hypothesized that exercise training would elicit greater skeletal muscle glycogen and ATP content that would be associated with lower plasma and soleus muscle ghrelin and AgRP concentrations.

2. Material and methods

2.1. Animals

All experiments involving the 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, Iran. Eighteen Wistar male rats (12 weeks old, 235-255 g) were acquired from Pasteur's Institute (Tehran, Iran) and maintained in the Central Animal House, School of Medical Sciences, TMU. The animals were housed 5 per cage (46-L volume) with a 12-hour:12-hour light-dark cycle. Temperature and humidity were maintained at 22°C ± 1.4°C and 55.6% ± 4.0%, respectively. Animals were provided food (a pellet rodent diet) and water ad libitum. Animals were randomly assigned into control (n = 8) and training (n = 10) groups. The control group remained sedentary, whereas the training group underwent a moderate running exercise program.

2.2. Exercise training protocol

First, the animals were familiarized with the rat treadmill apparatus each day for 4 days (the 14-lane motorized-driven treadmill was designed by the primary author; TMU, Tehran, Iran). The exercise group was trained for 6 weeks using the same training methods previously described [30,31]. The rats were run at 25 m/min for 60 minutes, 5 d/wk. The animals were killed 48 hours after the last exercise session. Food but not water was removed from the rat cages 4 hours before the experiment.

2.3. Tissue biopsies and blood samples

Forty-eight hours after the last training session (sixth week), rats were anesthetized with intraperitoneal administration of a mixture of ketamine (30-50 mg/kg body weight) and xylazine (3-5 mg/kg body weight). Afterward, soleus muscles were excised, cleaned, divided into 2 pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen for determination of soleus ghrelin, AgRP, ATP, and glycogen concentrations. Frozen soleus muscle was stored at −80°C until analyses were performed. Blood was collected directly from the heart into test tubes containing EDTA. Plasma samples were separated via centrifugation and then stored at −80°C until biochemical analyses were performed.

2.4. Soleus ghrelin, AgRP, ATP, and glycogen; plasma ghrelin and AgRP concentrations

Soleus and plasma total ghrelin levels were determined by a rat enzyme immunoassay method (SPIbio, Montigny le Bretonneux, France). The intraassay coefficient of variation and sensitivity of the method were 5.9% and 4 pg/mL, respectively. Soleus and plasma AgRP levels were determined by an enzyme immunoassay method (R&D Systems, Minneapolis, MN). Sensitivity was 0.68 pg/mL, and intraassay coefficient of variation was 3.4%. Soleus ATP concentration was determined using an ATP-sensitive bioluminescence kit (Bioaffin GmBH & Co KG, Kassel, Germany), and glycogen was determined via a commercial kit (glycogen colorimetric kit; Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5. Plasma glucose, insulin, and cortisol concentration

Plasma glucose was determined by an enzymatic, colorimetric method (glucose oxidase–amino antipyrine [GOD-PAP]; Pars Azmoun, Tehran, Iran); and the intraassay coefficient of variation and sensitivity of the method were 1.3% and 1 mg/dL, respectively. Plasma insulin was determined by enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden); and the intraassay coefficient of variation and sensitivity were 4.1% and 0.07 μg/L, respectively. Plasma cortisol was determined by enzyme-linked immunosorbent assay (Diagnostic Biochem Canada, London, Ontario, Canada); and the intraassay coefficient of variation and sensitivity were 7.6% and 0.4 μg/dL, respectively.

2.6. Statistics

All results are expressed as means ± SEM. All variables were compared using unpaired *t* tests. Correlations were calculated using a Pearson product moment correlation. All statistical analyses were performed using SPSS (Version 13; SPSS, Chicago, IL). All P values < .05 were considered significant.

