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RESEARCH LETTER

Effects of prolonged exercise on agouti-related protein: a pilot study

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Abstract Agouti-related protein (AgRP), is a signaling peptide that affects feeding behavior, energy homeostasis, and has also been shown to stimulate the hypothalamic–pituitary–adrenal axis. The purpose of this study was to determine the effects of 90 min of treadmill exercise on circulating AgRP concentrations and the relationship of AgRP responses to cortisol. Seven young males completed a preliminary trial followed by counterbalanced experimental and control trials 4–5 weeks apart. The experimental trial began 2.5 h after consumption of a standard nutrient beverage and consisted of treadmill exercise at 60 % of previously determined VO_{2max} for 90 min. Blood samples were collected before (−30 and 0 min), during (18, 36, 54, 72, and 90 min), and following exercise (20, 40, and 60 min). Blood samples were collected in a resting, control trial at the same time points as the experimental trial. Plasma lactate was significantly higher in the exercise than the control trial. Although AgRP increased from 18 min of exercise to peak at 90 min, these increases were not significantly different than values in the control trial. Cortisol responses during the exercise trial were significantly higher than the control trial. AgRP concentrations during early exercise were positively correlated with cortisol levels later in recovery. The obtained data suggest that AgRP concentrations during prolonged steady-state exercise are associated with subsequent cortisol increases, but further study is required to determine whether there is a causal effect.

Keywords Orexigenic peptide · HPA-axis · Steady-state exercise · Cortisol

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Introduction

Agouti-related protein (AgRP), an α-melanocyte stimulating hormone antagonist, is a signaling peptide that affects feeding behavior, energy homeostasis, and adiposity [9]. AgRP acts as an inverse agonist of melanocortin receptor 4 (MCR4) and mutations of MCR4 appear to be associated with early onset obesity [15]. AgRP has also been shown to stimulate the hypothalamic–pituitary–adrenal (HPA) axis and augment the HPA axis response to interleukin-1 [10, 19]. In humans, AgRP is mainly expressed in the hypothalamus and adrenal tissue; there are lower levels of expression in the kidneys, testes, and lungs [6, 17]. Circulating AgRP concentration is increased in humans and rats with fasting [3, 16], and has been shown to increase in male rats after training [8]. AgRP has been shown to increase after circuit training exercise in young men and women [5, 7], but studies are lacking regarding acute effects of prolonged exercise on AgRP in humans.

Circulating AgRP concentration is increased in obese and fasting humans [14], and is reduced in response to insulin, leptin, and glucose [1, 3]. Changes in glucoregulatory hormone concentrations can alter metabolism that

could ultimately affect caloric balance and metabolic health. Thus, understanding how prolonged exercise affects AgRP is important in this regard. We recently reported the effects of prolonged exercise on glucoregulatory hormone responses to exercise [11]. The purpose of the present study was to determine the effects of prolonged exercise on AgRP responses in a subset of the same subjects. Since there are data suggesting that AgRP has a stimulatory effect on the HPA-axis, we compared the exercise-induced AgRP responses to those of cortisol. We hypothesized that prolonged exercise would stimulate a gradual increase in AgRP and cortisol and that increases in AgRP concentrations would be related to increases in circulating cortisol.

Methods

Subjects and study design

The study was approved by the Southeastern Louisiana University Institutional Review Board. Seven young (mean ± SE, 22.57 ± 1.62 y) male subjects were recruited for the study and gave informed consent to participate in the study. Descriptive data are shown in Table 1. Based upon a medical history and a 3-day food record, subjects met the required criteria: 1) between the ages of 18–35 y, 2) not on any prescription medications, 3) absence of cardiovascular or metabolic disease, and 4) adherence to a normal diet that would not affect metabolic responses to exercise. Each of the subjects was considered to be physically active and currently engaged in regular weekly aerobic exercise defined as a minimum frequency of 3–4 × per week, 30–60 min per session. The general design of the study included a preliminary session to collect anthropometric and physiological data, followed by an experimental (exercise) session and a control (resting) session to determine endocrine responses in a counterbalanced manner with 1 month between trials [Fig. 1].

