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Original Article

Effect of carbohydrate intake on endogenous hormones: anabolic and catabolic orientation content of highly qualified sportsmen–combat athletes

ANDREY GRYAZNYKH[1], MARINA BUTAKOVA[2], LIUDMILA GREBENYUK[3], MARIYA KISELEVA[4], TIMUR NASYROV[5], MIKHAIL KOLOKOLTSEV[6], ANTON VOROZHEIKIN[7], ELENA ROMANOVA[8], OLEG BAYANKIN[9], WITOLD KOWALSKI[10], PAVEL TYUPA[11]
[1]Humanities Institute of Northern Studies, Yugra State University, RUSSIA
[2]Expert and Forensic Center of the Ministry of Internal Affairs of Russia in the Kurgan region, RUSSIA
[3]Laboratory of Purulent Osteology, National Ilizarov Medical Research Centre for Traumatology and Orthopedics, Ministry Healthcare, RUSSIA
[4]Department of Defectology, Institute of Pedagogy, Psychology and Physical Culture, Kurgan State University, RUSSIA
[5]Clinical Resident of Ilizarov Centre Education, National Ilizarov Medical Research Centre for Traumatology and Orthopedics, Ministry Healthcare, RUSSIA
[6]Department of Physical Culture, Irkutsk National Research Technical University, RUSSIA
[7]Department of Information Technologies, Kaliningrad Institute of Management, Kaliningrad, RUSSIA
[8]Department of Physical Education, Altai State University, RUSSIA
[9]Department of Theoretical Foundations of Physical Education, Institute of Physical Culture and Sports, Altai State Pedagogical University, RUSSIA
[10]Institute of Sport and Physical Culture, University of Economy in Bydgoszcz, POLAND
[11]Kickboxing and hand-to-hand fighting Sports School No 13, Kaliningrad, RUSSIA

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Abstract:
Hormonal mechanisms of metabolic reactions regulation during long-term muscle work (during doing sports) determine the subsequent course of recovery processes after exercise. Food of different composition intake (mainly carbohydrate) affects the hormonal relationships in the series of anabolic/catabolic hormones and is a factor in correcting the damaging effect of hyperkinesia on the human body, having a protective effect. These issues have not been sufficiently studied and need to be clarified. In addition, eating before exercise, during exercise, and in the post-stress period is able to control physiological recovery reactions dynamics. *The purpose* of this study is to analyze the postprandial (after taking carbohydrate food) dynamics of changes in anabolic and catabolic hormones content and the anabolic index (IA) in the blood serum in various functional states in highly qualified athletes – wrestlers. *Materials and methods.* The study involved 10 highly qualified athletes - wrestlers. In the subjects under conditions of relative muscle rest, after 60 minutes of physical activity, the content of hormones in the blood serum was determined: cortisol, testosterone and testosterone/cortisol index. In the empty stomach dynamics, after the combined effect of a carbohydrate breakfast and muscle load, these hormones content was studied in the investigated conditions after 15, 45, 75 and 105 minutes. *Reserarch results.* Carbohydrate food contributes to the high 736.1±56.4 nmol/l fasting cortisol concentration reduction. This effect becomes significant in 75 minutes after eating - 498.37 ± 36.6 nmol/l. The carbohydrates intake did not cause a significant change in cortisol concentration in blood serum after the 60-minute muscle cycling exercise. The cortisol content remained high 981.0 ± 71.7 nmol/l during the entire inter-digestive period (105 minutes). There were no significant changes in testosterone concentration when taking carbohydrate food, both in a relative muscle rest state, and under the muscle dynamic load action. The anabolism index was low in the background conditions of 0.037 ± 0.003, carbohydrate food intake led to a significant increase in this indicator to 0.057 ± 0.005 after 105 minutes of the inter-digestive period. *Conclusion.* It is established that carbohydrate food is able to have a protective effect, being a metabolic mechanism, affecting tension/recovery hormonal processes of the athletes' body.
Key Words: **specific dynamic effect of food (carbohydrate breakfast), anabolism index, athletes-wrestlers, muscle loading**

Introduction

Achieving maximum results in modern sports is ensured by systematic, targeted muscle loadings, which requires the maximum functional reserves mobilization of the athletes' body (Diachenko et al., 2020; Shynkaruk et al., 2020). An increase in training loadings volume and intensity contributes to the structural and functional improvement of blood circulation and the strengthening of the nervous system's trophic functions, a sufficient

Corresponding Author: ELENA ROMANOVA, E-mail: romanovaev.2007@mail.ru

energy reserve formation, and an increase in skeletal and cardiac muscles capillarization (Bishop et al., 2019). These body changes are aimed at developing its potential capabilities, increasing its functional reserve, adequate adaptation to muscle loadings, energy mitochondrial support, and accelerating recovery. The faster the recovery is, the more the more abilities the body has for performing subsequent work, and, consequently, the higher its functional capabilities and efficiency are.

