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ORIGINAL SCIENTIFIC ARTICLE

EFFECT OF INTENSIVE PHYSICAL LOADS ON PLASMA TESTOSTERONE AND CORTISOL CONCENTRATION IN ELITE ATHLETES

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Abstract

The study purpose was to investigate changes in the content of testosterone and cortisol in the blood of qualified athletes after testing them to determine their maximal oxygen consumption (VO_2max).

Materials and methods. Thirty-seven international level athletes: 15 biathletes, 12 rowers, 6 boxers, 4 modern pentathletes participated in this study. The test to evaluate VO_2max used exercise of increasing intensity on a rowing ergometer for rowers and on a treadmill for biathletes, boxers, modern pentathletes. Total testosterone and cortisol concentrations were determined in blood serum by an immunoenzyme analyzer before and after the test for determination of VO_2max.

Results. The concentration of total testosterone has been found to decrease significantly on average by 24.4% and the concentration of cortisol has increased by 34% due to physical activity. The highest level of maximum oxygen consumption was found in athletes with high levels of basal testosterone and cortisol in the blood. There is a high correlation between the basal content of total testosterone and the maximum consumption of oxygen in athletes ($r = 0.58$).

Conclusions. The high content of basal testosterone and cortisol is an important factor for the athlete's special working efficiency: higher level of basal cortisol has been accompanied by smaller increases in cortisol per load and high basal level of testosterone makes is possible to keep its concentration sufficient. The concentration of total testosterone and cortisol can be used as a marker of the effect of a training load.

Keywords: testosterone, cortisol, athletes.

Introduction

The endocrine system plays an essential role in the maintenance of the normal functioning of organisms' various systems, as well as in the reconstruction of metabolism during the development of urgent and long-term adaptation of the organism to physical loads and in recovery processes after stress, etc. (Hammami et al., 2017; Strahorn et al., 2017). During physical loads, the functions of many endocrine glands, especially those which participate in the formation of organisms' general adaptation to stress factors are changed. The magnitude and direction of these changes depend on their participation in providing metabolic processes during training, as well as on the intensity, duration of physical loads, and body training. The content of hormones in blood plasma depends on muscle fibers, the amount included in the work, the volume of performed muscle work, physical training direction, level of physical preparation, duration of rest intervals between workouts, etc. (Viru A. & Viru M., 2004; Hansen et al., 2001; Popovic et al., 2019).

The most attention is paid to hormones testosterone and cortisol. In practice, they are used primarily as markers of fatigue (Gaviglio, 2018). Also, the possibility of using basal concentrations of hormones and their changes under conditions of physical loads as markers of overstressing syndrome is investigated (Cadegiani & Kater, 2019). However, accurate data on the expression of changes in these indicators are absent, which creates difficulties in its interpretation for control and correction of training and competition loads, as well as the process of recovery in the athletes' bodies.

According to the results of modern scientific data, cortisol level is increased in response to intensive physical loading (Jacks et al., 2002; Vingren et al., 2016; Suay et al., 1999). As for the contents of the testosterone in conditions of physical loading: 30-minute exercises on endurance increase

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its content in blood serum, as well as intensive exercises, but without an increase in cortisol concentration. The peak of testosterone content connected with physical exercises should be considered an adaptive phenomenon, but the data about their short-term and long-term effect is not enough (Sgrò et al., 2014).

Our preliminary research shows that physical exercise is accompanied by the levels of testosterone and cortisol (Maydanyuk & Vdovenko, 2021). The expression of their changes depends on the orientation of loading, functional state of health, and individual organism characteristics of athletes.

The contents of cortisol and testosterone are used as an indicator of success in competitions: the larger it is during competitions, the higher the performance of the athlete is. Research with the participation of representatives of different sports showed that the winners of the competition had higher concentrations of cortisol (Lautenbach & Lobinger, 2018; Papacosta et al., 2016; Vingren et al., 2016) and testosterone (Suay et al., 1999; Crewther et al., 2016). With a higher basal level of cortisol athletes need less stimulation to reach peak levels, which may be one of the forms of athletes' adaptation to repeated physical loads (Popovic et al., 2019). Thus, in the last work of Muscella and co-authors (Muscella et al., 2021), a reliable correlation of cortisol and testosterone concentrations in plasma with the level of functional preparation of judges is established, namely, with maximal oxygen consumption (VO_2max, $ml·min^{-1}·kg^{-1}$).

