Short Communication

Effect of gender and jumping exercise on leukocyte number, dopamine and prolactin levels in horses

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Abstract

Physical exercise is one of the most physiologically stressful stimuli which cause animal to experience reversible alterations in various homeostatic variables. The aim of this study was to evaluate the effect of gender and jumping exercise on leukocyte, dopamine and prolactin values and the correlation among these parameters in 20 clinically healthy and regularly trained horses. The horses took part in a three-day jumping competition. Blood sampling was performed before the first day of competition (T0), within 10 minutes from the end of each race (J1, J2, J3) and 24 h after the end of J3 race (T24) in order to assess dopamine, prolactin and leukocyte values. Two-way repeated measures analysis of variance showed significant effect of gender ($P<0.05$) on dopamine and prolactin levels and of exercise ($P<0.0001$) on all studied parameters in the geldings and mares. Dopamine values negatively correlated with prolactin and leukocyte values in the mares and geldings throughout the monitoring period, whereas a significant positive correlation between prolactin and leukocyte values was found in the mares and geldings. The findings of this study suggest that the gonads likely influence the activity of dopaminergic system and the degree of prolactin secretion. The exercise-induced changes in the studied parameters are probably related to the dynamic physiological adaptations to exercise that allow re-establishment of the homeostatic equilibrium of the organism. The correlation found among the studied parameters suggests a possible interaction among immune cells and these hormones, supporting the existence of intercommunication between the endocrine and immune systems.

Keywords: athletic horse, exercise, leukocytes, plasma dopamine, plasma prolactin

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Introduction

Physical exercise induces various stress responses leading to a disturbance of homeostasis and a number of regulatory systems are called upon to return the body to a new level of homeostasis (Arfuso et al., 2016). In particular, the endocrine and nervous systems work in concert to initiate and control movement and all involved physiological processes. Studies carried out on human species (Rojas Vega et al., 2011; Hackney, 2008; Sutoo and Akiyama, 2003; Melis et al., 2003) have shown that, depending on the intensity and duration, exercise stimulates the release of the hormone prolactin as well as the dopamine synthesis, resulting in behavioral and physiological changes. Prolactin is a peptide hormone secreted by the anterior pituitary gland involved in many physiological process including reproduction, homeostasis, growth and development, metabolism, immunoregulation and behavior (Bole-Feysot et al., 1999; Freeman et al., 2000). The neuroendocrine control of prolactin secretion from the anterior pituitary gland involves multiple factors including prolactin releasing (PRFs) and inhibiting (PIFs) factors (Chang and Shin, 1999). Prolactin-releasing stimuli include suckling, light, audition, olfaction and stress; whereas the main physiological Prolactin-inhibiting factor is dopamine (Rojas Vega et al., 2011). It has been stated that the effects of exercise on prolactin are probably mediated by the release of PRFs rather than the inhibition of PIF activity in human studies (Rojas Vega et al., 2011), suggesting that, during exercise, stimulatory input from PRFs overrides the strong inhibitory effect of dopamine. Interestingly, increased prolactin levels are often accompanied by a rise in circulating leukocyte numbers following exercise in human and murine species (Field et al., 1991; Luger et al., 1992; Ortega et al., 1997). Thus, greater numbers of immune cells could interact with increased levels of prolactin in the blood during exercise, leading to overall stimulation of the immune system (Ortega et al., 1997). On the contrary, dopamine has been proposed to be an inhibitor of leukocyte proliferation in humans (Basu and Dasgupta, 2000). *In vivo* studies showed that animals treated with dopamine agonist developed anemia, leukopenia, thrombocytopenia and suppressed antibody response (Nagy et al., 1983). Dopamine is a member of the catecholamine family of neurotransmitters whose cell bodies are located in the pars compacta region of the substantia nigra, an area implicated in the initiation of movement (Meeusen et al., 2001). One of the most consistent findings in animal literature is the observation that, during physical activity, dopamine content and release are increased in brain areas rich in dopamine innervation (Bailey et al., 1993; Hasegawa et al., 2000; Hattori et al., 1994); whereas little information is available regarding its circulating plasma levels following exercise. Although physical exercise has been shown to stimulate prolactin secretion in equine species (Clavier et al., 2012), what is not known is whether the exercise-induced prolactin response is due to an immediate reduction in dopamine values and whether the levels of these hormones are associated with the post-exercise inflammatory process. The aim of this study was to investigate changes in the levels of prolactin, dopamine and white blood cell count in athletic horse after jumping competition in order to improve the knowledge about the relationship between the endocrine and immune systems.