The modern multidisciplinary approach to the study of the human body adaptive capabilities under chronic stress conditions (muscle loading) has revealed a fairly wide range of research areas that determine not only the study of organs and tissues local reactions, but also the systemic response of the body. Thanks to the multi-factor analysis, the researchers identified the mechanisms and ways of formation, the definition of evaluation criteria and the search for optimal methods to increase the athletes' body reserve capabilities.

At the same time, there is insufficient knowledge of the hormonal metabolic reactions regulation mechanisms in chronic stress in the form of constant muscle tension issue. There are conflicting data on the levels of anabolic and catabolic hormones in the adrenal cortex and sex glands in chronic stress caused by hyperkinesia (Rutherfurd-Markwick et al., 2017; Doeven et al., 2018; Saad et al., 2020; Kraemer et al., 2020; Schwanbeck et al., 2020; Foretic et al., 2020). There is also conflicting information about the humoral-hormonal mechanisms (factors) of regulation involvement in ensuring the body's resistance to the adverse effects of hyperkinesia (Banfi et al., 2012). The question of hormonal relationships in the conditions of food specific dynamic effect (conditionally carbohydrate, protein and fat breakfast) as a factor of the damaging effect of hyperkinesia correction over the human body and affecting the dynamics and degree of recovery after exercise remains open. (Wilk et al., 2018; Poulios et al., 2019; Rosset et al., 2017; Rollo et al., 2020).

Research aim was to study the effect of taking a carbohydrate breakfast on anabolic and catabolic hormones content in blood serum, the anabolism index in highly qualified martial artists in various functional conditions.

Material & methods

A group of athletes was examined. A total of 10 male volunteers participated in the research. The main conditions for participation in the study were voluntary and written informed consent, taking into account bioethical norms. All the subjects were screened and, for health reasons, belonged to the main medical group.

The subjects were highly qualified athletes aged 21.3±2.8 years, developing speed and strength qualities (wrestling, sambo (unarmed self-defence) wrestling), whose muscle activity is implemented mainly in an anaerobic energy regime, which training loadings duration was at least 9±1 hours per week.

Data Collection and Research Design.

The days of the examination fell on the preparatory period of the training cycle. All studies were conducted in the morning, on an empty stomach. Previously, two days before the examination, the training activity was minimized. As an acute physical activity model, we proposed working on a cycle ergometer for 60 minutes with a cadence of 60-65 revolutions per 1 minute. The loading power was defined as 2 watts per kilogram of body weight. We used standard certified equipment: the Aerofit MaxFit B7 cycle ergometer, the BIO-RAD 550 biochemical analyzer, and the «IFA-BEST» hormone detection kits.

To study the endogenous hormone secretion dynamics in blood serum under the influence of food and muscle loads, under the conditions of food specific dynamic action in various conditions of the body functioning, a food load in the form of a trial (test) carbohydrate breakfast consisting of 100 g of semolina /semolina porridge with butter and 200 ml of unsweetened tea was used. The volume of the breakfast was 400 ml, the caloric content was 200 kcal.

Two series of studies were conducted. In the first series, the subjects took a carbohydrate breakfast in the morning on an empty stomach. In the second series of studies, the subjects performed physical activity in the morning on an empty stomach, after which they immediately took a carbohydrate breakfast. In all cases, the content of hormones in the blood serum was determined on an empty stomach and in dynamics in 15, 45, 75 and 105 minutes after taking a food breakfast. The concentration of Steroid EIA-cortisol-01 cortisol («Alcor Bio», Russia) and testosterone was determined in blood serum by the enzyme immunoassay method using industrial kits, and the anabolism index (AI) was calculated using the formula: AI (%) = (Testosterone/Cortisol). The reference values of the index are 5-8% (Crawford et al., 2015). The conducted research does not infringe on the rights and does not endanger the athletes' well - being in accordance with the ethical standards of the Human Rights Committee of the Helsinki Declaration of 2008 (WMA Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects).