The correlation between testosterone and cortisol levels is rather diverse and contradictory (Kraemer & Ratamess, 2005; Cadegiani & Kater, 2018). Some scientists interpret them as an indicator of physiological stress during training or an index of anabolism (Andrzejewski et al., 2021), others as a predictor of competition results (Gaviglio, 2018).

Thus, it is extremely interesting and promising to study changes in the testosterone and cortisol concentrations under conditions of different physical loads, as this will allow for the future assessment, control, and regulation of the training process in time.

The purpose of our work was to study changes in the content of the testosterone and cortisol in the blood of qualified athletes after the performance of the test for the determination of the VO_2max.

Materials and methods

Study participants

Thirty-seven international level athletes: 15 biathletes, 12 rowers, 6 boxers, 4 modern pentathletes (age 27.0 ± 0.7 years old, height 182.0 ± 1.8 cm, body mass 75.2 ± 1.9 kg, and body fat percentage 9.8 ± 0.5%) participated in this study.

Laboratory research

Total testosterone and cortisol concentrations were determined in blood serum by immunoenzyme analyzer ChemWell (awareness Technology, USA) using test systems AccuBind ELISA (Monobind Inc., USA). The concentration of lactate was measured enzymatically on variophotometer Diaglobal (Diaglobal GmbH, Germany) with the use of LAC142 standard reagent sets (Diaglobal GmbH, Germany).

Study organization

The research design included three steps (Figure 1).

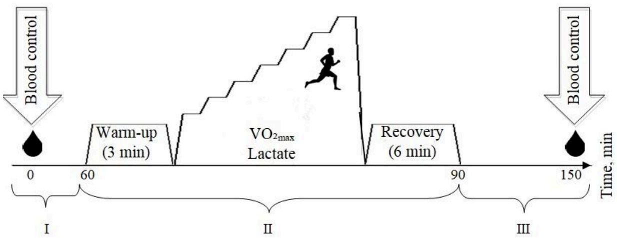

Fig. 1. Experimental design

The first step (I) involved the taking of blood samples for evaluation of the initial concentration of total testosterone and cortisol concentrations. The first blood draws before the physical load was carried out at 7 a.m. on an empty stomach. The second step (II) included the load test to evaluate the maximal oxygen consumption (VO_2max, $ml·min^{-1}·kg^{-1}$) and blood lactate concentration (Lactate, $mmol·l^{-1}$). The third step (III) involved the taking of blood samples for evaluation of total testosterone and cortisol concentration after test load. The second blood draw was taken one hour after the test load.

All participants did not take any food 2 hours before and during the study.

Load testing

Test to evaluate the VO_2max used exercise of increasing intensity on the rowing ergometer Concept II (USA) for rowers and on the LE 500 treadmill (Vyaire Medical GmbH, Germany) for biathletes, boxers, modern pentathletes. The load test lasted until the athletes reached the VO_2max. The sign of reaching VO_2max was the lack of increase in oxygen consumption with increasing power or its fluctuations within 100 $ml·min^{-1}$. Respiratory system parameters were recorded using an Oxycon mobile gas analyzer (CareFusion, USA; Jeager, Germany). Calibration of the test system was carried out following the recommendations of the producer. Before performing the test load, a 6-minute warming up was performed, and after the test was completed, the rate of recovery processes was assessed for three minutes. The degree of the initial exercise load on the Concept rowing ergometer was 1.5 $W·kg^{-1}$ body mass, at each subsequent stage the load increased by 30 W; the initial load on the running ergometer was 2.0 W per kg body mass (running speed – 10 $km·h^{-1}$). The duration of each step of the test load was two minutes (testing on a treadmill) or three minutes (testing on a rowing ergometer).

Blood draws for evaluation of the maximum concentration of lactate were performed immediately (within 1 to 7 seconds) after the test load. Samples of capillary blood were taken from the lateral surface top phalange of the ring finger.