3. Results

3.1. Soleus, ghrelin, AgRP, ATP, and glycogen concentrations

No significant differences between trained and control animals were found for body weight, plasma glucose, insulin, cortisol, and soleus ATP concentrations (Table 1). Data revealed that total ghrelin concentrations in the soleus muscle were significantly lower in trained rats vs controls (87.1 ± 3.4 compared with 70.2 ± 4.28 pg/mL, $P < .01$). In addition, total ghrelin levels in plasma were lower in trained compared with control animals (26.21 ± 1.21 vs 22.27 ± 1.35 pg/mL, $P < .045$) (Fig. 1). Resting plasma total AgRP concentrations were significantly higher in trained compared with control animals (88.15 ± 3.66 to 155.8 ± 24.4 pg/mL, $P < .024$), but higher total AgRP concentrations in trained compared with untrained rat soleus muscle were not significantly different ($P < .16$) (Fig. 2). Greater glycogen concentrations were found in trained rat soleus muscle when compared with control animals (5.0 ± 0.41 vs 3.55 ± 0.51 mg/g) (Fig. 2). There were significant inverse correlations between plasma ghrelin and plasma AgRP concentrations as well as between plasma ghrelin and soleus glycogen concentrations ($r = -0.613$, $P < .009$ and $r = -0.472$, $P < .05$, respectively) (Table 2).

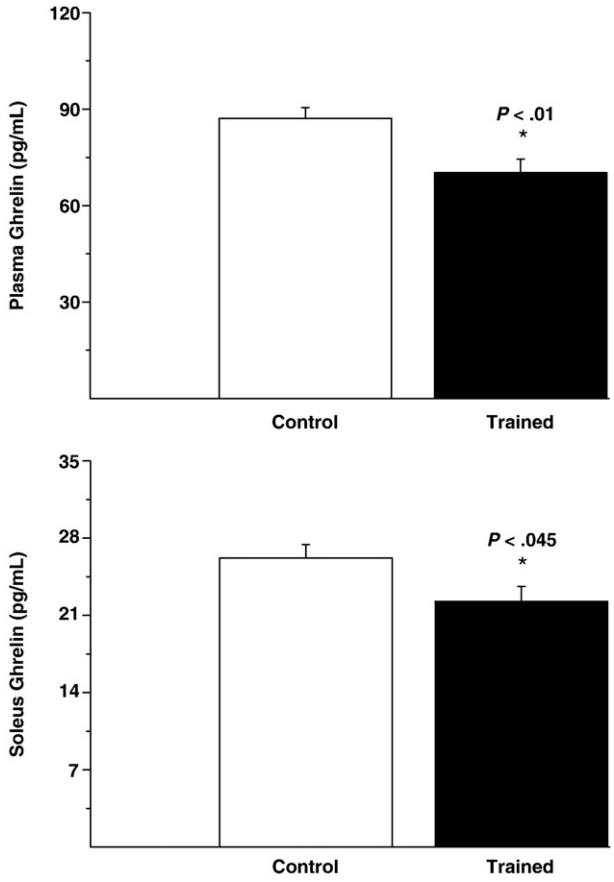

Fig. 1. Plasma and soleus ghrelin concentrations in control and trained wild-type male rats. *Trained vs control. Data are expressed as mean ± SE for 8 to 10 animals per group.

4. Discussion

The major findings of the present study were that 6 weeks of treadmill running reduced rat ghrelin levels in rat soleus muscle and plasma, whereas the concentrations of AgRP were elevated in plasma, but not in soleus muscle. Higher resting soleus muscle glycogen concentrations were also observed. Regarding the effect of exercise on plasma ghrelin concentrations, our findings are consistent with one rodent exercise training study [26] and are in disagreement with some human studies in which training was associated with weight loss [2,14,22,29]; there was no difference in body weights of exercised and control animals in the present study. It should be noted that information concerning the effect of exercise training on skeletal muscle, particularly soleus muscle ghrelin concentrations, is lacking. However, in a study by Wang et al [28], a short- (acute) and long-term (8 weeks) treadmill exercise training regimen (20 m/min, 5 degrees of grade, and 40 minutes per session) reduced hypothalamic total ghrelin and obestatin in rats. In the present study, the level of total ghrelin was significantly lower in trained rat soleus muscle at the end of the 6-week treadmill exercise program (25 m/min, 60 minutes per session).

