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Original article

The effect of interval versus continuous exercise on plasma leptin and ghrelin concentration in young trotters

S. Kowalik, W. Kędzierski

Department of Biochemistry and Animal Physiology, Faculty of Veterinary Medicine
University of Life Sciences in Lublin, Akademicka 12, 20-033 Lublin, Poland

Abstract

The effect of interval vs. continuous exercise on plasma leptin and ghrelin concentration in young Standardbred horses was studied. The experiment was conducted on 27 trotters, in the age between 2 and 3 years. They were divided into two groups according to the type of exercise. Blood samples were collected through jugular venipuncture in the following experimental conditions: at rest, immediately after exercise and 30 minutes after the end of the effort. Plasma leptin and ghrelin concentrations were determined using RIA tests. The continuous exercise induced an increase in plasma leptin concentration whereas the interval type of exercise did not influence the level of this hormone (3.47 ± 0.78 vs. 4.07 ± 0.94 and 2.31 ± 0.15 vs. 2.36 ± 0.21 ng/mL, respectively). The plasma ghrelin concentration measured after the continuous exercise, significantly increased (720 ± 27.4 vs. 814 ± 13.8 ; $p \leq 0.05$) whereas concentration of this hormone assessed after the interval exercise, significantly dropped (982 ± 56.5 vs. 842 ± 35.6 pg/mL; $p \leq 0.05$). The changes in plasma ghrelin concentration measured after the end of the effort correlated inversely with blood lactic acid concentration. In conclusion, the obtained results showed that medium-intensive type of exercise, such as trot, interval or continuous, slightly affected plasma leptin level but significantly affected plasma ghrelin concentration in young Standardbred trotters.

Key words: leptin, ghrelin, hormones, horses, energy metabolism, exercise

Introduction

An improvement of energy production is a basic part of the adaptive mechanisms of race horses to training. A potential role in this process may be played by the gut hormones, leptin and ghrelin, which are related to the control of energy metabolism through a neuroendocrine pathway (Gao and Horvath 2008). Leptin, secreted mainly by white adipocytes, is known as an important regulator of various aspects

of the state of satiety and hunger, feed intake, and reproduction ability (Gentry et al. 2002, Prentice et al. 2002, Waller et al. 2006). It also promotes the oxidation of lipids increasing energy expenditure (Dotsch et al. 2003). In horses, the peripheral concentration of plasma leptin is related to fat mass and body condition score (Buff et al. 2002, Kearns et al. 2006). The level of this hormone showed the diurnal variation attributed to feeding time and insulin response (Gordon and McKeever 2005, Steelman et al. 2006). Feed

deprivation or other states of negative energy balance led to a decrease in plasma leptin concentration in mares (McManus and Fitzgerald 2000, Buff et al. 2005, Buff et al. 2006, Kędzierski et al. 2008). Nevertheless, the effect of exercise on plasma leptin level in horses is not clear. A few studies revealed that there were no changes in leptin level during exercise tests. However, some modulation in concentration of this hormone was observed a few hours after effort (Carmill et al. 2003, Piccione et al. 2005, Gordon et al. 2007a).

Ghrelin, which is produced mainly in the stomach, as well as in other gastrointestinal segments and selected areas of the brain, plays an opposite role (Gil-Campos et al. 2006). This hormone increases appetite, food intake, energy balance and as a result, body weight (Hayashida et al. 2001, Gil-Campos et al. 2006). In cases of low-caloric diets or chronic exercise the plasma ghrelin level in horses increased (Gordon and McKeever 2005). Moreover, in states of satiety or after glucose challenge the concentration of this peptide decreased (Gordon and McKeever 2006). The influence of exercise on the plasma ghrelin level in horses was less studied. In the study of Gordon et al. (2007a), the exercise tests led to changes in plasma ghrelin concentrations in old mares a few hours after the end of effort. On the other hand, an increase in plasma ghrelin level was shown during exercise tests in trained trotters (Gordon et al. 2006). Hence, leptin and ghrelin play a major role in energy homeostasis. There are new data on the functional role of these hormones and their possible interactions with each other, with glucose – insulin dependent metabolism, and with cortisol as well (Gordon et al. 2007b).

