

Influence of short-term exercise on serum leptin concentration in the horse

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ABSTRACT

Leptin is a protein hormone produced predominantly by adipocytes. The influence of physical exercise on serum leptin levels was not studied in the horse. The aim of our research is to evaluate, in this species, the variations of leptin and glucose concentration during show jumping. Moreover, blood lactate concentration was determined in order to evaluate the intensity of the effort. Eight Sella Italiana horses were used for this study. The test consisted of six jumping trials at different heights (between 100 and 140 cm). Blood samples were collected through jugular venipuncture on the following experimental conditions: at rest, immediately after the trial, 30 min after the trial and 24 h after the trial. Blood lactate concentration was immediately analyzed with a field meter (Accusport Boehringer Mannheim, Monza, Italy). Glucose concentration was determined by the enzymatic colorimetric method GOD-PAP, while serum leptin concentration was quantified by using ELISA kits. From analysis of obtained results, applying repeated measures ANOVA, P value for lactate was $P < 0.0001$, with a $F_{(7,21)} = 34.35$ and for glucose was $P < 0.0003$, with a $F_{(7,21)} = 8.706$, while no statistical significance was observed for serum leptin concentration. Obtained results showed that short duration exercise, as show jumping, does not affect serum leptin levels.

Key words: physical exercise, lactate, glucose, leptin, jumping horse

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Introduction

Since the cloning of murine and human obese genes in 1994 (ZHANG et al., 1994), much work has been devoted to elucidating the biology and physiological role of leptin. Leptin has an impact on several physiological systems, including neuroendocrine and immune function, as well as being involved in growth and development (DURSTINE et al., 2001). It is known that plasma leptin concentrations are associated with satiety and that leptin stimulates the oxidation of lipids, increasing energy expenditure (TRAYHURN et al., 1998; BRYSON et al., 1999; BAILE et al., 2000; MEISTER, 2000). These effects suggest that leptin plays a major role in energy homeostasis, providing to hypothalamus information on the amount of body fat and limiting excessive energy storage in adipose tissue of mammals. Although the role of leptin in several physiological areas is only partially understood at best, even less is known about the effect of exercise on plasma leptin concentrations. Studies have been carried out in man (VAN AGGEL-LEIJSEN et al., 1999; ESSIG et al., 2000); some authors have shown that circulating leptin levels in athletes are lower than in sedentary subjects, according to the lower body fat content of the sportsmen and probably as a result of the complex neurohormonal adaptations on the long-term physical training (HALUZIKOVA et al., 2000). The effects of exercise on circulating leptin have been investigated, in man, in response to short- (HOUMARD et al., 2000) and long-term exercise training (PERUSSE et al., 1997; THONG et al., 2000; RESELAND et al., 2001) and following single bouts of exercise (maximal, submaximal, short duration and long duration) (RACETTE et al., 1997; TORJMAN et al., 1999; ESSIG et al., 2000; WELTMAN et al., 2000; FISHER et al., 2001; NINDL et al., 2002). Studies examining the leptin response to exercise have produced conflicting results (PERUSSE et al., 1997; DIRLEWANGER et al., 1999; ESSIG et al., 2000; HILTON and LOUCKS, 2000), but it appears that circulating leptin levels are only decreased by bouts of exercise with considerably high intensity (ELIAS et al., 2000) and long duration (LANDT et al., 1997; LEAL-CERRO et al., 1998; DUCLOS et al., 1999; OLIVE and MILLER, 2001; ZACCARIA et al., 2002). Data from previous investigations suggest that altered plasma leptin concentrations are due to change in energy intake and expenditure balance, as circulating leptin is influenced by physical activity if the exercise session meets an energy expenditure threshold (HICKEY and CALSBEEK, 2001). Thus, the aim of this study is to discuss the influence of exercise on leptin, in particular to examine the effects of jumping on serum leptin concentrations in the athletic horse.