Materials and Methods

The study was carried out on 20 regularly trained Royal Warmblood Horse Studbook of the Netherlands (KWPN) horses (10 geldings and 10 mares, 10-12 years old, 500±20 kg mean body weight). All horses were managed equally, housed in individual boxes, under natural photoperiod and environmental condition (mean temperature of 25±6°C and mean relative humidity of 67±3%). The horses were fed standard rations constituted of hay (first cut meadow hay, sun cured, late cut) and a mixture of cereals (oats and barley, 50% each). The ration was administered three times a day and water was available *ad libitum*. Protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and Directive 2010/63/EU for animal experiments. Each horse enrolled in the current study was transported to the competition site the day before the first competition in order to avoid stress. All horses enrolled in the current study had the same level of training and the same experience of jumping competition. The horses took part in a jumping competition constituted of three days. Each race session was preceded by a 20 minute warm-up consisting of walk, trot and gallop with six jumps (height: from 100 to 140 cm). During the first day the horses competed for the following technical specifications: total length, 550 m; obstacle height, 140 cm; and total efforts, 13 (7 verticals, 6 oxer, 1 triple combination). During the second day the horses competed for the following technical specifications: total length, 600 m; obstacle height, 145 cm; and mixed competition including efforts, 15 (8 verticals, 7 oxer, 1 double combination, 1 triple combination). During the third day of competition the horses competed for the following technical specifications: total length, 600 m; obstacle height, 145 cm; and mixed competition including efforts, 15 (9 verticals, 6 oxer, 1 double combination, 1 triple combination). From each animal blood samples were collected by jugular venipuncture into two vacutainer tubes with cloth activator (Terumo Co., Tokyo, Japan) for prolactin and dopamine analyses, and into vacutainer tubes containing EDTA (Terumo Co., Leuven, Belgium) for evaluation of leukocyte concentration. Blood sampling was performed before the first day of competition (T0), within 10 minutes from the end of each race (J1, J2, J3) and 24 h after the end of J3 race (T24). Immediately after collection, the blood samples were placed in refrigerated bags and transported to the laboratory for analysis. For the assessment of serum dopamine concentration from each horse one tube with cloth activator was centrifuged at 1000 x g for 20 minutes, whereas for the measurement of serum prolactin concentration the other tube with cloth activator was centrifuged at 1000 x g for 15 minutes. Serum dopamine concentration was assessed using a commercially available kit by quantitative Sandwich ELISA method with a sensitivity of 1.0 pg/mL, a detection range of 6.25-200 pg/mL, and both intra-assay and inter-assay coefficient of variability of less than 15%. Serum prolactin concentration was assessed using a commercially available kit by quantitative Sandwich ELISA method with a sensitivity of 3.1 ng/mL, a detection range of 6.25-400 ng/mL, intra-assay coefficient of variability of less than 8%, and inter-assay coefficient of variability of less than 12%. The EDTA whole blood samples were delivered to the laboratory and processed within 2 hours. Values of leukocytes were assessed using an automated hematology analyzer (HeCo Vet C, SEAC, Florence, Italy). All data are expressed as mean ± standard error of the mean (SEM). Two-way repeated measures analysis of variance (ANOVA) was applied to determine statistically significant effect of gender and jumping exercise on the levels of prolactin, dopamine and leukocytes. Bonferroni multiple comparison test was applied for post hoc comparison. The Pearson's Correlation test was performed to assess significant correlations among prolactin, dopamine and leukocyte values obtained from the jumper horses throughout the monitoring period. P values <0.05 were considered statistically significant. Statistical analysis was performed using the STATISTICA software package (STATISTICA 7 Stat Software Inc., Tulsa, Oklahoma).