Expression of AgRP occurs in the hypothalamus of the brain as well as in the periphery (adrenal glands, testis, lungs, and skeletal muscle) [12,32,33]. A major mode of action is in antagonistic binding of melanocortin receptors 3 and 4, targets of α-melanocyte stimulating hormone, an appetite suppressor [1]. As such, existing studies of the effects of exercise on tissue AgRP expression have focused upon brain AgRP messenger RNA (mRNA) expression. Levin and Dunn-Meynell [27] reported no effect of exercise on AgRP expression in the ARC in wheel-running and food-restricted rats. In another study, AgRP mRNA expression in ARC was significantly increased in both sedentary and running food-restricted rats as compared with freely fed rats [11]. The investigators of that study reported that this effect was greater in the running food-restricted animals.

Table 1
Animal weight, plasma glucose, insulin, cortisol, and soleus muscle ATP concentrations in control and trained wild-type male rats

Variables	Control group (n = 8)	Trained group (n = 10)	*P* values
Weight (g)	247.5 ± 6.64	251.3 ± 6.47	.69
Glucose (mg/dL)	183.7 ± 13.7	186.4 ± 8.9	.86
Cortisol (mg/dL)	4.71 ± 0.38	3.90 ± 0.23	.06
Insulin (mIU/L)	979 ± 183	974 ± 112	.80
Soleus ATP (μmol/g)	2.69 ± 0.041	2.61 ± 0.046	.186

Data are expressed as mean ± SE for 8 to 10 animals per group.

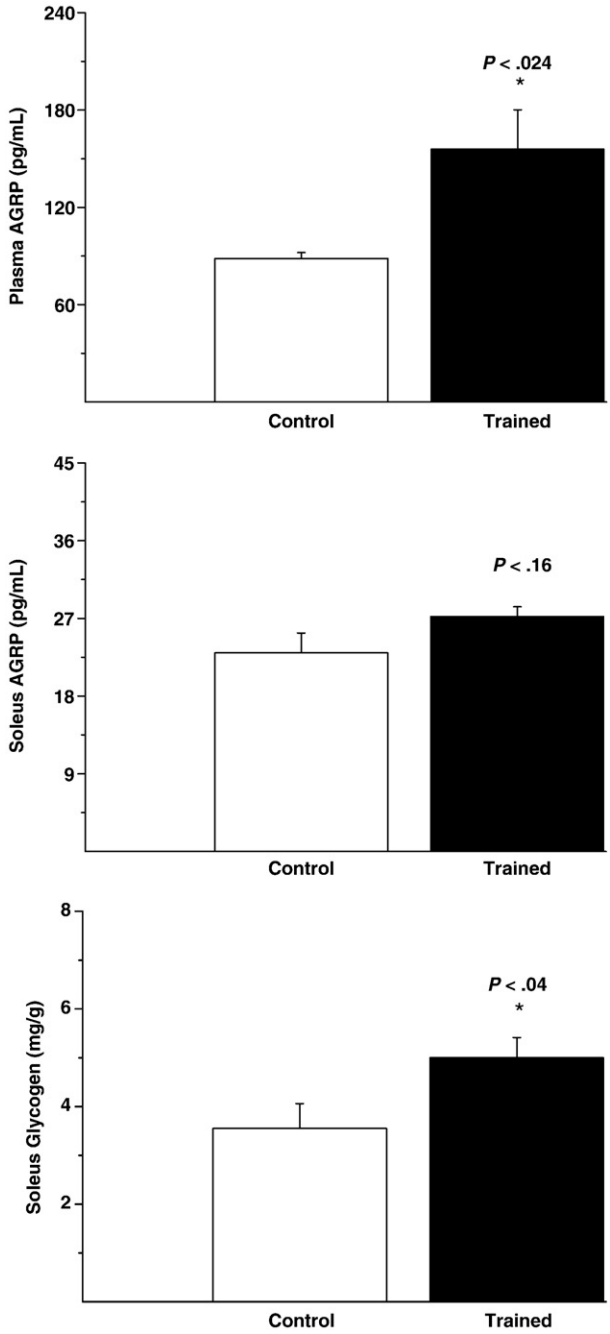

Fig. 2. Plasma and soleus AgRP and soleus glycogen concentrations in control and trained wild-type male rats. *Trained vs control. Data are expressed as mean ± SE for 8 to 10 animals per group.

