Monitoring steroid hormones in horses performing intense exercise loads

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Abstract. Performance of loads of different intensity and power by horses is accompanied by changes in the balance of metabolic processes provoked by the corresponding hormones. In this regard, the aim of the study was to evaluate the dynamics of changes in the level of steroid hormones when horses perform high-speed and speed-strength loads. The study involved 8 Russian trotters (6 stallions and 2 mares) of the running class 2.05 and faster, as well as 8 stallions of draft breeds (5 Soviet heavy draft breeds and 1 Vladimir breed, as well as 2 representatives of the Vyatka breed) prepared for testing under the heavy draft eventing program. Trotters performed a sweeping movement over a distance of 1600 m with a speed of more than 3 minutes. Draft horses trotted 2000 m with a traction force of 50 kg (heavy draft horses) and 30 kg (Vyatka stallions). The testosterone and cortisol levels were determined in the horses' blood taken before the start and after the finish. The data obtained showed that high-speed and high-speed-strength loads caused similar changes in the hormonal status of trotters and harness horses. The background and post-load testosterone levels in the horses' blood are more significantly affected by the length of the preliminary training than by the intensity and power of the load itself. Older and high-class trotters are capable of repeatedly increasing the level of cortisol in the blood in response to a high-speed (swinging) load. The muscle work performed by trotters and harness horses did not cause an imbalance of anabolic and catabolic processes in their bodies, which reflects the adequacy of the load presented to the current state of their training.

1 Introduction

Selection and exploitation of horses, for the most part, pursues the goal of obtaining muscle strength in any form. All candidates for the breeding production staff of horses of almost all breeds undergo (or are required to undergo) long-term training of varying intensity. Tightening approaches to issuing licenses for obtaining the status of a breeding farm requires owners to make a mandatory assessment of the performance of both stallions and mares recommended for reproduction. In this case, it is natural that interest in studying the impact of training and competitive loads of various types on the body of horses of different

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ages and sexes increases. Domestic scientists have clarified the issue of the consequences of the exploitation of fast-paced horses for their fruitful career, mainly hippodrome [1-8]. Statistically significant evidence was obtained of a decrease in the efficiency of fruitful activity in mares of fast-paced and sport breeds after a long period of training and performances. At present, the fact of the depressing effect of stressors of various nature on the reproductive functions of horses of both sexes is not in doubt. Although, according to sports physiologists, physical activity cannot be considered stressful, the same organs and systems are involved in the body's response to it, in particular the hormones of the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-cortical links of endocrine regulation, which play an important role in the implementation of reproductive activity. The stress reaction implies the activation of the functional system designed to adapt the body to the action of a damaging agent at the cellular and molecular level, which is associated with the suppression of the neurohumoral regulation system of its reproductive capacity. Considering the multifaceted role of sex hormones in the processes of anabolism, the production of proteins that bind other hormones, as well as their influence on the activity of many enzymes , there is no doubt that they do not participate in the implementation of muscle work. Since males are more often subjected to longer training and testing in horse breeding, and there is clearly not enough scientific work on the effect of such exploitation on their body, it is advisable to consider the reaction of the pituitary-adrenocortical system of the body to stress, in particular muscle, loads. Since the close cooperation of the pituitary-adrenal and pituitary-gonadal systems is an established fact, their mutual influence is assessed as reciprocal, when increased activity of adrenal hormones has a depressing effect on the reproductive abilities of the body. Suppression of sexual function under the influence of various strong stimuli is biologically expedient , since the body is forced to minimize energy-consuming expenditures on functions that are not relevant for survival at a particular moment, in particular, reproduction in unfavorable living conditions. When performing muscle loads, adaptive changes occur in the work of the endocrine system, which result in a change in the ratio of the production of steroid hormones and gonadotropins. Modern research aimed at identifying the features of the body's adaptation to physical activity is based on the establishment of endocrine mechanisms that regulate reproductive function. People who regularly engage in sports have been shown to have a decrease in testosterone levels against the background of an increase in the content of hormones that provide catabolic processes. When performing muscle work, the secretion of adrenocorticotropic hormone and, accordingly, cortisol increases proportionally to its intensity, which, in turn, inhibits the secretion of testosterone produced by the testicles. Thus, studying the activity of the adrenal glands - sex glands system is promising for establishing the features of the body's adaptation to both physical activity and fruitful activity. There is no doubt about the close connection between testosterone production and the ability to perform intense muscle work. In sports physiology, it has been established that at a high level of training, a low testosterone level can be registered, which is associated with a higher level of cortisol in the blood. Testosterone is considered the main androgenic hormone that has an anabolic effect. The result of increasing its content in the blood is an increase in the hemoglobin level, a decrease in the fat content, the development of the musculoskeletal system, an increase in myocardial contractility, and also inhibition of glucocorticoid receptor expression and reduction of cortisol binding. Freely circulating testosterone enters the cell cytoplasm, where it binds to androgen receptors, forming ligands that penetrate the cell nucleus. The result is translation of mRNA androgen receptors, and this response to sports training can last 1-2 days. During intense physical activity, testosterone levels decrease, since its synthesis is associated with significant energy expenditure. Excessive physical work can still stimulate a significant but short-term increase in testosterone concentration. In the absence of subsequent equally pronounced