Results and Discussion

Figure 1 shows the mean values ± SEM of prolactin, dopamine and leukocyte trends recorded in the geldings and mares throughout the monitoring period. The results obtained in the present study showed significant differences in dopamine ($P<0.05$) and prolactin ($P<0.05$) levels due to gender, whereas no change in leukocyte values was found between the mares and geldings ($P>0.05$). In particular, the mares showed lower dopamine and higher prolactin levels than the geldings, suggesting that the gonads likely influence the activity of tuberoinfundibular dopaminergic system and the degree of prolactin secretion (Ben-Jonathan and Hnasko, 2001). This confirms the data obtained by other authors (Colborn et al., 1991; Thompson et al., 2015) who reported different levels of circulating prolactin among mares and geldings. Stimulatory effects on prolactin production and secretion of estrogen in females and androgen in males have been reported in other species and various stimulatory effects of estradiol, testosterone, and dihydrotestosterone on prolactin characteristics in ovariectomized mares were previously demonstrated (Thompson et al., 1991). Significant effect of physical exercise on dopamine and prolactin levels was found in both the mares and geldings ($P<0.0001$). Particularly, decreased dopamine values were found in J1, J2 and J3; whereas an opposite trend was observed for dopamine levels as confirmed by the significant negative correlation found between these hormones. Dopamine, acting on specific brain areas, is related to the motivation for locomotion (Sallis, 2000). Exercise has been mentioned as influencing dopaminergic pathways and receptors (Melis et al., 2003). Exercise modifies brain function, but the mechanism by which it does so is unknown. Animal studies indicate that calcium ions affect brain function and calcium activates tyrosine hydroxylase (Sutoo and Akiyama, 2003), the rate-limiting enzyme for catecholamine synthesis, in the brain through a calmodulin-dependent system. The experiments demonstrated that exercise increased serum calcium levels and serum calcium was transported to the brain where it stimulated dopamine synthesis (Sutoo and Akiyama, 2003). Although it is quite known what occurs in the central nervous system, little information is available from literature about the effect of exercise on dopamine levels at the peripheral level. The decreased dopamine values found in the present study in J1, J2 and J3 at T0 and T24 in both the jumper mares and geldings could be due to the transition of circulating dopamine in the spleen likely to occur during a stressful condition (Rothman et al., 2003). In the spleen, the noradrenergic sympathetic fibers can take up circulating dopamine, which could then be released on sympathetic activation to be taken up in turn by leukocytes through active transport (Gordon and Barnes, 2003). According to a previous study carried out on human and equine athletes (Rojas Vega et al., 2011; Hackney, 2008; Di Giovanni et al., 2015), increased prolactin values were found after physical exercise (J1, J2 and J3) at rest (T0) and recovery (T24) in both mares and geldings. This prolactin response to exercise hypothetically could be due to an immediate antagonism of hypothalamic dopamine input to the pituitary, or to some other stimulation factors related the hypothalamic-hypophysial portal system (Rojas Vega et al., 2011; Hackney, 2008; Di Giovanni et al., 2015). Thus, we could assume that the higher prolactin values after jumping sessions than at rest and recovery conditions is related to the superimposition of stimulatory input of prolactin releasing factors such as thyrotrophin-releasing hormone (TRH), arginine vasopressin (AVP), vasoactive intestinal peptide (VIP) and serotonin (5-HT). According to our hypothesis some authors proposed that exercise-induced hyperprolactinaemia was related to changes in peripheral modulators of serotonergic function in human species (Strüder et al., 1997); other researches carried out in horse showed that the secretion of prolactin was stimulated after administration of VIP and AVP (DiGiovanni et al., 2015). The hyperprolactinemic states following exercise could have a functional explanation since it is well stated that high prolactin levels could have suppressive effects on the reproductive systems in men and women (Ben-Jonathan et al., 2008). In humans as well as many other species, reproductive function is linked to energy reserves and availability (Ben-Jonathan et al., 2008; Brandebourg and Ben-Jonathan, 2007). It is conceivable that prolactin rise during exercise serves as a signal for a reduction in reproductive function due to the reduced or limited energy available induced by strenuous activity (Kelesidis and Mantzoros, 2006; Sone and Osamura, 2001). Moreover, since some types of immune cells synthesize and secrete prolactin (Ben-Jonathan et al., 2008; Brandebourg and Ben-Jonathan, 2007; Dimitrov et al., 2004), it is possible that prolactin plays a key role in the activation of the immune system following exercise. This hormone might serve as a mediator to the post-exercise inflammatory process as well as a mean to initiate aspects of the process in order to allow recovery regeneration and adaptation to exercise (Hackney, 2008). This hypothesis seems to be reinforced by the significant positive correlation found between the values of prolactin and leukocytes throughout the monitoring period. The concentration of circulating leukocytes, as prolactin levels, was increased following exercise in both the mares and geldings. On the contrary, the leukocyte values negatively correlated with the dopamine levels, suggesting an opposite effect of dopamine on the immune system compared to prolactin (Table 1). This hypothesis agrees with previous studies showing an inhibitory effect of dopamine on leukocyte proliferation (Basu and Dasgupta, 2000; Nagy et al., 1983). The functional significance of dopamine and prolactin on the immune system is strengthened by the presence of their receptors and specific endogenous transport system in leukocytes (Gordon and Barnes, 2003; Suzuki et al., 1999). The results of the present study showed a statistically significant increase in leukocyte levels following exercise, although it remained within the reference range throughout the experimental period (Weiss and Wardrop, 2010). The leukocytosis represents one of the most consistent effects of exercise on the immune system (Horohov, 2004). Following high intensity exercise, changes in leukocyte count are likely due to splenic contraction and catecholamine release (Zobba et al., 2011). In exciting conditions including exercise activity, the spleen releases both erythrocytes and leukocytes into peripheral circulation and catecholamines increase cardiac output, hydrostatic blood pressure, and muscular activity. The leukocytes are subsequently swept from the marginated into the circulating pool, transiently increasing the leukocytes concentration. In addition, the mobilization of granulocytes from the marrow pool caused by exercise-induced increases in cortisol concentration may play a role in the higher leukocyte values found after exercise compared to rest condition (Horohov, 2004).