Because the active isoform of human AgRP crosses the blood-brain barrier (although it is slow to do so) [34], expression of AgRP in the periphery could have direct effects on melanocortin receptors and thus increase hunger. Regarding effects of exercise on plasma AgRP, only acute exercise effects have been investigated. We recently reported that a single session of circuit resistance exercise at 35% 1-RM increased plasma AgRP immediately after the exercise bout in humans [16]. In the present study, we found that 6 weeks of exercise training increased circulating levels of AgRP at rest in rats. To our knowledge, this is the first study to report training-induced changes in plasma AgRP. In addition to the new information concerning plasma AgRP, this is the first report that exercise training did not change the levels of AgRP in the soleus muscle.

The mechanisms by which treadmill exercise alters total ghrelin and AgRP concentrations in plasma and rat soleus muscle are not known. The expression of ghrelin and AgRP mRNA in muscle has been previously reported [3,4,7,12]. On the other hand, it is believed that release of AgRP from hypothalamic tissues could be observed in plasma. Li et al [32] reported that fasting elicited a 3-fold increase in AgRP-LI release from hypothalami of rats. A single peak of hypothalamic AgRP-LI was very close to a peak identified in rat serum. Thus, exercise-induced AgRP mRNA expression in the rat brain may explain higher plasma AgRP levels in trained rats [25,27]. An increase in AgRP level in soleus muscle could also be explained by higher AgRP expression and its uptake by tissues, particularly in the soleus muscle [25,27].

Lower soleus and plasma total ghrelin levels in this study might be explained by higher soleus muscle glycogen concentrations in trained rats. It is well documented that endurance exercise training increases liver and muscle glycogen content with or without carbohydrate loading in rats [10,30,35,36]. Moreover, Andersson et al [24] reported that a single session of treadmill running (22 m/min for 60 minutes at 10% grade) resulted in a significant reduction in liver and gastrocnemius (white and red) muscle glycogen content. They also showed that 1 hour of running these animals followed by 1 hour of postexercise glucose feeding (3g/kg of body weight) restored exercise-induced liver and muscle glycogen depletion and reduced plasma total ghrelin. Thus, it could be speculated that the training-induced increases in muscle glycogen contributed to lower circulating ghrelin levels.

In summary, this is the first study demonstrating the effects of exercise training on soleus muscle ghrelin, AgRP,

Table 2
Correlations between plasma ghrelin and AgRP levels with other measured variables

Variables	Plasma ghrelin *R* values	*P*	Plasma AgRP *R* values	*P*
Plasma ghrelin (pg/mL)	–	–	−0.613	.009
Plasma AgRP (pg/mL)	−0.613	.009	–	–
Soleus ghrelin (pg/mL)	0.057	.82	−0.386	.13
Soleus AgRP (pg/mL)	0.026	.9	0.031	.90
Plasma glucose (mg/dL)	0.049	.84	−0.257	.30
Plasma cortisol (mg/dL)	0.213	.39	−0.269	.28
Plasma insulin (mIU/L)	−0.041	.87	0.226	.36
Soleus ATP (μmol/g)	0.47	.125	0.031	.90
Soleus glycogen (mg/g)	−0.472	.050	0.255	.34

Data are expressed as mean ± SE for 8 to 10 animals per group.

and plasma AgRP concentrations. The present study is also the first demonstrating that plasma AgRP is not related to the glycogen supercompensation mechanism but is inversely related to plasma ghrelin levels in trained animals. The ghrelin findings are consistent with the general concept of intra- and intertissue appetite signaling. Specifically, the exercise training increased muscle glycogen synthesis; with greater intramuscular carbohydrate stores, there were reductions in intramuscular and plasma ghrelin concentrations resulting in what seems to be reduced orexigenic signaling. Further investigation of the effects of muscle ATP and glycogen deficiency on concentrations of these peptides and their expression in skeletal muscles is warranted.

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