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Peripheral modulators of the central fatigue development and their relationship with athletic performance in jumper horses

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Simple Summary: 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 equilibrium. The aim of this study was to evaluate the peripheral modulators of serotoninergic function and neurohumoral factors changes in athletic horses during an official jumping competition, and to evaluate their relationship with the physical performance of competing horses. The findings obtained in the current survey showed that jumping exercise influenced the levels of tryptophan, leucine, vaine, non-esterified fatty acids (NEFAs), dopamine and prolactin and that these changes are related with physical performance of competing horses. These findings suggest that the serotoninergic system may be involved in fatigue during jumping exercise.

Abstract: The current study aimed to investigate whether peripheral modulators of serotoninergic function and neurohumoral factors changes in athletic horses during an official jumping competition, and to evaluate their relationship with the physical performance of competing horses. From 7 Italian Saddle mares (6-9 years; mean body weight 440 ± 15 kg), performing the same standardized warm-up and jumping course during an official class, heart rate (HR) was monitored throughout the competition. Rectal temperature (RT) measurement, blood lactate and glucose, serum tryptophan, leucine, valine, the tryptophan/branched-chain amino-acids ratio (Try/BCAAs), dopamine, prolactin and non-esterified fatty acids (NEFAs) were assessed before the exercise event (T0), at the end of competition stage (5 min ± 10 sec following the cessation of the exercise, T_{POST5}), and 30 min after the end of competition (T_{POST30}). Highest HR values were recorded during the course and at the outbound ($P<0.0001$); blood lactate and RT increased after exercise respect to rest condition ($P<0.0001$). Lower leucine and valine levels ($P<0.01$), and higher tryptophan, Try/BCAAs ratio and NEFAs values were found at T_{POST5} and T_{POST30} respect to T0 ($P<0.0001$). Higher prolactin concentration was found atT_{POST5} and T_{POST30} compared to T0 ($P<0.0001$), whereas dopamine showed decreased values after exercise compared to rest ($P<0.0001$). Statistical significant correlations among the peripheral indices of serotoninergic function, neurohumoral factors and athletic performance parameters were found throughout monitoring period. The findings provide indirect evidence that the serotoninergic system may be involved in fatigue during jumper exercise under stressful situation as competition, in which, in addition to physical effort, athletic horses experience emotional reactivity and mental stress.

Keywords: jumping exercise; horses; amino-acids; athletic performance; serum dopamine; serum prolactin

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1. Introduction

Animals competing in equestrian disciplines are required to have high technical skills. Among the parameters considered for the evaluation of athletic performance in

horse, heart rate and blood lactate are the most studied as these parameters are good indices of the fitness level and of workload effort. Cardiovascular, respiratory, metabolic responses and musculoskeletal adaptations to exercise have been well studied in equine species allowing specific exercise testing for racehorses [1]. However, few studies have dealt with exercise testing for event [2], dressage [3] and jumper horses [4], thus a paucity of information is available on physical performance and on fatigue signals arising in athletic horse during official competition.

Despite its current popularity, the cause(s) of fatigue and its underlying mechanism(s) have only recently begun to be explored in the athlete horse. Fatigue is an important factor affecting exercise and sporting performances. It is defined physiologically as the inability to maintain power output, [5,6] and the organism uses it as a defense mechanism to avoid irreversible damage due to excessive exertion. Fatigue during exercise has traditionally been considered to be the result of events localized within skeletal muscle [7]. However, fatigue cannot always be ascribed to events residing within skeletal muscle, indeed, it is a complex multifactorial element with peripheral and central components. Central fatigue develops in the central nervous system and involves brain serotonin level [8]. Although it seems naive to assume that the activity of serotoninergic neurons should be determined by substrate availability alone, the central fatigue hypothesis proposes that this situation may arise during periods of stress, such as those occurring during exercise and/or competition. The serotonergic system is associated with numerous brain functions that can positively or negatively affect endurance [9] Accordingly, the synthesis and metabolism of serotonin in the brain increases in response to exercise [10]. Furthermore, the rise of brain serotonin concentration is associated with markers of central fatigue such as decreased motivation, lethargy, tiredness and loss of motor coordination [9]. Increase of serotonin synthesis in the brain is correlated with high levels of blood-borne tryptophan, the amino-acid precursor to serotonin. The rate limiting step in the synthesis of serotonin is the transport of tryptophan across the blood-brain barrier into the brain [11].