Ethics

The research was conducted following the basic bioethical norms of the Helsinki Declaration of the World Medical Association on Ethical Principles of Scientific and Medical Research, as amended (2000, as amended in 2008), the Universal Declaration on Bioethics and Human Rights (1997), and the Council of Europe Convention on Human Rights and Biomedicine (1997). Each study participant provided written informed consent to participate in the study.

Statistical analysis

All statistical analyses were performed using "STATISTICA 12" with a significance level set at 0.05 unless stated to the contrary. One-way ANOVA, cluster analysis (k-means), correlation analysis (Spearman correlation) were used to assess cortisol and testosterone levels change during CPET. The Dunkan method (post hoc analysis) was used for differences estimation between groups. The correspondence of the sample to the normal distribution was checked for asymmetries and excesses, which indicated the proximity of the distribution to the normal curve.

Results

The main anthropometric characteristics (height, weight, percentage of body fat) and results of the athletes' tests are presented in Table 1.

It was found that the concentration of basal testosterone in the athletes` blood serum was within the reference values for men (Maydanyuk, & Vdovenko, 2021). The main attention in this study is devoted to studying the influence of test loading on the levels of total testosterone and cortisol in blood plasma. Total testosterone levels decreased on average by 24,4% after the physical loading (Table 2).

It is necessary to note sufficient variation of total testosterone concentration in an hour after loading compared with the values registered in rest. For example, the decrease in the total testosterone content was registered in 73% of cases (27 athletes): including a slight rise (5-20%) in 24% (9 athletes), significant increase (more than 50%) – 30% of cases (11 athletes) and 19% (7 athletes) cases of total testosterone concentration decreased by 20-50%.

In response to the impact of the load, cortisol level in blood plasma an average significant increase of 34% was detected ($p = 0.025$). The testosterone/cortisol (T/C) ratio per hour after loading was reduced by an average of 36% ($p = 0.00012$). For example, the average T/C ratio values were 6.1 ± 0.61% (4.9-7.4%) before the load and 3.8 ± 0.47% (2.9-4.8%) after it. It is necessary to note the correlation between the content of basal testosterone and VO_2max ($r = 0.58$, $p = 0.0001$), which is coordinated with the data Muscella and co-authors (2021). At the same time, there is no reliable correlation between the basal level of cortisol and the VO_2max. A reliable correlation between concentrations of total testosterone and cortisol after loading ($r = - 0.47$, $p = 0.028$) is also established. The results of the study show the importance of metabolism activation, including anabolic processes, to achieve high performance.

Thus, physical loads are accompanied by changes in levels of testosterone and cortisol. The expression and direction of changes in these hormones depend on the condition and individual athletes' characteristics. For more detailed analysis, we used clustering and conditionally divided the group

Table 1. Anthropometric characteristics and results of athletes' tests (n = 37)

Indicator	M ± m	CI 95%*
Age, years	27.0 ± 0.7	25.5 – 28.6
Height, sm	182.0 ± 1.8	178.3 – 185.6
Weight, kg	75.2 ± 1.9	71.3 – 79.1
Body fat, %	9.8 ± 0.5	8.9 - 10.8
Power, $W{\cdot}kg^{-1}$	5.3 ± 0.2	4.9 – 5.6
Maximal oxygen consumption, $ml{\cdot}min^{-1}{\cdot}kg^{-1}$	59.2 ± 1.3	56.5 – 61.83
Lactate, $mmol{\cdot}l^{-1}$	11.0 ± 0.5	9.9 – 12.0

CI 95% – confidence interval.

Table 2. Total testosterone and cortisol levels in athletes' blood before and after the test load (n = 37)

Indicator	M ± m (CI 95%)		P
	Before load	After load	
Total testosterone, $nmol{\cdot}l^{-1}$	25.4 ± 1.94 (21.5 – 29.3)	17.23 ± 1.3 (14.6 – 19.9)	0.00001
Cortisol, $nmol{\cdot}l^{-1}$	487.4 ± 42.3 (401.6 – 573.2)	578.1 ± 44.3 (488.1 – 668.2)	0.025
Testosterone/ cortisol ratio, %	6.1 ± 0.6 (4.9 –7.4)	3.8 ± 0.5 (2.9 – 4.8)	0.00012

CI 95% – confidence interval.