Data Analysis

The summary data tables' formation was carried out in the Microsoft Office Excel 2016 software environment, statistical processing of the material and plotting was carried out using the STATISTICA 10 program of StatSoft. The choice of the statistical analysis method was based on the study of the sample distribution normality. According to the results of the Kolmogorov-Smirnov and Shapiro-Wilk tests, a suitable parametric and nonparametric statistics method was selected. With a normal distribution of the sample ($p > 0.20$ according to the Kolmogorov-Smirnov normality criterion, $p > 0.05$ according to Shapiro-Wilk), statistical analysis and evaluation of the relationship between the parameters were performed using parametric statistics (the Student's t-test, the Pearson correlation coefficient). In the opposite case they were performed by methods of nonparametric statistics (Mann-Whitney U-test, sign test, Spearman correlation coefficient). Statistically significant differences and correlations were considered at the level of $p < 0.05$. It is generally assumed that for medical and biological studies, this value of «**p**» is sufficient. At $0.05 < p < 0.1$, we concluded that there was an unreliable trend. The correlation strength was evaluated according to the generally accepted ranking table, according to which a weak relationship is observed with a correlation coefficient from 0 to ± 0.299, an average from ± 0.3 to ± 0.699, and a strong relationship from ± 0.7 to ± 1.

Results

Muscle load, depending on the intensity, duration, physical condition of the body and other factors, leads to a significant change in the intensity of metabolic (endocrine) processes throughout the body, which is manifested both by the release and suppression of various hormones', having complex mechanisms of mutual influence secretion.

In the examined athletes, in relative muscle rest conditions, the level of cortisol in the fasting blood exceeds the reference values (180-660 nmol/l), which corresponds to previous studies (Crawford, et al., 2015). Carbohydrate intake leads to a decrease in cortisol concentration in the blood during the entire postprandial period. If at 15 and 45 minutes these changes are weakly expressed, then by 75 and 105 minutes of the inter-digestive period there is a decrease of 1.5 times compared to the data obtained on an empty stomach ($p < 0, 001$) (Table 1)

Table 1. Cortisol and testosterone content in the blood serum of the examined athletes in various functional conditions and when taking carbohydrates

	T	15’	45’	75’	105’
Cortisol (180-660 nmol/l)					
Profile	736,1±56,4	646,93±26,2	609,09±54,3	498,37±36,6$^{\#\#}$	446,9±33,2$^{\#\#\#}$
Loading	757,5±43,5	847,8±60,8*	879,7±51,3**	981,0±71,7***/$^{\#}$	645,4±57,4*
Testosterone (12,1-38,2 nmol/l)					
Profile	26,7±2,1	25,3±2,3	25,2±1,9	25,3±1,4	24,3±1,9
Loading	33,0±2,5	30,4±2,6	26,5±2,3	25,4±2,1$^{\#}$	28,3±2,1
Anabolism index (AI) (Testosterone/Cortisol)					
Profile	0,037±0,003	0,039±0,003	0,044±0,004	0,053±0,004	0,057±0,005
Loading	0,046±0,005	0,039±0,005	0,032±0,003	0,028±0,003**	0,048±0,005

Note: Profile – endocrine profile indicators; Loading – immediately after muscle loadings, T – on an empty stomach; * - differences are significant relative to background values $p<0.05$; ** - $p < 0.01$; *** - $p < 0.001$; # - differences are significant relative to fasting values $p < 0.05$; ## - $p < 0.01$; ### - $p < 0.001$.

The muscle load did not cause significant changes in the serum cortisol content in the subjects. Immediately after performing the cycle ergometric loading, there is a slight increase in cortisol level in the subjects' blood. And further, during the entire inter-digestive period, there was a tendency to increase cortisol concentration in the examined athletes-wrestlers' blood with a peak at 75th minute ($p < 0,001$) and a decrease in this indicator at 105th minute ($p < 0,05$). The fact of a significant excess of the studied indicator relative to similar conditions in relative muscle rest state was also established (Fig. 1).

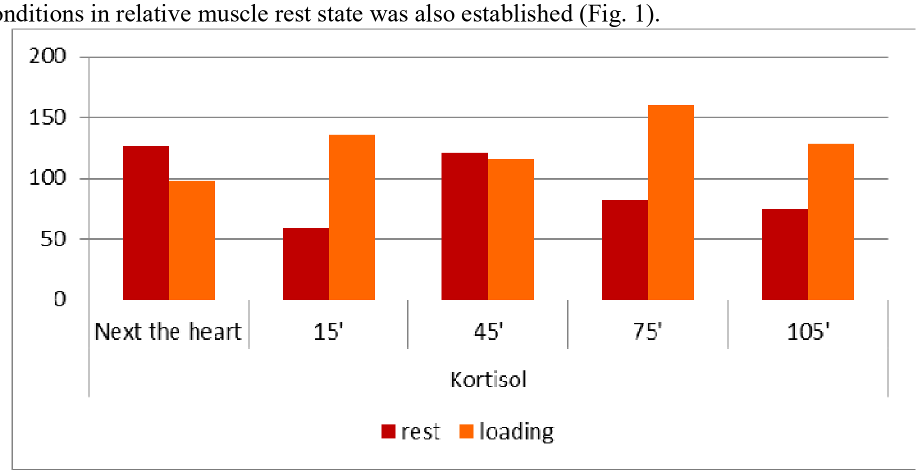

Fig. 1. Dynamics of changes in the cortisol concentration in the studied conditions (nmol/l)