Besides to tryptophan, other compounds in the bloodstream, such as branched-chain amino-acids (BCAAs), lipids, hormones and neurotransmitters can orchestrate the fine mechanism of the onset of fatigue. During and after exercise, the BCAAs intervene in the muscle protein synthesis by stimulating mRNA translation [12] and they prevent muscle proteolysis by inhibiting mechanisms that involve the mammalian target of rapamycin [13,14]. The increased uptake of BCAAs and their oxidation by skeletal muscle will reduce their concentration of available for competition with tryptophan for transport across the blood–brain barrier resulting to an increase in the uptake of tryptophan to the brain, and therefore synthesis and release of serotonin by the brain [15]. Studies carried out on human species [16,17,18,19] 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. Indeed, emotional or physical stress can temporarily increase prolactin levels. Prolactin also interacts with the dopaminergic system and has been linked to anxiety in several species. Influence of neurotransmitters on fatigue has also been proposed, and dopamine seems to be linked to the 'central' component of fatigue for its well-known role on motivation and motor behavior and it is therefore thought to have an enhancing effect on performance [20,21].

Although show jumping is part of the Olympic equestrian disciplines, and despite the need to monitor fitness and workload, there is a paucity of information about field exercise testing in jumper horses during official competition [4,22] and none of these tests assessed the indices of serotoninergic function and their relationship with exercise performance.

In view of these considerations, the current study aimed to investigate the effects of jumping exercise on some peripheral modulators of serotoninergic function (e.g. tryptophan, Tryptophan/BCAAs ratio, NEFAs) and neurohumoral factors (e.g dopamine, prolactin) as well as their relationship with physical performance in high-level jumper horses

performing the same standardized warm-up and jumping course during an official competition.

2. Materials and Methods

Animals and experimental design. All treatments, housing, and animal care were carried out in accordance with the standards recommended by the European Directive 2010/63/EU for animal experiments. This study was carried out on 7 Italian Saddle mares (6-9 years; mean body weight 448 ± 15 kg), after the informed consent of the owners. All horses were managed equally at the same horse training center in Sicily, Italy (latitude 38°10′ 35′′N; longitude 13°18′14′′E), housed in individual boxes (3.5 x 3.5 m), under natural photoperiod and environmental conditions (mean temperature of 26±5ºC and mean relative humidity of 66±4%). All horses were trained and ridden by the same trainer and rider, respectively. Diet composition for horses was formulated according to their training requirements. In particular, animals were fed twice a day (07.00 AM; 05.00 PM), with a total food amount of about 2.5% dry-mater of horse body weight (forage:concentrate ratio 70:30) and water was available ad libitum. All animals were clinically healthy (based on a thorough clinical examination) and free from internal and external parasites. The horses took part in an outdoor jumping competition. The session was preceded by a warm-up on the flat consisting of walk 1 (1 min), trot (3 min), walk 2 (1 min), canter (2 min) and walk 3 (1 min). the next stage of standardized warm-up included 4 vertical and 4 oxers jumps of increasing height (height: from 100 to 140 cm). The trainer timed each warm-up stage and used the same sequence of jumps for all subjects (alternating verticals and oxers with the same eight). After the warm-up, horses competed in the same jumping course with the following technical specifications: total length, 500 m; obstacle height, 140 cm; and total efforts, 15 (9 verticals, 6 oxer, 1 double combination, 1 triple combination). The competition stage was made up of four phases including: inbound (waiting time inside the arena before competing), course (time of the jumping phase), outbound (time lapse between the end of the course and the exit from the arena), end (time lapse between the exit and the arrival to the stable).

Rectal temperature (RT) and heart rate (HR) measurement. The RT, taken as representative of body temperature, was measured by a means of a digital thermometer (HI92704, Hanna Instruments Bedfordshire, UK), inserted 15 cm in the rectum before the exercise event (T0), at the end of competition stage (5 min ± 10 sec following the cessation of the exercise, TPOST5), and 30 min after the end of competition (TPOST30).