increases in testosterone concentrations after subsequent training effects, it is assumed that muscle adaptation to physical activity is associated to a greater extent with the synthesis of androgen receptors than with an increase in the content of free testosterone. Thus, to date, many studies have been carried out to establish the dynamics of hormonal changes, in particular the content of cortisol and testosterone, as well as their effect on athletes during high-intensity muscle activity. Cortisol is a steroid hormone that stimulates gluconeogenesis , enhances lipolysis , has an anti-inflammatory effect and helps preserve energy resources in the body, increasing the amount of glycogen in the liver, reducing the breakdown of glucose in the muscles, and involving adipose tissue in energy supply. Cortisol limits protein synthesis, its effect is associated with protein catabolism, limiting hypertrophy. During intense exercise, cortisol concentration increases, and testosterone is more involved in the recovery process. Currently, there is evidence of the absence of significant dynamics in the level of a number of steroid, tropic hormones and catecholamines during training and competitive loads in athletes and people not involved in sports. At the same time, there is information about the absence of a decrease in testosterone levels during high-intensity training loads. Thus, studying the problem of developing strength qualities and endurance is impossible without taking into account the work of the hormonal sphere accompanying training activities and covering the regulation of protein metabolism in muscles. There is no doubt that the hormonal response to training exposure is largely associated with its type, taking into account the direction of exposure - to the development of endurance or strength. Despite the great interest shown in the problem of hormonal regulation of muscle activity, there are still many unidentified features occurring in the body of horses undergoing training, since the data obtained by different researchers are relatively few and ambiguous. The results of previous studies have shown that muscle work of varying intensity, depending on the degree of training of horses, can affect the levels of testosterone, cortisol and, accordingly, the value of their ratio, that is, reflect the state of metabolism and the balance of anabolism and catabolism in their bodies. At the same time, the issue of comparative monitoring of steroid hormone levels in draft horses performing competitive loads has been studied rather poorly, in connection with which, the purpose of the study was to establish changes in the content of steroid hormones in the blood of horses performing high-speed and high-speed-strength loads.

2 Material and methods of the study

The experiment was conducted on 8 older (5 years and older) Russian trotter horses of both sexes undergoing hippodrome tests, as well as on 8 purebred horses over 5 years old (6 heavy draft stallions and 2 Vyatka stallions) participating in tests under the heavy draft eventing program. The motor load for trotters was submaximal intensity flapping work over a distance of 1600 m, and heavy draft horses and Vyatka horses covered a 2000 m distance at a trot with a draft load of 50 kg (heavy draft horses) and 30 kg (Vyatka horses). Blood for the study was taken from horses at rest the day before the tests and within 15-20 minutes after the finish. Hormones in the blood were determined in a veterinary laboratory with the appropriate equipment. The obtained digital data were calculated using the method of statistical calculations; the reliability of the difference in the compared values was determined using the Student criterion.

3 Research results

Distinctive features of adaptive reactions of the equine organism are determined by the peculiarities of hormonal regulation of the functions of the stress-realizing system, the

range of fluctuations of physiological responses to the action of stimuli of different magnitude and strength. Meanwhile, the characteristics of training and competitive loads with a long experience of training sessions to a certain extent modify the type of response, and the features of the conditions of the work performed affect the ratio of steroid hormones to each other. Analyzing the content of testosterone in the blood of horses at rest, it should be noted that its average level is 12.24 nmol / l, with a range of fluctuations from 2.346 to 24.340 nmol / l (Table 1). An interesting fact is that in one of the two trotter mares the level of this hormone reached 4.228 nmol / l, which is only 1.3 nmol / l less than in two stallions of the same age. Four stallions aged 6-10 years had a fairly high testosterone level from 14.63 to 24.39 nmol /l and were in the 2.05 min and faster speed class. This fact confirms the previously established pattern that there is a relationship between the testosterone level and the ability to perform intense muscle work. During high-intensity muscle work, the testosterone content in the blood can decrease, since its synthesis requires a lot of energy. If the level of this hormone is reduced even during rest, this reflects the development of a state of overtraining. In trotting horses, after a swing load, the testosterone content in the blood increases reliably, and especially significantly (about 9 times!) in the mare with the lowest background level. The increase in testosterone content in the remaining trotting horses ranged from 1.5 to 3 times. An increase in testosterone content in response to a fast trot indicates the absence of signs of overtraining in horses and suppression of their sexual sphere.