Preliminary session

As in our previous study [11], body composition (7-site skinfold assessment, [18]) and maximal oxygen consumption (VO_{2max}) were determined for each subject in the first session. VO_{2max} was determined from a graded treadmill exercise test starting at 2.5 mile per hour and 4 % grade and progressing by 1 mile/hour every 2 min until exhaustion (Kraemer Protocol, [11]). A metabolic cart (Parvo-Medics 2400, Sandy UT) was used to collect ventilatory volumes, as well as F_EO_2, and F_ECO_2 to determine VO_2. Criteria for determining that VO_{2max} was reached included: the primary criterion of a plateau in VO_2 with an increase in workload or 2 of 3 secondary criteria (1) reaching predicted maximal heart rate, (2) respiratory exchange ratio greater than 1.1, or (3) a rating of perceived exertion (15 point Borg Scale) of 19 or 20.

Table 1 Descriptive data for male subjects. $N = 7$

Measure	Mean ± SD
Age	22.57 ± 1.61 y
Height	176.29 ± 7.61 cm
Weight	71.83 ± 7.61 kg
Body mass index	23.26 ± 3.81 kg/m^2
Percent body fat	11.77 ± 6.2 %
VO_{2max}	54.61 ± 9.44 ml/kg/min

Exercise and control sessions

For both the exercise and control sessions, subjects were required to 1) maintain their normal diet, 2) not exercise for 48 h prior to sessions, 3) not drink alcohol for 48 h before sessions, 4) fast from 12:00 pm the night prior to the sessions, 5) at 7:00 am ingest a liquid meal (Ensure Plus^{TM}), and 6) report to the lab at 8:00 am. The 350 kcal liquid meal consisted of a macronutrient percentage of 15 % protein, 28 % fat, and 57 % carbohydrate. Its consumption 2 h before the control and exercise sessions insured the AgRP responses to control and exercise sessions under practical conditions without an effect of prolonged fasting on metabolic and endocrine responses. Before study onset, calculations indicated that the pre-exercise time period would be associated with normal daily insulin and blood glucose concentrations and that the 350 kcal liquid meal would enable the subjects to complete prolonged exercise in which they would expend an estimated 700–900 kcals.

Intravenous catheter insertion into a dorsal vein of the hand was performed at 8:30 am and a saline lock was used to maintain patency and allow access for blood collection. At 9:00 am (−30 min) and 9:30 am (0 min) before exercise, resting blood samples (28 mL/sample) were collected. Subjects then began 90 min of steady-state exercise at 60 % VO_{2max} with continual monitoring of VO_2,while treadmill speed and grade were adjusted to maintain the intensity of 60 % VO_{2max}. Blood samples were collected at 18, 36, 54, 72, and 90 min of exercise, and 20, 40, and 60 min following exercise. The 18-min window during exercise and 20-min window during recovery were chosen to capture meaningful changes in hormone concentrations during exercise and recovery. During the control session, an identical blood sampling protocol was used while subjects rested in a seated position without exercise or VO_2 measurement. Blood samples for AgRP and cortisol analysis were collected in chilled EDTA tubes, whereas blood samples for lactate analysis were collected in a sodium oxalate/potassium fluoride tube.