In order to evaluate the workload during the competition, each horse was equipped with equine HR monitors (Polar Horse Trainer, S 610, Polar Electro Europe BV, Fleurier Branch, Switzerland) to record HR during each step of warm-up and competition. Two electrodes were placed against the horse's wet coat: the positive electrode was first placed under the saddlepad, the negative electrode was then fixed to the saddle girth on the left side of thorax, and finally the electrodes were connected to a transmitter (T51H), fixed to a breast strap, that transmitted data to a watch-type data logger (Polar S-610I), placed near the electrodes. Recorded data were then downloaded on a personal computer, by using the Polar Equine 4.0 software, to be analyzed. In particular, HR was logged every 5 seconds during the whole exercise. The values of HR measured before the exercise event at rest (T0), during each step of warm-up including walk, trot, canter, jumps 1-8, and competition including inbound, course, outbound, end of competition stage (5 min ± 10 sec) following the cessation of the exercise, T_{POST5}), and 30 minutes after the end of competition (T_{POST30}).

Blood sampling procedures and laboratory analysis. Blood collection was performed by the same operator before the exercise event (T0), at the end of competition stage (5 min ± 10 sec following the cessation of the exercise, T_{POST5}), and 30 min after the end of competition (T_{POST30}). The samples were collected by jugular venipuncture into EDTA vacuum test tubes (Terumo Corporation, Tokyo, Japan) to assess blood lactate and glucose concentration immediately after collection using a small handheld meter (Accutrend Plus,

Roche Diagnostics, Switzerland). Moreover, from each subject blood samples were collected into three vacuum tubes with clot activator (Terumo Corporation, Tokyo, Japan) which were placed in refrigerated bags and transported to the laboratory for analysis. One tube with clot activator was centrifuged at 1,300g for 10 minutes and the individual serum samples obtained were deproteinized with 5-sulfosalicylic acid and leucine, valine, and tryptophan concentrations were assessed by highperformance liquid chromatography method. The ratio between tryptophan and BCAAs levels (Try/BCAAs) has been then calculated for each sample. On serum samples, the concentration of serum total proteins (biuret method) and NEFAs was determined by means of commercially available kits (total proteins, Byosistems, Reagents and Instruments, Barcelona, Spain; NEFAs, Randox, Crumlin, UK) by means of an automated analyzer ultraviolet–visible spectrophotometer (model Slim SEAC, Florence, Italy).

The second tube with cloth activator was centrifuged at 1000 x g for 20 minutes for the assessment of serum dopamine concentration, whereas for the measurement of serum prolactin concentration the third 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%.

Statistical analysis. Before statistical analysis, all data were tested for normality of distribution using the Kolmogorov–Smirnov test. All data resulted normally distributed ($P > 0.05$) and parametric statistical analysis was performed. One-way repeated measures analysis of variance (ANOVA) was applied to determine statistically significant differences in the values of RT, HR, blood lactate and glucose, serum tryptophan, leucine, valine, Try/BCAAs, total proteins, NEFAs, dopamine and prolactin obtained from each horse during monitoring period. Bonferroni multiple comparison test was applied for post hoc comparison.

The Pearson's Correlation test was performed to assess whether the peripheral modulators of serotorinergic function (e.g. tryptophan, leucine, valine, Try/BCCAs, NEFAs) correlated with themselves, and with neurohumoral factors (e.g. domanine and prolactin), as well as with indices of athletic performance in jumper horses throughout monitoring period.

P values < 0.05were considered statistically significant. Data were analyzed using statistical software Prism v. 4.00 (Graphpad Software Ldt, USA, 2003).

3. Results

All the results are expressed as mean values ± standard deviation (± SD).