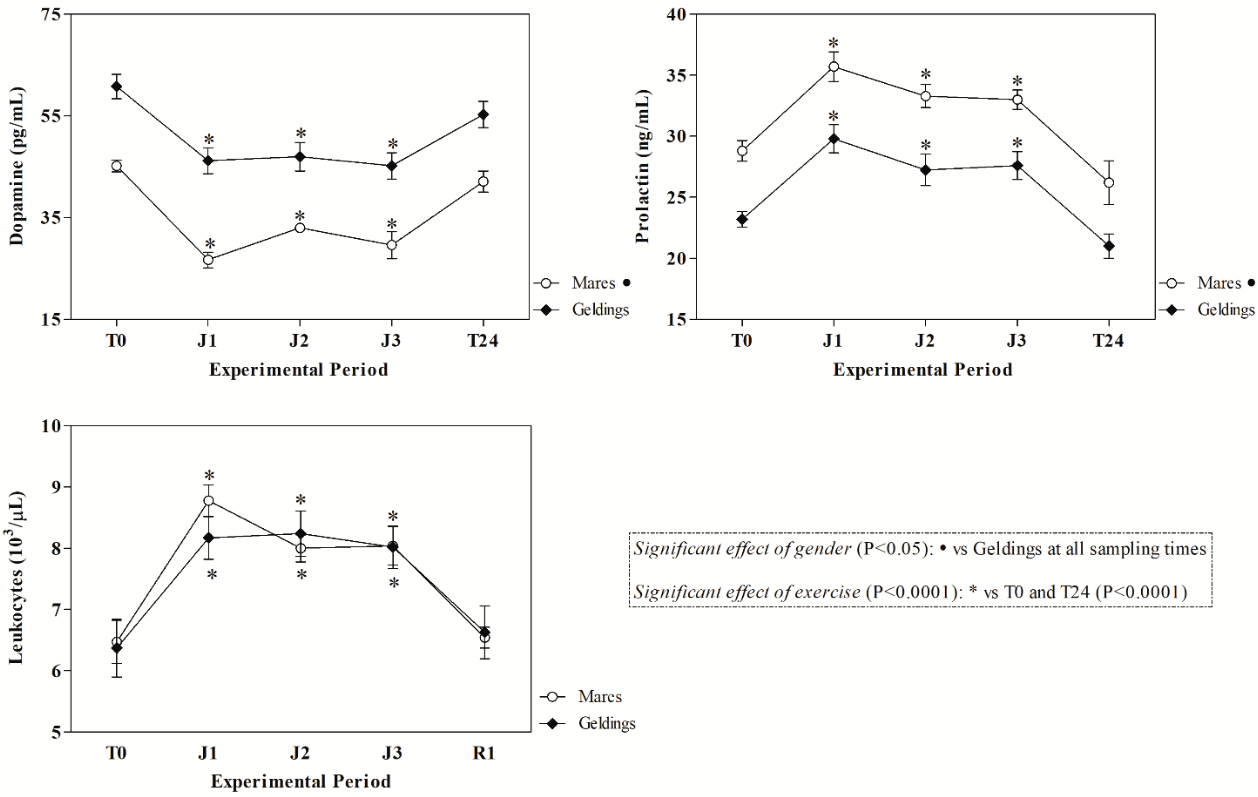

Figure 1 Mean values ± standard error of the mean (±SEM) of serum dopamine, prolactin and leukocyte values obtained from mares and geldings on the first day of competition (T0), within 10 minutes from the end of each race (J1, J2, J3) and 24 h after the end of J3 race (T24)

Table 1 Coefficients of correlation among values of dopamine, prolactin and leukocytes calculated for mares and geldings during experimental period. P values <0.05 were considered statistically significant.