Materials and methods

Eight Sella Italiana horses (4 females and 4 geldings, average age 10 ± 2 years, average body weight 400 ± 30 kg), clinically healthy and specifically trained, were used. Animals were fed three times a day, at 07:00 a.m. on hay, at 1:00 p.m. on commercial pellets and at 6:00 p.m. on both foods, with water ad libitum. Blood samples were collected through

jugular venipuncture using Vacutainer (Terumo corporation) tubes with no additive, on the following experimental conditions: at rest, immediately after the trial, 30 min after the trial and 24 h after the trial. Blood samples were clotted at room temperature for 1h. Samples were then centrifuged at $2000 \times g$ for 20 minutes and sera were stored at -20°C until being assayed for leptin. Blood lactate concentration was immediately determined by a field meter (Accusport Boehringer Mannheim, Monza, Italy), glucose concentration was determined by the GOD-PAP method (TRINDER, 1969), with a standard kit (code 90003026 SEAC, Florence, Italy) and a spectrophotometer (model DU-40, Beckam Instruments, Fullerton, CA); leptin serum concentration was quantified in duplicate, using ELISA (Sandwich) kits (EIA-2395, DRG Instruments GmbH, Germany); we used the procedure suggested by the company. The test (mean duration of $2 \text{ min} \pm 10 \text{ sec}$) consisted of six jumping trials at different heights (between 100 and 140 cm). Before the beginning of the trial, animals underwent a pre-agonistic warm-up consisting of a 5-minute walk, 5 minutes of trot and 5 minutes of gallop. The trial consisted of a 610 meter-long distance, with three wide and five straight 140 cm-high fences, 2 gates (upright-upright and wide-upright) and 1 wall.

Results

All results were expressed as mean \pm SD. Analysis of variance (one-way and repeated measures ANOVA) was used and Bonferroni's test was applied for post hoc comparison. Table 1. shows the mean values of the parameters considered, together with their standard deviations.

Table 1. Average values (Mean \pm SD) of the hematochemical parameters considered, during a show jumping in 8 jumpers

Parameters	Experimental conditions			
	At rest	After trial		
		0 min	30 min	24 h
Lactate (mmol/L)	2.15 \pm 0.50	5.41 \pm 1.26	2.39 \pm 0.40	1.99 \pm 0.41
Glucose (mmol/L)	3.25 \pm 0.24	2.76 \pm 0.20	3.13 \pm 0.24	3.24 \pm 0.20
Leptin (ng/ml)	3.81 \pm 0.45	3.86 \pm 0.39	3.81 \pm 0.19	3.85 \pm 0.19

From analysis of obtained results, applying repeated measures ANOVA, P value for lactate was $P < 0.0001$, with a $F_{(7,21)} = 34.35$ and for glucose was $P < 0.0003$, with a $F_{(7,21)} = 8.706$. By applying Bonferroni's Multiple Comparison Test, lactate showed a statistically significant increase immediately after the trial compared to the value at rest ($P < 0.001$) and

a statistically significant decrease 30 min and 24 h after the trial, compared to the value immediately after the trial ($P < 0.001$); Glucose showed a statistically significant decrease immediately after the trial compared to rest ($P < 0.001$) and a statistically significant increase 24 h after the trial, compared to the value immediately after the trial ($P < 0.001$). No statistically significant differences were observed for leptin. Figure 1. shows the pattern of serum glucose and leptin concentrations observed in the different experimental conditions (at rest, immediately after the trial, 30 min and 24 h after the trial) in 8 jumpers.

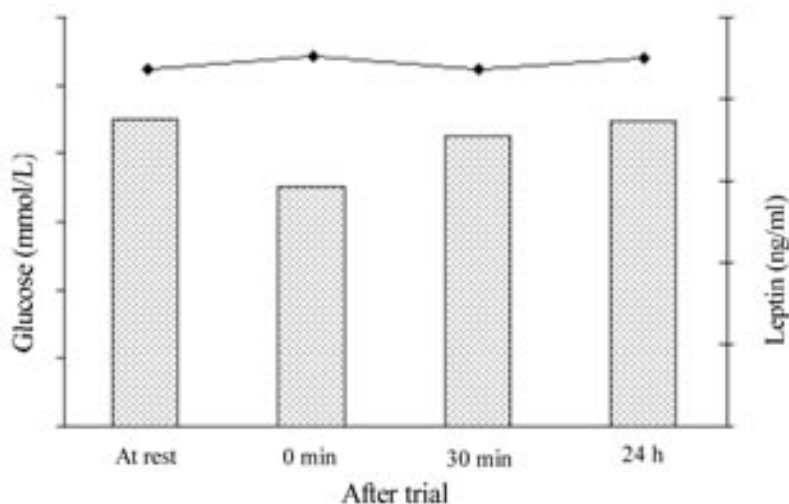


Fig. 1. Glucose (■) and leptin (—◆—) concentrations pattern during the different experimental conditions in 8 jumpers