Note: Profile – endocrine profile indicators; Loading – immediately after muscle loadings, T - on an empty stomach. * - the differences are significant relative to the profile values of $p < 0.05$; ** - $p<0.01$; *** - $p < 0.001$; # - differences are significant relative to fasting values $p < 0.05$; ## - $p < 0.01$; ### - $p < 0.001$.

Normally, testosterone concentrations range in the blood is from 12.1 to 38.3 nmol/l. The testosterone level in the examined wrestlers' blood was within the reference values. This correlates with the researchers' data indicating that athletes (n = 8) had significantly higher testosterone concentrations during low-intensity training (early December) than during heavy sprint-specific training (late March) (mean value ± SD; 23.37 ± 5.28 vs. 20.99 ± 4.74 nmol • l (-1), respectively, P=0.04) (Grandys et al., 2015).

Assessing the dynamics of changes in testosterone concentration in the examined wrestlers' blood after taking carbohydrate food, no significant changes were found in our work. There is a tendency to decrease this indicator in the postprandial period (Table 1).

Examining the effect of muscle tension on testosterone level in the subjects' blood, we did not find significant differences. At the same time, there is a slight excess of this hormone concentration in the subjects' blood, compared to the data obtained in the profile conditions.

Carbohydrate food intake after performing a muscle loading causes a gradual decrease in testosterone concentration, reaching a minimum by the 75th minute of the inter-digestive period in relation to the data obtained on an empty stomach, $p < 0,05$ (Fig. 2).

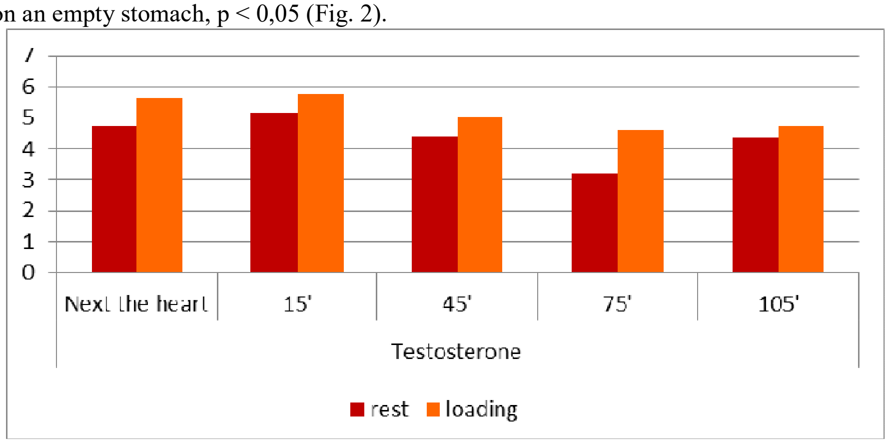

Fig. 2. Dynamics of changes in testosterone concentration in the studied conditions (nmol/l)

Note: Profile – endocrine profile indicators; Loading – immediately after muscle loadings, T - on an empty stomach. * - the differences are significant relative to the profile values of $p < 0.05$; ** - $p<0.01$; *** - $p < 0.001$; # - differences are significant relative to fasting values $p < 0.05$; ## - $p < 0.01$; ### - $p < 0.001$.

To study metabolic transformations peculiarities, clarify the reactions of anabolic/catabolic processes course, as well as physiological stress during training, the testosterone/cortisol coefficient (anabolism index – AI) was determined in this study. In relative muscle rest conditions, this indicator in the postprandial period increased slightly in the examined athletes during the entire postprandial period (Table 1).

Immediately after performing the muscle loading, there is a decrease in the stress index - testosterone/cortisol throughout the entire inter-digestive period with a minimum at 75th minute ($p < 0,01$) (Fig. 3).