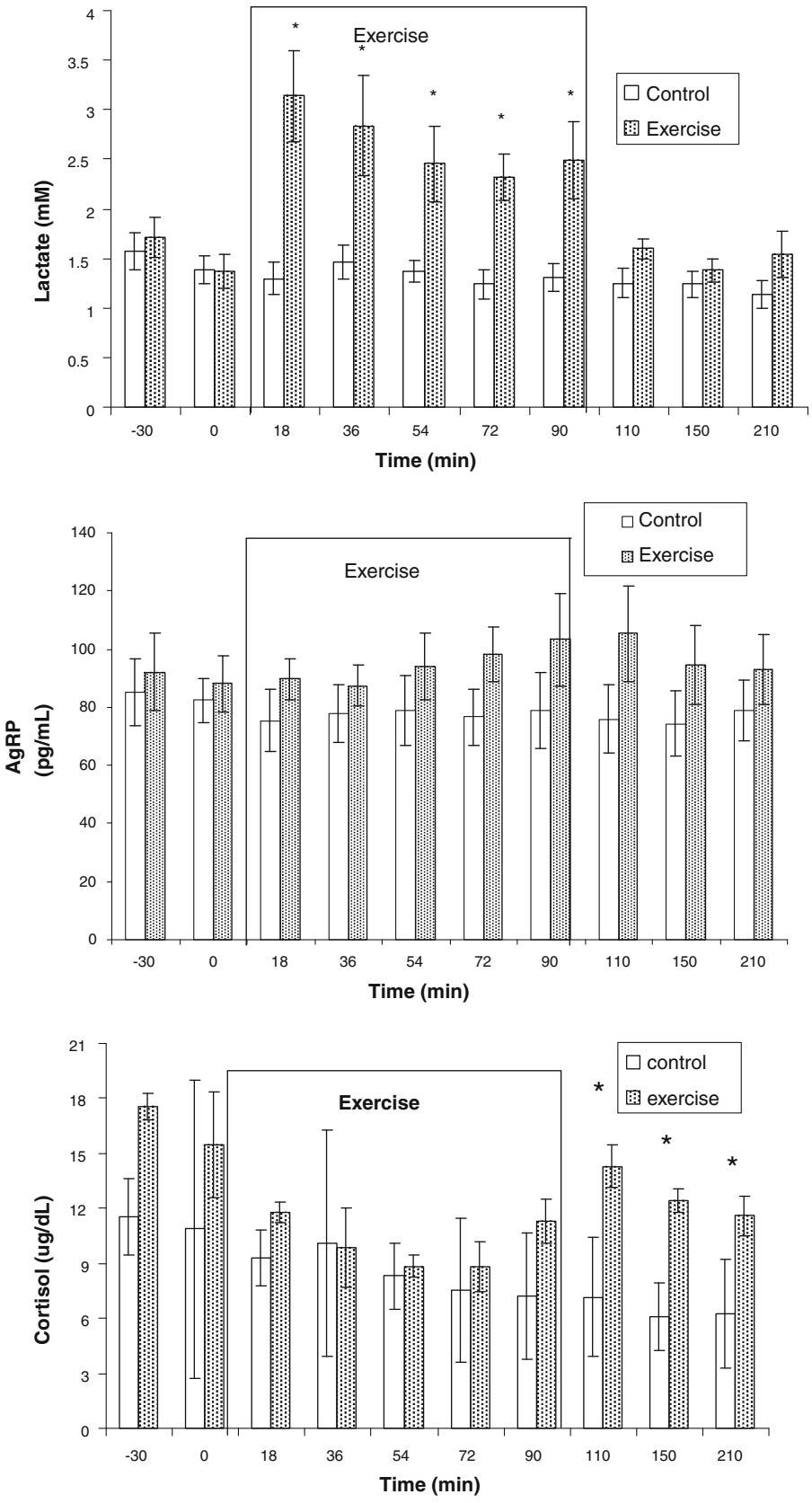

Fig. 1 *Top panel* represents mean ± SE lactate concentrations for exercise and control trials for seven subjects before exercise, during exercise, and for 1 h of recovery. *Middle panel* represents mean ± SE AgRP concentrations for exercise and control trials for seven subjects before exercise, during exercise, and for 1 h of recovery. *Bottom panel* represents mean ± SE cortisol concentrations for exercise and control trials for seven subjects before exercise, during exercise, and for 1 h of recovery. *Significantly different values for the exercise compared with the control trial at the same time point