Discussion and conclusions

Studies which have investigated the effects of physical activity on circulating leptin in man range from designs that employed short duration exercise at varying degrees of intensity to very long duration bouts of considerably high volume. Several investigations suggest that serum leptin concentrations are unaltered by short duration exercise (41 minutes or less) (PERUSSE et al., 1997; WELTMAN et al., 2000; FISHER et al., 2001); in contrast, other studies, which employed exercise bouts of considerably high volume have resulted in decreased leptin (LANDT et al., 1997; LEAL-CERRO et al., 1998; DUCLOS et al., 1999; ESSIG et al., 2000). These data provide support for the notion that an exercise-induced energy expenditure threshold must be achieved in order to have influential effects on circulating leptin concentrations. The delayed response of decreased leptin to exercise (24-48 h) (HICKEY et al., 1996; LANDT et al., 1997; PERUSSE et al., 1997; RACETTE et al.,

1997; TORJMAN et al., 1999) led to the hypothesis that exercise-induced reductions in leptin are probably associated to alterations in nutrient availability at the level of adipocytes, the primary site of leptin production and secretion (HULVER and HOUMARD, 2003). With the support of several studies (OLIVE and MILLER, 2001) a negative energy balance (induced by calorie restriction or physical activity) may result in a reduced glucose flux to the adipocyte and in a decreased synthesis of leptin. In the study of horse athletic performance, knowledge of metabolic factors involved during physical exercise (which vary according to the type of effort required) is important. Our study was carried out on jumpers. With regard to the studied parameters, we observed that they maintained the pattern observed by several authors in previous researches (ART et al., 1990; CAOLA et al., 1991; LEKEUX et al., 1991; PICCIONE et al., 1995; ASSENZA et al., 1996; SCRIBANO et al., 1997): blood lactate concentration showed a maximum value immediately after the trial, confirming that jumping induces an effort which involves both anaerobic and aerobic metabolism. Rapid recovery of athletes (with lactate returning to basal levels 30 min after the trial) shows the good performance of the subjects chosen for our study. As for serum leptin concentrations, the observed values are included within ranges reported in the literature for this species (McMANUS and FITZGERALD, 2000; BUFF et al., 2002). Leptin showed no statistically significant differences by comparing the different experimental conditions, leading to the conclusion that a 2-minute exercise, as in show jumping, does not influence circulating leptin in the jumping horse. Among the parameters studied, glucose returns to basal levels 30 min after the trial, according to previous studies carried out on jumpers (CAOLA et al., 1991; PICCIONE et al., 1995). These data led us to associate no changes in serum leptin observed in our research to rapid reversion of glucose to basal levels after the trial, confirming that short duration exercise, such as a jumping trial, does not affect serum leptin concentrations, according to that observed in the athletic man. Further investigations need to be carried out in order to gain a better understanding of the relationship between leptin and physical activity during long duration and high intensity exercise in athletic horses.

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SAŽETAK

Leptin je hormon koji uglavnom proizvode adipociti. Utjecaj tjelesne vježbe na razinu serumskog leptina u konja dosada nije istražen. U ovom istraživanju cilj je bio utvrditi razlike u koncentraciji leptina i glukoze u tijeku preskakanja prepona. Osim toga, određivana je i koncentracija laktata u krvi radi procjene jačine opterećenja. U istraživanje je bilo uključeno 8 talijanskih Sella konja. Vježba je uključivala 6 preskakivanja različitih visina (između 100 i 140 cm). Uzorci krvi uzeti su iz jugularne vene u sljedećim vremenskim razmacima: u tijeku odmora, neposredno nakon vježbe, 30 min nakon vježbe i 24 sata nakon vježbe. Koncentracija laktata u krvi utvrđena je terenskim instrumentom (Accusport Boehringer Mannheim, Monza, Italy), koncentracija glukoze pomoću enzimske kolorimetrijske metode GOD-PAP, a koncentracija serumskog leptina pomoću ELISA kitova. Primjenom analize ponovljenih mjerenja ANOVA, utvrđene su statistički značajne vrijednosti za laktat $F_{(7,21)} = 34.35$ ($P < 0.0001$) i za glukozu $F_{(7,21)} = 8.706$ ($P < 0.0003$). Razlike u vrijednostima za koncentraciju serumskog leptina nisu bile statistički značajne. Polučeni rezultati pokazuju da kratkotrajna vježba, poput preskakanja prepona ne utječe na koncentraciju serumskog leptina.

Ključne riječi: tjelesna vježba, laktat, glukoza, leptin, konji preponaši