As displayed in Figure 1, HR values recorded from each horse showed a statistically significant dynamic change throughout the official competition ($P<0.0001$) with higher values at each step of competition compared to T0 and T_{POST30} ($P<0.0001$). Moreover, highest HR values were recorded during the course and at the outbound. The statistical analysis of data of HR recorded during the jumping stage of the warm-up showed a significant increase of HR values when shifting from 100 to 125 cm jumps ($P<0.001$). As showed in Figure 2 blood lactate showed higher values at T_{POST5} respect to T0 and TPOST30 ($P<0.001$), also RT increased after exercise (T_{POST5} and T_{POST30}) respect to rest condition ($P<0.0001$), whereas total proteins and glucose showed unchanged values after exercise compared to rest condition ($P>0.05$). According to the findings gathered from the application of one-way ANOVA and as showed in Figure 3, leucine and valine showed lower levels ($P<0.01$) after exercise (T_{POST5} and T_{POST30}) compared to baseline values measured at rest (T0), whereas higher tryptophan, Try/BCAAs ratio and NEFAs values were found at T_{POST5} and T_{POST30} respect to T0 ($P<0.0001$). Higher prolactin concentration was found atT_{POST5} and T_{POST30} compared to T0 ($P<0.0001$), whereas dopamine showed lower values after exercise compared to rest ($P<0.0001$).

Statistical significant correlations among the peripheral indices of serotoninergic function, neurohumoral factors and athletic performance parameters were found throughout monitoring period (Table 1).

Table 1. Coefficients of correlation among peripheral modulators of serotorinergic function (e.g. tryptophan; leucine; valine; Try/BCCAs ratio; NEFAs), neurohumoral factors (e.g. domanine and prolactin), and indices of athletic performance (e.g. heart rate, HR; rectal temperature, RT; blood lactate; blood glucose) in jumper horses throughout monitoring period. values and age calculated for males and females of foals and for males and females of horses during the monitoring period. P values <0.05 were considered statistically significant.

	Try/BCAAs	**Prolactin (ng/mL)**	**Dopamine (pg/mL)**	**NEFAs (μmol/L)**	**HR (beats/min)**	**RT (°C)**	**Lactate (mmol/L)**	**Glucose (mmol/L)**
Tryptophan (mmol/L)	r = 0.99 P < 0.0001	r = -0.26 P = 0.29	r = -0-97 P < 0.0001	r = 0.75 P < 0.0001	r = 0.69 P = 0.002	r = 0.88 P < 0.0001	r = 0.75 P < 0.0001	r = -0.26 P = 0.29
Leucine (mmol/L)	r = -0.84 P < 0.0001	r = -0.26 P = 0.29	r = 0.79 P = 0.0001	r = 0.75 P < 0.0001	r = -0.57 P = 0.01	r = -0.66 P = 0.002	r = -0.69 P = 0.001	r = 0.5 P = 0.84
Valine (mmol/L)	r = -0.90 P < 0.0001	r = -0.26 P = 0.29	r = 0.89 P < 0.0001	r = 0.75 P < 0.0001	r = -0.69 P = 0.001	r = -0.81 P < 0.0001	r = -0.68 P = 0.002	r = 0.09 P = 0.70
Try/BCAAs		r = 0.89 P < 0.0001	r = -0.97 P < 0.0001	r = 0.97 P < 0.0001	r = 0.69 P = 0.002	r = 0.88 P < 0.0001	r = 0.75 P < 0.0001	r = -0.26 P = 0.29
Prolactin (ng/mL)	r = 0.89 P < 0.0001		r = -0.91 P < 0.0001	r = 0.93 P < 0.0001	r = 0.86 P < 0.0001	r = 0.92 P < 0.0001	r = 0.87 P < 0.0001	r = -0.36 P = 0.014
Dopamine (pg/mL)	r = -0-97 P < 0.0001	r = -0.91 P < 0.0001		r = - 0.99 P < 0.0001	r = -0.78 P = 0.0001	r = -0.93 P < 0.0001	r = -0.80 P < 0.0001	r = 0.27 P = 0.29