Table 3. Content of total testosterone, cortisol and the maximum oxygen consumption in athletes of different clusters (M ± m)

Indicator		Cluster A (n = 13)	Cluster B (n = 13)	Cluster C (n = 11)
Testosterone, $nmol{\cdot}l^{-1}$	1	32.0 ± 2.7	18.9 ± 1.1*	10.7 ± 1.5*, **
	2	17.1 ± 2.1	16.9 ± 1.6	10.8 ± 1.9*, **
Cortisol, $nmol{\cdot}l^{-1}$	1	790.2 ± 34.0	334.3 ± 20.9*	251.3 ± 35.1*
	2	755.0 ± 63.7	462.1 ± 72.3*	428.0 ± 72.5*
Testosterone/cortisol ratio, %	1	4.1 ± 0.4	6.0 ± 0.6*	4.6 ± 0.8*
	2	2.6 ± 0.4	4.8 ± 0.8*	2.9 ± 0.8*
Maximal oxygen consumption, $ml{\cdot}min^{-1}{\cdot}kg^{-1}$		62.0 ± 1.9	59.8 ± 1.6	52.2 ± 2.4*, **

Note: clustering is based on basal testosterone and cortisol contents. 1 – basal level, 2 – one hour after the test load, * – differences with cluster 1 are statistically significant ($p \le 0.05$), ** – differences with cluster 2 are statistically significant ($p \le 0.05$).

Table 4. Total testosterone and cortisol concentrations and VO_{2max} in athletes of different clusters (M ± m)

Indicator		Cluster I (n = 11)	Cluster II (n = 14)	Cluster III (n = 11)
Testosterone, $nmol \cdot l^{-1}$	1	32.2 ± 2.8	27.8 ± 1.5	13.4 ± 1.0*, **
	2	21.4 ± 2.5	15.8 ± 1.0	12.0 ± 1.3
Cortisol, $nmol \cdot l^{-1}$	1	509.4 ± 81.8	605.0 ± 70.9*	345.5 ± 50.4*, **
	2	587.0 ± 76.9	616.6 ± 68.1*	530.6 ± 86.2**
Testosterone/cortisol ratio, %	1	6.5 ± 1.3	5.7 ± 1.0	4.5 ± 0.4*, **
	2	3.7 ± 1.4	3.2 ± 0.5	3.1 ± 0.5
Maximal oxygen consumption, $ml \cdot min^{-1} \cdot kg^{-1}$		66.3 ± 1.6	62.6 ± 1.2	55.1 ± 1.5*, **

Note: clustering is based on total testosterone before and after test load. 1 – basal level, 2 – one hour after the test load, * – differences with cluster 1 are statistically significant ($p \leq 0.05$). ** – differences with cluster 2 are statistically significant ($p \leq 0.05$).

into three clusters: clusters “A” and “B” included 13 people, and cluster “C” – 11 people. Clustering is based on basal testosterone and cortisol contents with the prior normalization of data (Table 3).

Cluster “A” is characterized by high cortisol (790.2 ±34.0 $nmol \cdot l^{-1}$) and total testosterone levels (32.0 ± 2.7 $nmol^{-1}$) in rest. After the test load, total testosterone content is significantly reduced on average by 47% and cortisol does not change (755.0± 63.7 $nmol^{-1}$). For cluster “B”, there are slight fluctuations in total testosterone concentration (within 10%) and average growth of 38% in cortisol content in response to test load. Cluster “C” is characterized by the lowest total testosterone level, which does not change after the test is performed and significant growth of cortisol content after the test load – on average by 72%. Three clusters are likely to differ in values in basal total testosterone concentrations. Cortisol concentrations both before and after one hour of loading are quite different from the rest only in cluster “A”, differences between clusters “B” and “C” are not statistically significant.

It is necessary to note statistical differences in athletes' VO_2max in cluster “C” with the other two clusters. For example, the average VO_2max values in cluster “A” and “B” were 62.0 ± 1.9 $ml \cdot min^{-1} \cdot kg^{-1}$, and 59.8 ± 1.6 $ml \cdot min^{-1} \cdot kg^{-1}$, and in cluster “C” were 52.2 ± 2.4 $ml \cdot min^{-1} \cdot kg^{-1}$.

