Short-, moderate-, and long-term treadmill training protocols reduce plasma, fundus, but not small intestine ghrelin concentrations in male rats

A. Ghanbari-Niaki1, A. Jafari2, M. Moradi3, and R.R. Kraemer4

1Exercise Biochemistry Division, Faculty of Physical Education & Sports Sciences, Mazandran University, Babolsar Mazandran; 2Department of Physical Education & Sports Sciences, Faculty of Humanities, Islamic Azad University, Shahrekord Branch; 3Department of Physical Education and Sport Sciences, Faculty of Literature and Humanities, Shahrekord University, Shahrekord, Iran; 4Department of Kinesiology and Health Studies, Southeastern Louisiana University, Hammond, LA, USA

ABSTRACT. Background: It has been suggested that circulating ghrelin levels are upregulated by fasting, hypoglycemic status, and a physical exercise-induced energy deficit. Aim: The purpose of the present study was to investigate the time-course adaptations of the plasma, fundus, and small intestine ghrelin concentrations as well as related hormones and liver ATP content to 3, 6, and 12 weeks of treadmill endurance running. Material/subjects and methods: Thirty-nine male Wistar rats (12-14 weeks old) were randomly assigned to 3 control (C3, n=5; C6, n=7 and C12, n=7) and 3 training groups (E3, n=6; E6, n=7 and E12, n=7). The rats in the 3 training groups were exercised on a motor-driven treadmill at 25 m/min (0% grade) for 60 min/day, 5 days/week for 3, 6, and 12 weeks, respectively. The animals were sacrificed 48 h after the last session of each training program and tissues were analyzed. Results: Total ghrelin concentrations were significantly (p<0.05) lower in trained rat plasma and fundus tissue after all treadmill endurance running programs. Small intestine ghrelin concentrations remained unchanged. Plasma GH concentrations and liver ATP content were significantly higher in E6 and E12 groups. Conclusion: Data indicate that as little as 3 weeks of moderate treadmill exercise reduces plasma and fundus total ghrelin concentrations with elevated plasma GH and liver ATP content occurring after 6 and 12 weeks of training. Exercise training-induced improvement of energy source availability and negative feedback from increased GH levels may play a role in reducing plasma and fundus ghrelin levels. (J. Endocrinol. Invest. 34: 439-443, 2011)

INTRODUCTION

Ghrelin is a 28-amino acid peptide that has been isolated from the human and rat stomach (mucosa and submucosal cells) and pancreas and is recognized as a novel player in the gut-brain regulation of GH and energy balance (1-5). Ghrelin is also secreted in neuuropeptide Y-containing neurons in the arcuate nucleus of the hypothalamus (6) and other extra-gastrointestinal tract tissues (7-10). It has been suggested that ghrelin acts as a multifunctional peptide (11), regulating a variety of bodily functions in mammalian and non-mammalian species (5, 11-14). However, most investigations have focused on the role of ghrelin in food intake, weight gain, energy/glucose homeostasis, and adiposity (15-19). Ghrelin concentrations in blood and other tissues are regulated by several factors including fasting, anorexia, insulin-induced hypoglycemia, weight reduction, and other negative energy balance circumstances (20-24). The effects of different forms of physical exercise on plasma and brain total and acylated ghrelin in humans (25-37) and rats (7, 38-41) have been studied by several investigators, with some conflicting findings. Both no change (28, 42) and reductions (29-31, 39) in plasma total ghrelin levels have been documented in response to acute exercise; however, exercise training studies have revealed significant increases in circulating ghrelin in women who lost weight (32), no change in obese subjects (34), and reductions in rats who lost weight (40, 42). However, to the best of our knowledge, no studies have investigated whether these disparate findings from previous training studies are due to differences in training time allowed for adequate endocrine adaptation. Thus, the first purpose of the current study was to investigate the time course alterations of plasma, fundus, and small intestine ghrelin concentrations from a treadmill endurance running program. The second purpose of the present study was to see whether changes in plasma ghrelin concentrations are accompanied by changes in fundus and small intestine ghrelin levels, liver ATP content, as well as GH, insulin, and cortisol levels.

MATERIALS AND METHODS

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. The protocol was approved by the Ethics Committee of the School of Medical Sciences, Tarbiat Modares University (TMU), Tehran, Iran. Thirty-nine male Wistar rats (12-14 weeks old) weighing 200-220 g were used for this study. Animals were...
obtained from Pasteur’s Institute (Tehran, Iran) and maintained in the Central of Animal House, School of Medical Sciences of TMU. The animals were housed 5 per cage 24.5 × 15 × 8 in. Light was controlled on a 12:12-h light-dark cycle. Temperature and humidity were maintained at 22±1.4 C and 55.6%±4.0%, respectively. Animals were fed a pellet rodent diet ad libitum and had free access to water. Animals were randomly assigned to control (no.=19) and training (no.=20) groups. Each group was further divided into 3 subgroups that served as a control group or that completed either 3, 6, or 12 weeks of training. Thus, the groups were comprised of 3 control groups (C3, no.=5; C6, no.=7; C12, no.=7) and 3 training groups (E3, no.=6; E6, no.=7; E12, no.=7).

**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 min, 5 days/week. The animals were killed 48 h after the last exercise session. Food but not water was removed from the rat cages 4 h before the animals were sacrificed.

**Tissue biopsies and blood samples**

Forty-eight hours after the last training session, rats were anesthetized with an ip injection of ketamine (30-50 mg/kg bw, ip) and xylazine (3-5 mg/kg bw, ip). A portion of the fundus, upper small intestine, and liver were excised, washed in saline, and immediately frozen in liquid nitrogen for determination of fundus and small intestine ghrelin and liver ATP concentrations. All frozen fundus, small intestine, and liver pieces were stored at –80 C until analyses were performed. Blood was collected directly from the heart in test tubes containing EDTA, separated by centrifugation, frozen, and stored at –80 C until the biochemical analyses were performed.

**Fundus and small intestine total ghrelin and liver ATP**

Fundus and small intestine total ghrelin content were measured using a rat enzyme immunoassay method (SPIbio, Montigny le Bretonneux, France). The intra-assay coefficient of variation and sensitivity of the method were 5.9% and 4 pg/ml, respectively. Liver ATP was determined by an ATP sensitive Bioluminescence kit (Bioaffin GmbH & Co KG, Kassel, Germany).

**Plasma GH, insulin, and cortisol concentrations**

Plasma GH was determined using an enzyme-linked immunoabsorbent assay (ELISA) (Diagnostic Systems Laboratories Inc, Texas, USA) with an intra-assay coefficient of variation and sensitivity of 8.2% and 0.13 ng/ml, respectively. Plasma insulin and cortisol were also determined by ELISA (Mercodia AB, Uppsala, Sweden; Diagnostic Biochem Canada Inc., Ontario, Canada) with intra-assay coefficients of variation of 4.1% and 7.6% and sensitivities of 0.07 μg/l and 0.4 μg/dl, respectively.

**Statistics**

All data are expressed as means±SEM. The data were analyzed using an unpaired t-student test, while relationships between measures were determined using a Pearson product moment correlation. Statistical significance was accepted at p<0.05.

**RESULTS**

**Plasma, fundus, and small intestine total ghrelin and liver ATP concentrations**

No significant differences were found for body weight, insulin, and cortisol concentrations between respective control and exercise groups (Table 1). Data revealed that plasma and fundus total ghrelin levels were significantly (p<0.05) lower in trained groups at all time points of measurement than the corresponding control group (Fig. 1). Resting small intestine total ghrelin content remained un-