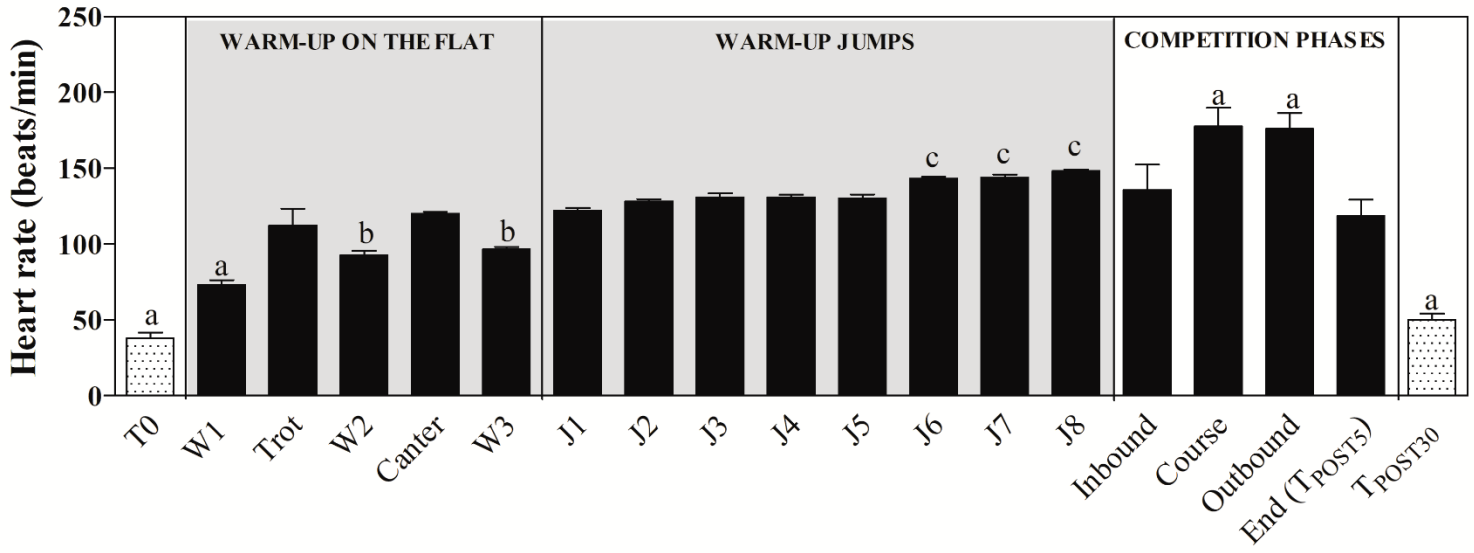


Figure 1. Heart rate values recorded from each horse during the monitoring period. The warm-up on the flat included walk (W1-W3), trot and canter section followed by 8 warm-up jumps (J1-J8). The competition in the show jumping arena include inbound (waiting time inside the arena before competing), course (time of the jumping phase), outbound (time lapse between the end of the course and the exit from the arena), end (time lapse between the exit and the arrival to the stable).