The range of energy expenditure during physical activity depends on many parameters like the intensity and duration time of exercise as well as horse current performance, age, breed, etc. (Kędzierski et al. 2009). In the process of conditioning trotters, two types of exercise schedules are used in practice. They are an interval type of exercise and a continuous type of exercise. The interval exercise produces a rise in performance in trotters but also appears to increase the risk of various injuries (Shearman et al. 2002). It is known that this type of effort alters also feed intake as well as the leptin and ghrelin concentrations in adult Standardbred mares (Gordon et al. 2006). However, the range of post-exercise changes in plasma leptin and ghrelin level seems to be depended on the age of studied trotters (Kędzierski and Kapica 2008). In European breeding practice, Standardbred horses are involved in a training routine when they are one-year-old. At this time, they are still in growing period and training and workload particularly loads their metabolism (Kędzierski et al. 2007b). Hence, the main goal of this study was to assess the effects of interval and continuous types of exercise on plasma

ghrelin and leptin concentration in young Standardbred horses. The relative intensity of exercise performed was assessed by the analysis of blood lactic acid (LA) concentration. It is important to note that concentration of leptin and ghrelin may serve as indicators of energy sufficiency, providing valuable information regarding the current energy state of the young trotters.

Materials and Methods

Animals

The effect of the type of exercise which was a part of the usual training routine was studied in a group of 27 clinically healthy young French Standardbred trotters aged 2-3 years. The animals were fed twice per day, at 6.00 and 16.00 with the standard diet designed for athletic horses. A mineralized salt block and fresh water were always available. The horses were divided into two groups according to the type of exercise performed: 10 of them realized the continuous type of exercise and 17 subjects performed the interval type of exercise of medium intensity. Prior to the start of the observation period, the horses had been conditioned in a conventional training program. The type of training, as well as its intensity, was consistent with the principles of the qualified trainer preparing the horses for races. The horses were exercised five days a week. On consecutive days they realized alternately the interval type of exercise and continuous type of effort. This training routine was applied for about 6 months. The study was accepted by the Local Ethical Review Committee for Animal Experiments of the University of Life Sciences in Lublin, and conducted according to the European Community regulations concerning the protection of experimental animals.

Procedures

The study was conducted during two consecutive days in summer time. All horses took part in training sessions which consisted of a 45 minute trot on a sand track. Each session started at least two hours after morning feeding. A group of 17 horses was administered the interval type of exercise. First, the horses had a warm-up of 15 min in trot. Then they were trotted at changeable speed during the next 15 min. Lastly, the horses were trotted for three steps of 500 m each, at increasing speeds: 5.1, 6.7 and 9.2 m/s. After every step they had a rest in walk of 1 min. The exercise was terminated by 10 min of a slow trot. The other group of ten horses realized the continuous exercise. They were continuously trotted on the distance of 11 000 m with the constant speed of 5.1 ± 1.1 m/s.

Three blood samples were collected from each horse via jugular venipuncture using tubes with tri-potassium EDTA. These samples were taken at rest (A), immediately after the end of exercise (B) and 30 minutes after the end of the effort (C). All samples were immediately centrifuged at $2000 \times g$ for 10 min and plasma was stored at -20°C until assayed.

Analyses

Plasma leptin concentration was determined using a multi-species leptin RIA kit (Linco Research, St. Louis, USA) previously validated for the use in horses (McManus and Fitzgerald 2000). The lowest level of leptin that can be detected by this assay is 1 ng/ml. The total ghrelin plasma concentration was determined using the RIA kit (Linco Research, St. Louis, USA) demonstrating the sensitivity at the level of 93 pg/ml. The results were expressed as human equivalents of immunoreactive ghrelin (Gordon and McKeever 2005). Samples were run in duplicate and counted for one minute in a gamma counter (Packard Instruments Company, USA). Blood LA concentration was measured using enzymatic diagnostic kit (Dr Lange, Berlin, Germany) and expressed as mmol/L.

Statistics

The results are presented as means \pm standard error (SE). Statistical analyses were performed using the statistical software package GraphPad Prism (Graph Pad Software, USA). Comparisons between the consecutive groups of horses were analyzed by the use of the unpaired Student's *t*-test and the effects of exercise using the Dunnett's *t*-test. The correlation coefficient was also assessed to compare plasma leptin, ghrelin, LA and glucose concentration, which was described previously (Kędzierski et al. 2007a). The statistical significance was accepted at the level of $p \leq 0.05$.