We also did not found the differences in T/C ratio between clusters and no statistically significant correlation between T/C ratio on the one hand and testosterone and cortisol levels on the other. Thus, T/C ratio does not correlate separately with testosterone or cortisol, at the same time the basal concentration of cortisol correlates (negative correlation) with the basal content of testosterone.

Due to the significant correlation between total testosterone concentration and VO_2max ($r = 0.58$, $p = 0.0001$), we also analyzed by cluster method the values of total testosterone before and after loading. Three clusters were allocated according to the results: I – 11 athletes (total testosterone level 33.2 ± 2.8 and 21.4 ± 2.5 $nmol \cdot l^{-1}$ (before and after loading)), to II – 14 athletes (27.8 ± 1.5 and 15.8 ± 1.0 $nmol \cdot l^{-1}$), to III – 12 athletes (13.4 ± 1.0 and 12.0 ± 1.3 $nmol \cdot l^{-1}$) (Table 4). It was found that the highest values of VO_2max were demonstrated by athletes with high or above-average basal total testosterone content, as well as the highest values of T/C ratio.

In our research statistically significant the higher cortisol level (basal and after test loading) in athletes I and II clusters relative to III was found. The greatest increase in cortisol content in response to the impact of test loading is observed in athletes in the III cluster: cortisol content increased by an average of 58%, at the same time by 26% and 15% in clusters I and II, respectively.

The best results of the test were demonstrated by athletes of I and II clusters (the highest total testosterone level before and after test load and basal cortisol content): the average values of VO_2max were 66.3 ± 1.6 and 62.6 ± 1.2 $ml \cdot min^{-1} \cdot kg^{-1}$, respectively. The lowest value of VO_2max is in the III cluster – 55.0 ± 1.5 (the lowest testosterone and basal cortisol values).

Discussion

The results of this study showed a significant ($p = 0.00001$) decrease (by an average of 36%) in total testosterone content and a significant ($p = 0.025$) increase (by an average of 34.2%) in cortisol level in an hour after intensive load with the achievement of VO_2max. This fact shows the considerable influence of intensive physical loads on steroid hormones. It is necessary to note variations of changes in total testosterone concentration under physical loading influence: its content was reduced in the majority of cases one hour after test load, at the same time in 27% of cases total testosterone was either unchanged or increased. The results obtained in this study coincide with Popovic et al. (2019) on changes in cortisol and testosterone levels in the near recovery period after test load. At the same time, the results of research by Sgrò et al. (2014) demonstrate an increase in total testosterone content after a test loading. Therefore, changes in testosterone levels in response to the impact of load are not unambiguous. The direction and expression of changes in testosterone and cortisol concentrations will depend on intensity, duration of loading and basal levels of hormones.

Jacks and co-authors (2002) were shown that cortisol content increased only due the intensity is 76% VO_2max and the duration of the load is not less than an hour and remains unchanged after load is 45% to 62% VO_2max. Also, the authors note that at 40-minute loads the level of cortisol is not changed. In turn, Toro-Román et al. (2021) indicates that after an incremental test in qualified cyclists the removal of cortisol with an urina is reduced. This can confirm the conclusion that acute fatigue or overstress are associated with a significant blood cortisol concentration decrease during physical loading (Viru, 2004). Popovic et al. (2019) note the positive effect on athletes' performance of the increasing basal cortisol levels. Authors explain this as an advantage of the intensive muscle activity energy supply, resulting from long-term adaptation to physical loads.

In the works of Papacosta et al. (2016) and Vingren et al. (2016) with co-authors, cortisol level is considered as one of the important factors influencing the competition's success and athletes' performance. The authors note that the highest cortisol content is typical for the most successful athletes. In our study, we found significantly the highest cortisol concentration (basal and after test loading) in athletes I and II clusters relative to III (depending on the content of the testosterone before and after loading test). The greatest cortisol level increase in response to the impact of test load is observed among III cluster representatives – on average by 58%, and by 26% and 15% respectively in clusters I and II. Thus the cortisol concentration growth under influence of loading is larger in case of its lower basal content.

