Original Article

MOLECULAR METABOLISM

Ghrelin mediates exercise endurance and the feeding response post-exercise

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ABSTRACT

Objective: Exercise training has several well-established health benefits, including many related to body weight, appetite control, and blood glucose homeostasis. However, the molecular mechanisms and, in particular, the hormonal systems that mediate and integrate these beneficial effects are poorly understood. In the current study, we aimed to investigate the role of the hormone ghrelin and its receptor, the growth hormone secretagogue receptor (GHSR; ghrelin receptor), in mediating the effects of exercise on food intake and blood glucose following exercise as well as in regulating exercise endurance capacity.

Methods: We used two mouse models of treadmill running to characterize the changes in plasma ghrelin with exercise. We also assessed the role of the ghrelin system to influence food intake and blood glucose after exercise, exercise endurance, and parameters potentially linked to responses to exercise. Mice lacking GHSRs (GHSR-null mice) and wild-type littermates were studied.

Results: An acute bout of exercise transiently elevated plasma acyl-ghrelin. Without the action of this increased ghrelin on GHSRs (as in GHSR-null mice), high intensity interval exercise markedly reduced food intake compared to control mice. The effect of exercise to acutely raise blood glucose remained unmodified in GHSR-null mice. Exercise-induced increases in plasma ghrelin positively correlated with endurance capacity, and time to exhaustion was reduced in GHSR-null mice as compared to wild-type littermates. In an effort to mechanistically explain their reduced exercise endurance, exercised GHSR-null mice exhibited an abrogated sympathoadrenal response, lower overall insulin-like growth factor-1 levels, and altered glycogen utilization.

Conclusions: Exercise transiently increases plasma ghrelin. GHSR-null mice exhibit decreased food intake following high intensity interval exercise and decreased endurance when submitted to an exercise endurance protocol. These data suggest that an intact ghrelin system limits the capacity of exercise to restrict food intake following exercise, although it enhances exercise endurance.

Keywords GHSR; Ghrelin; Exercise; Treadmill; Endurance; Food intake

1. INTRODUCTION

Ghrelin is a stomach-derived hormone that acts to stimulate growth hormone (GH) secretion as well as to affect various processes related to eating, body weight and blood glucose regulation [1]. In contrast to most other metabolically-acting gastrointestinal hormones, ghrelin acutely stimulates eating and also induces body weight gain upon repeated administration as a result of its orexigenic actions and its effects to reduce energy expenditure and preserve fat mass [2–6]. The actions of ghrelin are mediated through the growth hormone secretagogue receptor (GHSR; ghrelin receptor), which is expressed in several brain sites, the pituitary, and several peripheral organs [7–9]. GHSR activation by ghrelin requires a unique acylation of the hormone that occurs during its synthesis, although unacyl-ghrelin, which has actions *via* an as-of-yet unknown receptor, also exists in circulation [10–12]. Opposite to what might be expected based on the effects of administered ghrelin, genetic mouse models lacking ghrelin or GHSR do not demonstrate substantial differences in food intake and body weight when given free access to standard chow diet [13–18]. As such, an intact endogenous ghrelin system does not appear to be

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Abbreviations: ACC, acetyl coA carboxylase; AMP 5′, adenosine monophosphate; AMPK, AMP-activated protein kinase; CNS, central nervous system; COX IV, cytochrome c oxidase subunit 4; G6P, glucose-6-phosphatase; GH, growth hormone; GHSR, growth hormone secretagogue receptor; HIIE, high intensity interval exercise; HNF4α, hepatocyte nuclear factor 4α; IGF-1, insulin-like growth factor-1; IGFBP-1, insulin-like growth factor binding protein-1; PC, pyruvate carboxylase; PCG1α, peroxisome proliferator-activated receptor gamma coactivator 1α; PEPCK, phosphoenolpyruvate carboxykinase; PYGL, glycogen phosphorylase, liver; RT-PCR, reverse transcriptase-polymerase chain reaction; VMH, ventromedial hypothalamus

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essential to maintain normal energy homeostasis in mice during standard housing conditions — e.g. *ad libidum* access to standard chow, minimal to absent psychosocial or other types of stress, and lack of forced physical activity.

Recent studies suggest that the biological importance of endogenous ghrelin becomes accentuated during exposure to more metabolically-constrained and stressful environments. Indeed, mice lacking either ghrelin or GHSR demonstrate impaired ability to adapt metabolically and/or behaviorally to caloric restriction and psychological challenges. As such, a functional ghrelin system ensures protection from life-threatening falls in blood glucose in adult mice subjected to severe caloric restriction and in juvenile mice subjected to acute fasting [15,16,19—22], minimizes depressive-like behaviors in mice subjected to chronic psychosocial stress, mediates the antidepressant-like and anxiolytic-like behavioral effects of caloric restriction [23,24], and restricts body weight loss and stalls mortality associated with chronic anorexia/cachexia conditions [25]. Elevation of plasma ghrelin is a consistent feature in those challenging conditions [3,23,26—29], suggesting that the ghrelin system is actively upregulated in those conditions as a protective measure. This upregulation of plasma ghrelin stands in contrast to the reduction in plasma ghrelin and resistance to ghrelin signaling to stimulate food intake in overly-abundant nutritional states such as obesity [30]. Therefore, an emerging notion is that the ghrelin system may serve as an essential response to metabolic and stressful challenges, minimizing perturbations to metabolic and psychological homeostasis to promote survival [12].