Results

Leptin

The mean concentration of leptin in the blood plasma of the horses investigated is presented in Table 1. During the continuous exercise, the concentration of this hormone tended to increase after the effort in comparison to the values obtained before exercise and amounted to 4.07 and 3.47 ng/mL, respectively. Moreover, the difference between values of leptin concentration determined immediately after

the end of effort and at rest ($\Delta\text{B-A}$) was significantly higher ($p \leq 0.05$) during continuous than during interval exercise.

Table 1. Plasma leptin concentration in young Standardbred trotters exposed to different types of exercise (means \pm SE, ng/mL).

Samples	Continuous exercise (n = 10)	Interval exercise (n = 17)
A	3.47 \pm 0.78	2.31 \pm 0.15
B	4.07 \pm 0.94	2.36 \pm 0.21
C	3.94 \pm 0.98	2.73 \pm 0.31
$\Delta\text{B-A}$	0.60 \pm 0.21 ^x	0.05 \pm 0.17 ^y
$\Delta\text{C-A}$	0.47 \pm 0.27	0.42 \pm 0.22

A – samples taken at rest/before training, B – just after the end of the effort, C – after 30 minutes rest, n – number of horses

^{x,y} – values compared with rows statistically differ at $p \leq 0.05$ according to *t*-Student test.

Ghrelin

In response to continuous exercise, plasma ghrelin concentrations determined just after the end of effort increased significantly ($p \leq 0.05$) as compared to resting values, and decreased afterwards. The 30 minutes rest, however, was insufficient to reach the resting level of this hormone (Table 2). In contrast, during the interval exercise plasma ghrelin concentration dropped slightly just after the effort as compared to resting values. It reached a significant decrease 30 minutes after the end of exercise ($p \leq 0.05$). The values of ghrelin levels obtained just after the exercise and at rest ($\Delta\text{B-A}$) and 30 minutes after the end of effort and at rest ($\Delta\text{C-A}$), differed significantly statistic-wise, between studied types of exercise ($p \leq 0.01$).

Table 2. Plasma ghrelin concentration in young Standardbred trotters exposed to different types of exercise (means \pm SE, pg/mL).

Samples	Continuous exercise (n = 10)	Interval exercise (n = 17)
A	720 \pm 27.4 ^a	982 \pm 56.5 ^a
B	814 \pm 13.8 ^b	907 \pm 39.4 ^{ab}
C	758 \pm 19.6 ^{ab}	842 \pm 35.6 ^b
$\Delta\text{B-A}$	94.2 \pm 25.4 ^x	-75.8 \pm 52.5 ^y
$\Delta\text{C-A}$	37.7 \pm 20.9 ^x	-140 \pm 61.3 ^y

A – samples taken at rest/before training, B – just after the end of the effort, C – after 30 minutes rest, n – number of horses

^{a,b} – values in columns statistically differ compared to sample A at $p \leq 0.05$ according to Dunnett's *t*-test

^{x,y} – values compared with rows statistically differ at $p \leq 0.05$ according to Student's *t*-test.

LA

The mean level of LA measured immediately after effort in the blood of horses in continuous exercise approximated 0.91 ± 0.03 mmol/L (in range from 0.80 to 1.08 mmol/L). It was significantly different ($p \leq 0.01$) in comparison to the level of interval-exercised horses, in which it raised to 2.36 ± 0.44 mmol/L (in range from 0.98 to 7.23 mmol/L).

The inverse correlation between ghrelin Δ B-A and LA blood concentration was stated on the level of $r = -0.60$. Moreover, the inverse correlation ($r = -0.51$) was also defined between ghrelin Δ B-A value obtained in this study and glucose Δ B-A value described in our previous study (27).

Discussion

The interval type of exercise is applied in training routine to improve horse performance (Shearman et al. 2002). However, the latest studies of Cottin et al. (2005, 2006) suggested the lack of positive effect of this type of training. Although, it is known that in adult mares the interval exercise test was associated with alterations of plasma leptin and ghrelin levels (Gordon et al. 2006). Hence, the major aim of the present study was to evaluate how the different types of exercise influence the leptin and ghrelin profile in young race trotters. The Standardbred horses used in this study were involved in specific types of moderate exercise, namely interval and continuous. The intensity of exercise performed in the present study was verified by measurement of blood LA. Lactate is a product of muscular glucose metabolism and it accumulates in muscle and blood when a high intensity of exercise takes place (Courouze 1999). Several papers revealed that the average blood LA concentration is strongly and positively correlated with the intensity of the effort and after the acute phase of training sessions it exceeded 4.0 mmol/L (Ferrante et al. 1994, Rainger et al. 1994, Guhl et al. 1996). The results of the present study, with the mean level of blood LA amounted to 2.36 mmol/L, proved that the proposed modes of exercise were not highly intensive.