The research data demonstrated different variants of steroid hormones changes due test loading. In 13 out of 37 athletes with high basal testosterone and cortisol levels (cluster "A") a significantly reduced testosterone content and a non-significant decrease in cortisol concentration. Testosterone concentration practically did not change in athletes with average levels of basal testosterone and cortisol (cluster "B"), at the same time cortisol content on average increased by 37.7%. The testosterone levels did not change and significantly increased cortisol concentration (on average by 72.3%) in 11 athletes (cluster "C") with extremely low basal testosterone levels. According to European Academy of Andrology (EAA) recommendations is defined as the lowest total testosterone serum concentration in men within 8-12 $nmol \cdot l^{-1}$. It should be noted that athletes with high and average basal testosterone values demonstrated the highest results of VO_2max. This fact and significant correlation between basal testosterone and VO_2max ($r = 0.58$) allow approving the importance of anabolic processes for achieving high VO_2max, which is coordinated with Muscella and co-authors (2021). At the same time, there is correlation between basal cortisol level and VO_2max.

Many research works are indicated to information value T/C ratio for estimation of anabolic-catabolic processes and also for the forecast of athletes' special performance (Gaviglio, 2018; Kraemer & Ratamess, 2005; Andrzejewski et al., 2021). In this study, the T/C ratio in an hour after loading has been significantly reduced by an average of 36%. We have not found any correlation between T/C ratio and total testosterone and cortisol contents. Thus, T/C ratio does not correlate separately with testosterone or cortisol concentrations. Similarly, there is no significant correlation between T/C ratio and VO_2max in this study.

Conclusion

1. A significant decrease in total testosterone content by an average of 24.4% and an increased plasma cortisol concentration by 34% in response to the impact of VO_2max test has been established.

2. It has been found that total testosterone and cortisol contents can be used as a marker for the training load effect. The relationship between testosterone concentrations and VO_2max was established 0.58 ($p = 0.0001$). The highest level of VO_2max was achieved by athletes with high basal testosterone and cortisol contents in the blood.

3. The higher basal cortisol content is accompanied by its less growth due test loading. High basal level of testosterone allows it to keep sufficient content during physical loading. This is an important factor in the athlete's special performance.

Conflict of interest

The authors state no conflict of interest.

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ВПЛИВ ІНТЕНСИВНИХ ФІЗИЧНИХ НАВАНТАЖЕНЬ НА КОНЦЕНТРАЦІЮ ТЕСТОСТЕРОНУ ТА КОРТИЗОЛУ В КРОВІ ЕЛІТНИХ СПОРТСМЕНІВ

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Авторський вклад: A – дизайн дослідження; B – збір даних; C – статаналіз; D – підготовка рукопису; E – збір коштів

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Мета дослідження – дослідити зміни вмісту тестостерону та кортизолу в крові кваліфікованих спортсменів після виконання тесту на визначення максимального споживання кисню (МСК).

Матеріали і методи. Дослідження проводили за участю 37 кваліфікованих спортсменів (чоловіки), що мали досвід виступів на Олімпійських іграх або інших міжнародних змаганнях. Серед них 15 спортсменів, які спеціалізуються в біатлоні, 12 – у веслуванні академічному, 6 – боксі, 4 – сучасному п’ятиборстві. Тест на визначення МСК передбачав виконання навантаження зростаючої потужності на веслувальному ергометрі (веслування академічне) або на біговій доріжці (біатлон, бокс, сучасне п’ятиборство). Вміст тестостерону, кортизолу визначали у сироватці крові спортсменів на імуноферментному аналізаторі до та після виконання тесту на визначення МСК.

Результати. Концентрація загального тестостерону значно знизилася в середньому на 24,4%, а концентрація кортизолу зросла на 34% через фізичну активність. Найвищий рівень максимального споживання кисню виявлено у спортсменів з високим рівнем базального тестостерону і кортизолу в крові. Існує висока кореляція між базальним вмістом загального тестостерону та максимальним споживанням кисню у спортсменів (r = 0,58).

Висновки. Високий вміст базального тестостерону і кортизолу є важливим фактором для особливої працездатності спортсмена: вищий рівень базального кортизолу супроводжується меншим збільшенням кортизолу на навантаження, а високий базальний рівень тестостерону дозволяє підтримувати його концентрацію достатньою. Концентрацію загального тестостерону і кортизолу можна використовувати як маркер ефекту тренувального навантаження.

Ключові слова: тестостерон, кортизол, спортсмени.

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