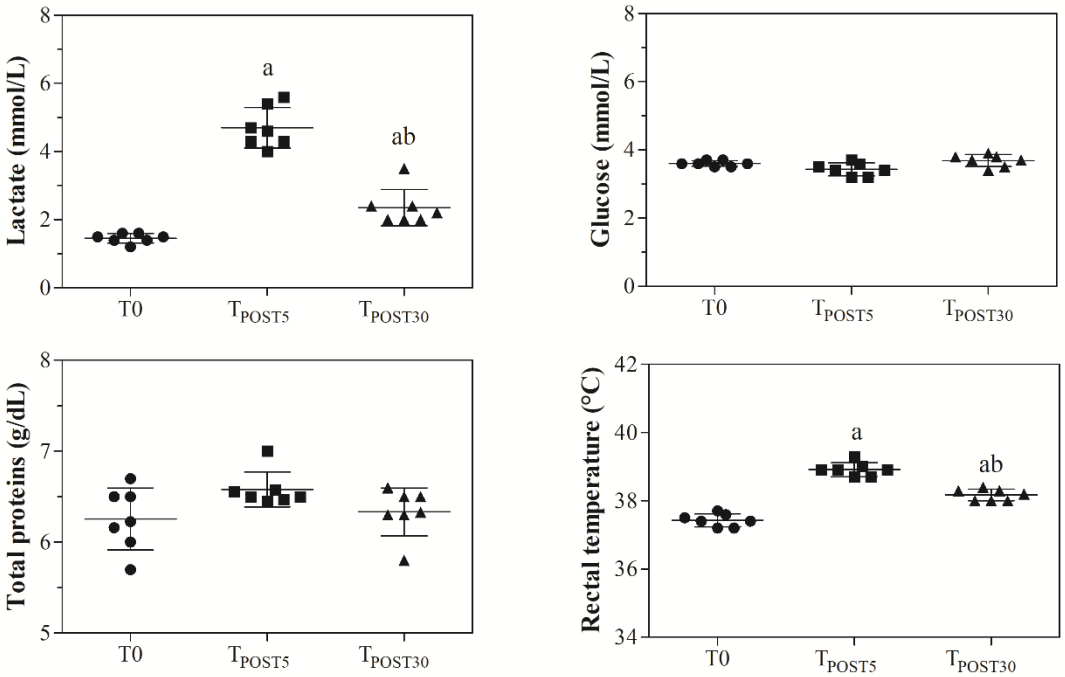


Figure 2. Blood lactate, blood glucose, serum total proteins and rectal temperature measured in each competing horse before the exercise event (T0), at the end of competition stage (5 min ± 10 sec) following the cessation of the exercise, T_{POST5}), and 30 minutes after the end of competition (T_{POST30}).