Analyses

Concentrations of plasma AgRP were determined by ELISA (Millipore Corp., St. Charles, MO). Cortisol concentrations were determined using Immulite (Siemens, Los Angeles CA). The inter-assay coefficients of variation for AgRP and cortisol were, 2.75 and 7.9 %, respectively. The intra-assay coefficient of variation for AgRP was 12.83 %. There was no intra-assay CV for cortisol since it was determined using Immulite. Lactate concentrations were determined by an enzymatic spectrophotometric analysis (Trinity Biotech, St. Louis, MO). Hematocrit was determined via the microhematocrit method, and whole-blood hemoglobin concentrations were determined by the cyanmethemoglobin method via spectrophotometry (Pointe Scientific, Canton, OH). The hematocrit and hemoglobin determinations were utilized to calculate plasma volume shifts across time [4].

A 2 × 10 repeated measures ANOVA was used to examine hormone changes over time and between trials and post hoc dependent *t* tests were applied where appropriate. In addition, the total response of the hormones and metabolites to exercise was determined by integrated area-under-the-curves (AUC), computed via a trapezoidal method after subtracting averaged baseline hormone concentrations for each subject; dependent *t* tests were used to determine differences between experimental and control trials. Pearson product moment correlations were performed to determine the relationship between AgRP and cortisol at different time points. Statistical analyses were performed by SPSS PASW Statistics 18 (IBM Corp, Somers, NY) with an alpha level of $P \leq 0.05$ considered as significant.

Results

Changes in plasma volume were not >7.5 % between the second resting blood sample and all subsequent exercise samples during exercise. For blood lactate, there was a significant main effect for time [$F(9,108) = 8.55$, $P = 0.00$] and trial × time point [$F(9,108) = 7.46$, $P = 0.001$] (Fig. 1). Plasma lactate concentrations were significantly greater during all five exercise time points compared to the control trial (see Fig. 1) revealing the metabolic stress produced by the exercise. Compared with the control trial, AgRP tended to increase during the exercise trial (Fig. 1); however, there was no significant effect for time [$F(9,108) = 1.107$, $P = 0.36$] or trial × time point [$F(9,108) = 1.83$, $P = 0.72$], nor were AgRP AUC values significantly different between exercise and control trials [578.37 ± 398.36 vs. −640.44 ± 466.36 μg min/dl, respectively, $P = 0.15$]. We have previously reported the cortisol data for $N = 8$ [11], and here we report the cortisol data for $N = 7$ to compare with the AgRP responses from the same subjects. For cortisol ($N = 7$), there was a significant effect for time [$F(9,108) = 6.77$, $P = 0.004$] and trial × time point [$F(9, 108) = 24.21$, $P = 0.006$] (Fig. 1) indicating a significant exercise effect. Moreover, there were positive correlations for different time points between AgRP during exercise and cortisol during recovery. AgRP concentrations for time points of 54, 72, and 90 min during exercise were significantly correlated with cortisol at time points 20, 40, and 60 min of recovery; significant positive correlations between AgRP and cortisol were also observed in recovery ($P < 0.05$, range of $r = 0.56$–0.74, Table 2).

Table 2 Significant correlations between AgRP and cortisol during exercise and recovery time points

AgRP concentration time point	Cortisol concentration time point	Correlation/ probability
E54 min	R20 min	0.48, $P = 0.08$
E54 min	R40 min	0.59 $P = 0.03$
E72 min	R20 min	0.60, $P = 0.02$
E72 min	R40 min	0.70, $P = 0.006$
E72 min	R60 min	0.36, $P = 0.21$
E90 min	R20 min	0.56, $P = 0.008$
E90 min	R40 min	0.71, $P = 0.005$
E90 min	R60 min	0.67, $P = 0.009$
R20 min	R20 min	0.65, $P = 0.012$
R20 min	R40 min	0.74 $P = 0.003$
R40 min	R20 min	0.58, $P = 0.030$
R40 min	R40 min	0.69, $P = 0.006$
R40 min	R60 min	0.62, $P = 0.018$