In this study, the performed exercise resulted in the increase in plasma leptin concentration, especially in the continuous type of effort in comparison to more intensive interval effort. In our previous study, acute exercise caused a significant increase in plasma leptin concentration in 1.5-year-old foals, but not in the group of older trotters (Kędzierski and Kapica 2008). Gordon et al. (2007a) did not state any changes in adult horses during exercise tests. On the other hand, no changes in the plasma leptin concentration in athletic horses were observed both during and after the jumping trial (Piccione et al. 2005). Moreover, Gor-

don and coworkers (2006) proved that plasma leptin concentration was not changed during the interval exercise tests, whereas it increased 30 min after the effort. Successive study of Gordon et al. (2007a) showed that short term, high intensive exercise led to a decrease in this hormone concentration in blood plasma 24 hours after the end of the exercise test. These observations are generally in agreement with the results of the present work where, however, only young trained trotters were studied.

The exercise performed by the studied horses also increased plasma free fatty acids (FFAs) concentration (Kędzierski et al. 2007b). FFAs are the principal substrates for the energy production during physical effort. Dotsch et al. (2003) showed that the main function of leptin is not only an anorectic effect but it also increases the hepatic FFAs oxidation. In this light, the increased level of leptin, as a hormone increasing the hepatic FFAs oxidation, can play a positive role in the process of energy metabolism regulation. The precise mechanism involving the rise in plasma leptin concentration during exercise still remains unclear.

In general, the changes in blood leptin concentration can have multidirectional effects. Recent studies in humans and horses have suggested that hypoleptinemia could interfere with proper immunity, bone metabolism, and reproductive function (Eleftheriou et al. 2005, Montez et al. 2005, Waller et al. 2006). This could finally lead to changes in food intake and reduction of body weight (Prentice et al. 2002, Gordon et al. 2007b). However, the results presented in this work showed that the mean concentrations of leptin at rest time in horses reached values higher in comparison to those presented in the study of Gordon et al. (2007b). On the basis of the results by Buff et al. (2005) and Van Weyenberg et al. (2007), which revealed that the leptin concentration is positively related to energy balance in animals, it was possible to conclude that the medium intensive exercise, applied in this experiment, positively influenced the energy homeostasis of the horses through the increase of plasma leptin concentration.

In the studied horses the exercise influenced also the plasma ghrelin concentration. Moreover, a relationship between the changes in the concentration of ghrelin and the intensity of training was found. The differences between values of this hormone obtained just after exercise and at rest were inversely correlated with blood LA level measured after effort. A similar correlation was found between the analogous changes in ghrelin and glucose level, as well. The results of plasma glucose level in studied horses were described previously (Kędzierski et al. 2007a). It is probable, that the intensive exercise, through increasing the plasma glucose level, results in a decrease in plasma ghrelin concentration. Gordon and McKeever (2006) reported that the concentration of this hormone in

horses was reduced after an oral or intravenous glucose challenge. Moreover, Gordon et al. (2007a) did not observe any changes in plasma ghrelin or glucose concentrations during an exercise test. In the light of these facts, a drop in plasma ghrelin concentration in horses takes place after high intensity exercise. It is also correlated with a rise in glucose level. The high level of glucose in exercise tests is a result of an intensification of the glycogenolysis rate. The results of the study by Broglio et al. (2003) suggested that ghrelin could directly stimulate glycogenolysis. The study of Barazzoni et al. (2005) showed that ghrelin might integrate energy balance by decreasing the lipid oxidation in the liver. The decrease in plasma ghrelin concentration, which was shown here after the interval exercise, can promote the use of lipids in the process of energy production. It leads simultaneously to more economical glucose utilization. On the other hand, ghrelin increases appetite and food intake. The lower value of this hormone after exercise can induce a decreased feed intake. This can lead also to losing of weight and condition in trained horses.

In conclusion, the intensity and type of effort influenced the value and the direction of the changes in plasma leptin and ghrelin concentrations. The meaning of the observed phenomenon and the eventual possibility of using the determination of these hormones as a diagnostic method of race trotters require further research.

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