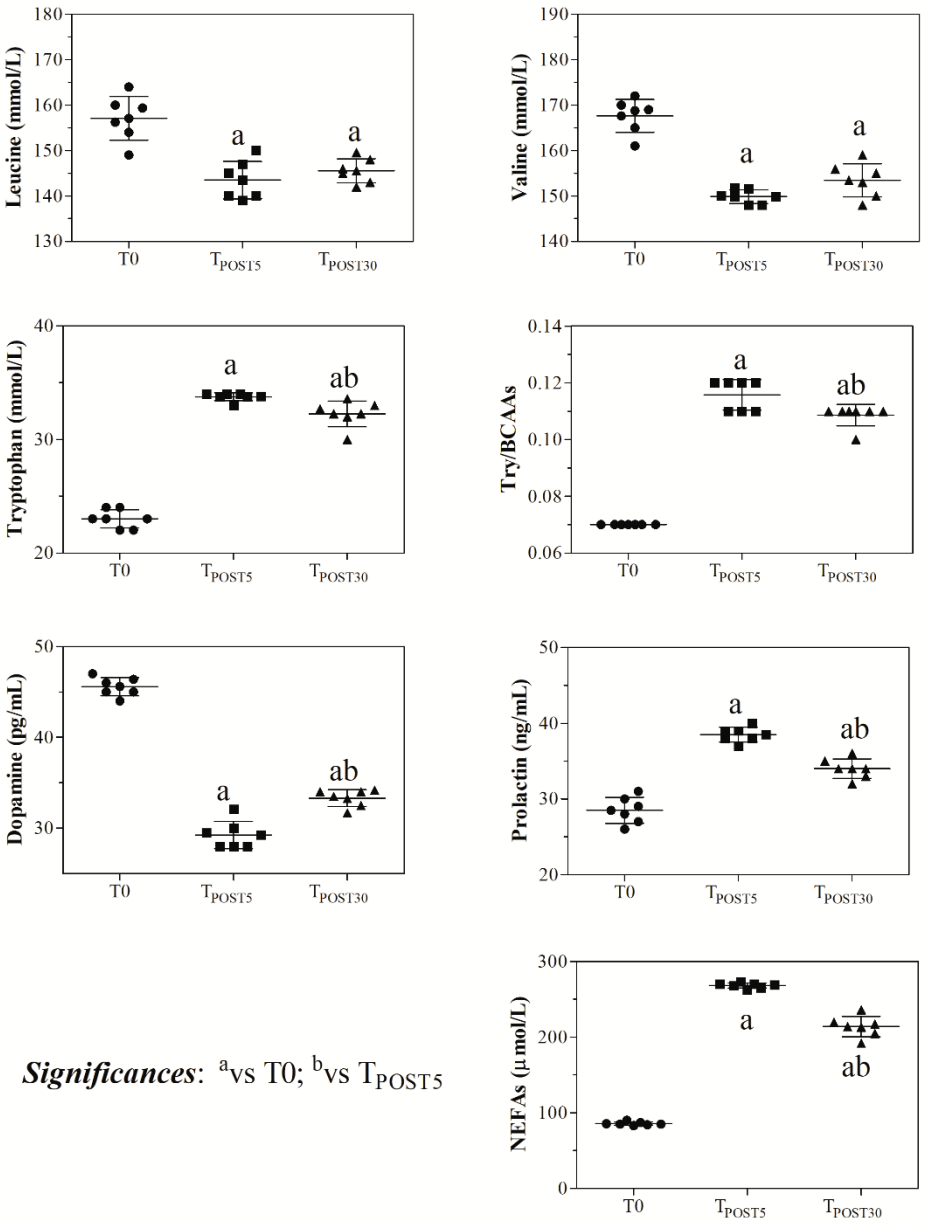


Figure 3. Serum amino-acids (e.g. tryptophan, leucine, valine), Try/BCAAs ratio, NEFAs, dopamine and prolactin values measured in each competing horse before the exercise event (T0), at the end of competition stage (5 min ± 10 sec) following the cessation of the exercise, T_{POST5}), and 30 minutes after the end of competition (T_{POST30}).

4. Discussion

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 equilibrium [23]. The nervous and endocrine systems work in concert to initiate and control movement and all involved physiological processes. According to the findings obtained in the current study the main indices of horses' fitness showed dynamic changes throughout monitoring period. Specifically, in agreement with previous studies investigating horse performance during official jumping competition [22,24], the results of the current study confirmed the significant contribution of anaerobic metabolism during jumping exercise [1] showing an increase of blood lactate 5 min after completing the exercise followed by a decline 30 min after the end of competition. As a matter of facts, although within a few minutes the lactate release start to decrease because removal from the circulation exceeds the release rate from the muscles [25], it is known that it takes 30 min to clear lactic acid from the muscles and bloodstream after exercise [26]. The HR of each subject is influenced by each step of both warm-up and jumping course of the competition showing highest values during and immediately after the jumping course. Noteworthy, the high HR values registered during the inbound to the show jumping arena highlighted that the official competition itself represented a contributing factor to HR increase as mental stress [27] and emotionality [28]. The HR may indicate the animal's internal physiological state, indeed, the rise in HR is not only caused by an increase in physical activity, but also reflects heightened emotional reactivity [28]. The trend of HR registered in jumper horses of the current survey agrees with the observations of a previous study carried out on Belgian Saddlebreeds competing in the Belgian Championship [22] in which, it has been suggested that horses experienced a need for accelerating near the finish of competition causing the HR increase due to the onset of fatigue. Furthermore, the results gathered in the present study highlighted that the obstacle height caused an increase of HR values in competing horses. The rise in HR could be due to the sympathetic nervous activity known to increase with increasing exercise intensity leading to a consistent rise of catecholamine levels. It is well established that the increase of the catecholamine concentration enhances the force of cardiac contraction and thus the cardiac output and the increase of HR. The results obtained in this study showed a significant increase of RT after physical exercise and this was not surprising, indeed, during exercise, horses' muscles work and a conversion of stored chemical energy into mechanical energy and thermal energy occurs [29,30]. The heat produced by muscle during exercise is such as to raise core temperature. Therefore, heat dissipation mechanisms should be activated. The primary physiologic mechanism leading to heat dissipation is represented by the increase in cardiac output that brings to heat transfer from core to the cutaneous blood circulation by eliciting reflex neurogenic vasodilatation in the skin resulting in increased skin temperature [29].

Body temperature is controlled by the opposing effects of adrenergic and serotoninergic neuronal activity in the hypothalamus. A rise in body temperature may be due to an augmented serotoninergic activity combined with the inhibition of adrenergic activity, or purely due to the increased serotoninergic activity [31]. Noteworthy, the results herein found seem to suggest that the potential peripheral modulators of serotoninergic function measured in athletic horses during a jumping competition tended to respond as predicted in the "central fatigue hypothesis" by Newsholme et al. [8]. The levels of leucine, valine and dopamine decreased after exercise in competing horses, whereas, tryptophan, Try/BCAAs ratio, prolactin and NEFAs showed increased concentration following jumping competition exercise. It could be hypothesized that the BCAAs were used by muscles, mainly active skeletal muscle, during short-term intense exercise as a source of energy for muscle contraction [6]. The increase of tryptophan found after exercise could be related to the rise of lipid content in the blood likely to occur during exercise as confirmed by the positive correlation found between the values of this amino-acid and NEFAs throughout monitoring period. During physical exercise, free fatty acids are mobilized from adipose