E54 min = after 54 min of exercise
E72 min = after 72 min of exercise
E90 min = after 90 min of exercise
R20 min = after 20 min of recovery
R40 min = after 40 min of recovery
R60 min = after 60 min of recovery

Discussion

This is the first study to determine the effects of prolonged, steady-state exercise on AgRP in humans. The major finding of this study was that AgRP concentrations did not rise significantly in response to the prolonged exercise session compared with the control session. However, there was a tendency for AgRP concentrations to be elevated in the later stages of exercise, and during this time period AgRP concentrations were significantly correlated with cortisol concentrations. Cortisol responses were typical of

responses during prolonged, steady-state exercise below 75 % VO_{2max} [11]. During the control trial cortisol declined, whereas during exercise, cortisol first declined, then rose to peak 20 min post exercise. Previous data has shown that 30 min of treadmill exercise at ~70 % VO_{2max} does not elicit a cortisol response [12], whereas exercise greater than 75 % VO_{2max} will increase cortisol concentrations [13]. It is known that moderate exercise intensities require longer duration to elicit a cortisol response [2].

Ghanbari-Niaki et al. [5, 7] reported acute effects of resistance exercise on circulating AgRP concentrations in humans. In a study of young males completing ten circuit training resistance exercises at 35 % of 1-repetition maximum (1-RM), it was reported that AgRP concentrations significantly increased immediately following exercise, but returned to nadir by 30 min of recovery [5]. The investigators concluded that greater AgRP concentrations post exercise could stimulate hyperphagia, compensating for caloric expenditure during exercise. Another study investigated the effects of muscle loading on AgRP expression in lymphocytes and circulating plasma concentrations in young females [7]. Subjects completed nine resistance exercises using loads of 40 % 1-RM, 60 % 1-RM, 80 % 1-RM, and a combination of the loads. Plasma AgRP concentrations were significantly elevated after all loading schemes except 40 % 1-RM. Collectively, these studies suggest that resistance exercise stimulates release of AgRP in humans and that increases in muscle loading enhance the effect. In the present study, there was not a significant plasma AgRP response to long-term aerobic exercise at a moderate intensity (60 % VO_{2max}); however, there was a trend for concentrations to become elevated during the latter portion of the exercise protocol. It is possible that greater exercise intensity would have resulted in a greater AgRP response.

It has been shown that AgRP can enhance the effect of a stimulated HPA-axis [10, 19]. In the present study, higher AgRP levels that occurred earlier during exercise were significantly correlated with elevated cortisol concentrations in recovery of the prolonged (90 min) exercise session. Xiao et al. [19] investigated the effects of intracerebroventricular (icv) administration of AgRP (20 μg) on stimulation of the HPA-axis in ovariectomized rhesus monkeys. Plasma cortisol concentrations began to rise after the 90 min post-administration sampling time point, with ACTH peaking at the 60 min time point. These data are generally in agreement with the data observed in the present investigation. Thus, results from the present study suggest that during prolonged, moderate-intensity exercise, elevations in AgRP may aid in stimulating cortisol responses, which could contribute to enhanced fat utilization and sparing of carbohydrate oxidation. These effects would help the individual sustain the metabolic demands of long-term exercise.

Conclusions

In summary, prolonged steady-state exercise at a moderate exercise intensity (60 % VO_{2max}) elicits substantial increases in blood lactate and cortisol concentrations without a significant change in AgRP. However, the tendency for AgRP concentrations to increase with exercise time correlated with cortisol concentrations at later time points. Given previous data indicating that AgRP enhances the HPA-axis response to stress, data suggest that AgRP may enhance cortisol responses to prolonged exercise allowing metabolic demands to be sustained, but further study is required for confirmation.

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Conflict of interest There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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