In this study, we aimed to study the biological significance of the ghrelin system in mice subjected to exercise as a metabolic challenge. Although the many health benefits of exercise — including weight maintenance, appetite control, improved insulin sensitivity, improved mental health, and secondary prevention of chronic diseases such as obesity, type II diabetes mellitus, cancer, and hypertension — are generally well-accepted, the molecular mechanisms that mediate and integrate these beneficial effects are poorly understood [31—35]. The potential role of the ghrelin system in mediating exercise capacity and the effects of exercise on food intake, body weight, and blood glucose are of particular interest given the central role of ghrelin in these processes [1,12]. The effect of exercise on plasma ghrelin levels has been investigated in multiple human and rodent studies although the results have been inconsistent, demonstrating either a decrease, increase, or no change [36—48]. Notwithstanding these discrepant observations on the changes in plasma ghrelin with exercise, the impact of the ghrelin system on performance of exercise, food intake after exercise, and, more broadly, the healthy metabolic outcomes of exercise is not well-established. Here, we use two mouse models of treadmill running to characterize the changes in plasma ghrelin with exercise as well as the function of the ghrelin system to influence exercise performance, food intake, and blood glucose acutely following exercise.

2. MATERIAL AND METHODS

2.1. Mice

All animal experiments were approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee. 10—16 wk-old male GHSR-null mice [18] maintained on a C57BL/6N background (by backcrossing to C57BL/6N for many more than 10 generations over the past 10+ years) and wild-type were used in the study. The mice were generated by crossing male and female mice heterozygous for the GHSR-null allele. Mice were housed at room temperature (22—24 °C) under a 12 h dark—light cycle with free access to water and standard chow diet [2016 Teklad Global 16% protein diet (Envigo, Indianapolis, IN)], except as indicated.

2.2. Exercise protocols

Motorized treadmills (Exer-6; Columbus Instruments, Columbus, OH) were used for exercise experiments. All mice were familiarized to the treadmills for 2 days prior to the exercise bout [Day 1: 5 min rest on the treadmill followed by running for 5 min at the speed of 8 m/min and then for 5 min at the speed of 10 m/min; Day 2: 5 min rest on the treadmill followed by running for 5 min at the speed of 10 m/min and then for 5 min at the speed of 12 m/min]. On Day 3, mice were subjected to a high intensity interval exercise (HIIE) bout (modified from Ref. [49]) to assess exercise-induced changes in plasma ghrelin, blood glucose, and food intake. Briefly, food was removed from all the mice at the start of the light cycle (7 AM) for a duration of 6 h, so as to eliminate any differences in food intake on the measured parameters (Figure 1A). Mice were rested on the treadmill for 5 min prior to performing the 1 h of exercise consisting of 3 × 20 min intervals (5 min at the speed of 12 m/min, followed by 10 min at the speed of 17 m/min, and then 5 min at the speed of 22 m/min), without rest between intervals. The 1 h exercise bout was performed either in the 6th h of food restriction ("6^{th}h EX") or the 2nd h of food restriction ("2^{nd}h EX"). The two-different time points for the 1 h-duration HIIE exercise bouts were chosen so that we could more fully characterize the post-exercise physiological changes — in particular, the duration of the effects. Yet a third group of animals was kept in their home cages on Day 3 instead of being submitted to the 1 h exercise bout (sedentary; "Sed"). The mice were coaxed to continue running on the treadmill by means of an electric stimulus (0.25 mA × 163 V and 1 Hz) generated by a shock grid present at the treadmill base and by manually tapping their tails using a soft nylon bottle brush, as needed to enable all the mice to complete the exercise bout.

Exercise endurance capacity was tested by subjecting *ad libitum*-fed 10—16 wk old naïve mice to a progressive running paradigm as described previously [50,51]. The mice were acclimatized to the treadmill for 2 days as described above. On Day 3, mice were placed on the treadmill for 5 min at rest, followed by running with a starting speed of 10 m/min for 40 min, next by running at speeds that were increased at the rate of 1 m/min every 10 min until the speed reached 13 m/min, and finally by running at speeds that were increased at the rate of 1 m/min every 5 min until exhaustion. The exhaustion time was noted as the time at which the mice stopped running and remained on the electric shock grid for 5 s, without attempting to resume running [50,51]. Of note, for the exercise endurance capacity studies, although the electric shock grids were in place, manual coaxing with the soft nylon bottle brush was only used for the group of GHSR-null mice who were time matched to run the same duration as wild-type littermates ("GHSR-null-TM"). Sedentary controls were similarly acclimatized to the treadmill on Days 1 and 2, but on Day 3 they were instead kept in their home cages without food for a duration matching that of exercise in the exercise group.

2.3. Blood and tissue collection

Blood samples were collected by a quick superficial temporal vein (submandibular) bleed in the HIIE bout experiment at the end of the 6 h caloric restriction period and by decapitation in the exercise endurance capacity experiment upon exhaustion. Blood was collected from the sedentary controls using a similar procedure at the same times of day as their exercised counterparts. Blood glucose and lactate concentrations were measured immediately using the hand-held Bayer Contour and Nova Biomedical Lactate Plus™ monitoring systems, respectively. Blood also was collected into EDTA-coated vaccutainer placed on ice and