tissue and are transported via the blood to the muscle where they serve as a fuel for muscle contraction [23]. Both free fatty acids and tryptophan compete for binding to albumin and thus, the increase of NEFAs concentration during exercise, resulting from an elevated adrenergic tone that stimulate subcutaneous lipolysis [32], could limit the binding of tryptophan and albumin, increasing the concentration of serum free tryptophan. The uptake of BCAAs by the active muscle as a source of energy yields to the increase of tryptophan/BCAA ratio after exercise [33]. The decrease in the BCAAs blood serum levels and the increase of the tryptophan level after jumper exercise could increase the rate of passage of tryptophan across the blood-brain barrier because BCAAs and tryptophan compete for the same transporter [34,35,36]. Thus, an enhanced entry of tryptophan into the brain would lead to increased serotonin levels that ultimately lead to anticipated central fatigue [33].The changes in the values of peripheral modulators of serotoninergic function found in competing horses after exercise showed a relationship with the considered indices of exercise performance, as emphasized by the positive correlation found between the serum levels of tryptophan, Try/BCCAs and NEFAs and the values of blood lactate, HR and RT. On the other hand, the amino-acids leucine and valine showed a negative correlation with the values of blood lactate, HR and RT measured in competing horses affetr exercise. Noteworthy, the peripheral modulators of serotoninergic function resulted negatively correlated with dopamine and a positively correlated with prolactin suggesting a possible linkage among the central fatigue and the neurohumoral factors. Dopamine, acting on specific brain areas, is related to the motivation for locomotion [21]. Exercise may elevate the global dopamine level in the brain without affecting the cerebral release via the jugular venous blood, because polar catecholamines do not readily penetrate the blood-brain barrier [37,38], and maybe only dopamine released into the hypophysial portal blood will appear in the jugular blood [39]. However, in healthy subjects, the brain demonstrates noradrenaline spillover into both the major and the minor jugular vein [40], and, although the passage of monoamines from the bloodstream to the brain is restricted by the blood-brain barrier, the existence of a barrier to movement in the opposite direction is less certain [41]. 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 after the competition conclusion could be due to the transition of circulating dopamine in the spleen likely to occur during a stressful condition [42]. 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 [43]. The finding of the current study showed an increase of prolactin levels after jumping competition that, 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 [16,44]. Thus, it could be assumed that the higher prolactin values recorded after competition is related to the superimposition of stimulatory input of prolactin releasing factors such as thyrotrophin-releasing hormone, arginine vasopressin, vasoactive intestinal peptide and serotonin. Noteworthy, the values of prolactin resulted positively correlated with the fitness' and stress indices, including HR, blood lactate and RT, recorded from competing horses, however, whether this hormone is involved in the increased perception of effort and the earlier onset of fatigue during exercise remains to be determined. It has been proposed that exercise-induced hyperprolactinaemia was related to changes in peripheral modulators of serotonergic function in human species [45]. However, studies on the possible relationship of prolactin secretion and peripheral modulators of serotoninergic function during exercise showed conflicting results. Fischer et al. [46] found an increase in prolactin concentration during exercise in proportion to the rise in the plasma free tryptophan concentration. On the other hand, prolactin secretion during exercise has subsequently been reported to be unrelated to the peripheral modulators of serotoninergic function [47].

5. Conclusions

To the best of authors' knowledge, this is the first study investigating the effect of jumping exercise on peripheral modulators and indices of serotoninergic function and their relationship to exercise performance in horses competing in an official jumping class. The findings provide indirect evidence that the serotoninergic system may be involved in fatigue during jumping exercise under stressful situation as competition, where, in addition to physical effort, athletic horses experienced emotional reactivity and mental stress. Although a relationship among peripheral modulators, neurohormones and indices of fitness seems to be exist during jumping exercise in horses, further studies are demanded to determine whether this linkage is responsible for the increased perception of effort and the earlier onset of fatigue during both training and competition in athletic horses. This would contribute to improve the knowledge on the horse's response to the jumping effort, and allow to better understand how a horse is coping with its training exercise or the emotional stress of a competition that will in turn contribute to improving animal welfare and athletic performance.

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