		Prolactin (ng/mL)	***Leukocytes (10³/μL)***
Mares	***Dopamine*** *(pg/mL)*	r = -0.49 P = 0.0003	r = -0.38 P = 0.006
	Prolactin *(ng/mL)*		r = 0.45 P = 0.001
Geldings	***Dopamine*** *(pg/mL)*	r = -0.32 P = 0.02	r = -0.28 P = 0.04
	Prolactin *(ng/mL)*		r = 0.46 P = 0.0009

Conclusion

The results of this study showed that exercise led to changes in the values of peripheral dopamine, prolactin and leukocytes in athletic jumper horses. These findings are probably related to the complex and dynamic physiological adaptations in response to exercise stressor. In addition, the correlations found between the values of leukocytes and dopamine or prolactin throughout the experimental period suggest a possible interaction between immune cells and these hormones, supporting the existence of intercommunication between the endocrine and immune systems.

Conflict of interest statement: The authors do not have any conflicts of interest with themselves or with other organizations.

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บทคัดย่อ

ผลของเพศและการออกกำลังกายแบบกระโดดต่อจำนวน leukocyte ระดับ dopamine และ prolactin ในม้า

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การออกกำลังกายเป็นสิ่งกระตุ้นทางสรีรวิทยาที่เครียดที่สุด และส่งผลให้เกิดการเปลี่ยนแปลงในตัวแปร homeostatic ต่างๆ ในตัวสัตว์ได้ การศึกษานี้มีวัตถุประสงค์ เพื่อประเมินผลของเพศและการออกกำลังกายแบบกระโดดต่อจำนวน leukocyte ระดับ dopamine และระดับ prolactin และความสัมพันธ์ระหว่างตัวแปรเหล่านี้ในม้าที่มีสุขภาพดีและม้าที่ได้รับการฝึก จำนวน 20 ตัว โดยม้าทุกตัวได้เข้าร่วมการแข่งขันกระโดดเป็นเวลา 3 วัน และเก็บตัวอย่างซีรัม ก่อนวันแข่งขัน (T0) ภายใน 10 นาทีนับจากสิ้นสุดการแข่งขันแต่ละครั้ง (J1, J2, J3) และ 24 ชั่วโมงหลังจากสิ้นสุดการแข่งขัน J3 (T24) เพื่อประเมินระดับ dopamine, ระดับ prolactin และจำนวน leukocyte ผลการวิเคราะห์ความแปรปรวนแบบสองทิศทางพบว่า เพศของม้า มีผลต่อระดับ dopamine และ prolactin ซึ่งมีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P <0.05$) และ สอดคล้องกันกับผลการออกกำลังกาย ($P <0.0001$) และพบว่าระดับ Dopamine มีความสัมพันธ์เชิงลบกับระดับ prolactin และจำนวน leukocyte ในขณะที่พบความสัมพันธ์เชิงบวกของระดับ prolactin และจำนวน leukocyte ในม้าตัวเมียและม้าตัวผู้ตอน ผลการศึกษานี้ชี้ให้เห็นว่า เพศมีผลต่อการทำงานของระบบ dopamine และระดับการหลั่งสาร prolactin และ การออกกำลังกายมีผลต่อทุกตัวแปรที่ศึกษา ซึ่งอาจเกี่ยวข้องกับการปรับตัวทางสรีรวิทยาแบบไดนามิก เพื่อสร้างสมดุลของ homeostatic โดยสรุปพบความสัมพันธ์ระหว่างจำนวนเซลล์ภูมิคุ้มกันและระดับฮอร์โมน ซึ่งสนับสนุนการเกี่ยวข้องกันของระบบต่อมไร้ท่อและระบบภูมิคุ้มกัน

คำสำคัญ: ม้าแข็งแรง การออกกำลังกาย leukocytes dopamine พลาสม่า พลาสม่า prolactin

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