Table 1. Concentration of steroid hormones and the value of the anabolic index in trotting and draft horses before and after exercise.

breed	testosterone, nmol /l	cortisol, nmol /l
Russian trotter	peace	
	12.24±3.200	94.83±11.723
	after exercise	
	28.37±6.952*	368.39±63.100***
harness	peace	
	6.66±1.174	111.10±18.125
	after exercise	
	10.45±2.140	170.56±28.300

Significant at * p ≥0.95; *** p≥0.999

Considering the multiple effects of sex hormones on the body, in particular testosterone, involved in anabolism processes and regulating the activity of many enzymes , its monitoring during significant strength loads by horses seems appropriate. Taking into account the fact that some of the harness stallions were used in reproduction, therefore their baseline testosterone level is of interest. On average, harness stallions have 84% lower blood testosterone levels than trotters, possibly due to a shorter period of regular training. The range of individual fluctuations in the content of this hormone in harness stallions was within the range of 1.3-12.3 ng / l. At the same time, at rest, two stallions (Soviet heavy draft and Vyatka breeds) recorded its highest value - 12.3 and 9.9 nmol / l, respectively. Trotting with a load requiring horses to demonstrate strength and endurance resulted in a slightly lower increase in testosterone levels than in trotters, an average of 57% in the group. However, if the winner of the heavy draft horse showed a 79% increase at the finish, the winner of the Vyatka breed, on the contrary, showed a rather significant (60%) drop in its content. It should be noted that under a draft load, an individually high - two to threefold increase in the testosterone hormone was observed only in three of the eight stallions. It can be assumed, therefore, that a brisk trot with a low draft force, requiring speed from the horse, is accompanied by a significant (even multiple!) increase in testosterone levels (in five of the eight trotters), while the simultaneous manifestation of both strength and speed,

on the contrary , provoked its significant increase in only three stallions out of eight. It is generally accepted that the level of cortisol reflects the degree of power of the performed muscular work, since its high content is observed during excessively intense training or overfatigue. The individual range of fluctuations in the level of cortisol in trotting horses was from 42.03 (in 1 horse) to 147.3 nmol / l, and in half of the individuals (including mares) the content of this hormone in the blood exceeded 103 nmol / l. Swing work, as expected, caused a sharp, on average, more than twofold, increase in the content of cortisol - from 197 to 677 nmol / l, and in the three fastest horses the increase was 400-500%. The background content of cortisol in the blood of draft horses averaged 111 nmol /l, which is slightly higher than that of trotters, but only three stallions had levels exceeding 120 nmol /l. It should be noted that two Soviet heavy draft horses that arrived from the same farm had fairly high cortisol levels - 173-198 nmol /l, which may indicate both intense preliminary training and an insufficient rest period before the performances. After the finish, the content of this hormone rose by an average of 54% in the group, with two stallions that showed mediocre results having a minimal increase - only 10-13%. The winners of the races were characterized by a 60-77% increase in the hormone level, and the recorded twofold increase in the cortisol content of the Vladimir stallion is explained by its strong excitement and active resistance when moving along the distance.

4 Discussion

There is no doubt that horses experience pre-start anxiety, which, together with the competitive load, has a significant effect on the physiological systems responsible for the stability of homeostasis. With appropriate adaptation of horses to loads of this kind, one should expect minor changes in the magnitude of the indicators of the activity of many systems, in particular, the endocrine system. Based on the analysis of data available in the literature and the results of our own research, we can state that the performance of muscle work of both speed and speed-strength nature is accompanied by a corresponding reaction from the hypothalamus - pituitary-gonadal and hypothalamic-pituitary-adrenocortical systems, expressed in changes in the concentration of steroid hormones in the blood - testosterone and cortisol. It has been established that minimal shifts in their content may indicate good adaptation of the body to the imposed loads, be it physical work or stress. Given the multifunctional nature of the effect of both hormones involved in the processes of anabolism and catabolism, it should be concluded that the higher background level of testosterone found in trotters, including mares, compared to stallions of draft breeds, indicates a predominant effect on this indicator of the duration of preliminary training, since in trotters it is at least 3 years, and in draft horses no more than 3 months. The performance of high-speed and high-speed-strength loads was accompanied by a significantly ($P \geq 0.95$) more significant increase in testosterone content in the blood of trotters compared to harness horses. Thus, our study confirms the fact of a greater increase in testosterone levels in the blood of horses during intense but short-term (less than 3 minutes) loads compared to high-power and longer-lasting (at least 10 minutes) work. Long-term training undoubtedly contributes to the ability of the horse's body to maintain the level of hormones required at the current moment. This is confirmed by the similar background concentration of cortisol in the blood of horses in both groups. However, trotters respond to muscle work with a significantly ($P \geq 0.95$) higher increase in cortisol levels compared to harness horses. Since trotters and harness horses showed a simultaneous increase in the concentration of both testosterone and cortisol, the work performed can be considered to correspond to their current state of training.

5 Conclusions

1. The performance of high-speed and high-speed-strength loads causes similar changes in the hormonal status in the body of trotters and harness horses.
2. The background and post-exercise testosterone levels in the blood of horses are more significantly influenced by the length of the preliminary training than by the intensity and power of the load itself.
3. Older, high-class trotters are capable of increasing cortisol levels many times in response to high-speed (swinging) loads.
4. The muscular work performed by trotters and draft horses did not lead to a disruption of the balance of anabolic and catabolic processes in their bodies, which reflects the adequacy of the load presented to the current state of their training.

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