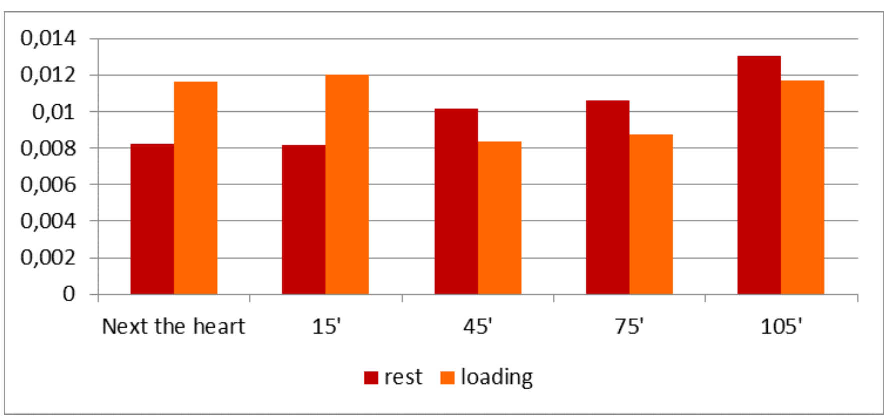

Fig. 3. Dynamics of changes in the anabolism index (testosterone/cortisol, nmol/l)

Note: Profile – endocrine profile indicators; Loading – immediately after muscle loadings, T - on an empty stomach. * - the differences are significant relative to the profile values of $p < 0.05$; ** - $p<0.01$; *** - $p < 0.001$; # - differences are significant relative to fasting values $p < 0.05$; ## - $p < 0.01$; ### - $p < 0.001$.

Dicussion

The researchers' works indicate that a small amount of carbohydrates consumed during exercise can increase the effectiveness of shorter (45-60 min), more intense (>75% of peak oxygen uptake; VO ($_{2}$peak)) exercises, despite the fact that endogenous carbohydrate reserves are unlikely to be limited. To maintain rapid glycogen saturation during the acute recovery phase after strenuous exercise, it is necessary to provide a large amount of exogenous carbohydrates (1.2 g · kg^{-1} · h^{-1}) (Cermak, van Loon, 2013).

In addition, it has been found that addition of carbohydrates during exercise reduces adaptation to training, but currently there is insufficient evidence to support this fact (Jeukendrup, McLaughlin, 2011). In contrast, carbohydrate intake during limited recovery has been shown to primarily determine muscle glycogen resynthesis and re-exercise capacity (Jeukendrup, 2014). To optimize repeated exercise after short-term recovery, carbohydrate intake of ≥ 1.2 g kg body weight˙ h^{-1} may maximize muscle glycogen replenishment (Algannam et al., 2018). An additional effect on isoenergetic carbohydrates amount recovery rates in adolescent swimmers is also known (McKinlay et al., 2020). Carbohydrates and proteins intake (CHO-PRO) after exercise increases muscle glycogen saturation providing recovery. (Margolis et al., 2021).

In the present study, all the detected changes have the same orientation. It concerns the reduction of the catabolic (damaging) effect of muscle loading on athletes' body when taking carbohydrate food.

We found in our study a significant excess of the main catabolic hormone - cortisol concentration, relative to the reference values, in the examined athletes - wrestlers on an empty stomach in a muscular rest state. It correlates with the results of a study, showing that wrestlers had significantly higher cortisol levels, both at rest and on a treadmill exercise test until complete exhaustion (Popovic et al., 2019). According to the researchers, intense and continuous physical exercise can cause changes in the hormones concentration in blood serum. When elevated laboratory levels lead to exceeding the reference range, further examinations are prescribed or participation in training is discontinued (Banfi et al., 2012).

Analyzing carbohydrate intake effect on cortisol content in blood serum, we found its steady decline in natural, non-loading conditions. The high lean concentration of the studied hormone was significantly higher than the normal values for the relative muscle rest state. Under these conditions, carbohydrate food intake reduced the catabolic hormone concentration to normal values, thus having a «smoothing» effect. Our findings are consistent with studies indicating that carbohydrate and protein intake was associated with reduced markers of muscle damage and improved recovery after exercise (Saunders, 2007).

This fact is considered important, because it allows us to talk about the possibility of correcting metabolic processes with the help of food, in this case, food of carbohydrate composition. Athletes-wrestlers, training mainly with a speed-strength orientation are characterized by chronically high levels of catabolic hormones. Therefore, it is important to note that carbohydrate food intake led to a significant decrease in the «stress hormone» - cortisol, which can be considered as one of the protective mechanisms for reducing functionally excessive stress.

The testosterone content in these conditions is in the normal distribution range of average values. Food intake does not have any significant effect on its concentration.

The hormones cortisol and testosterone are competitive agonists at muscle cell receptors level and the testosterone/cortisol ratio is used as an indicator of anabolic / catabolic balance. The testosterone/cortisol ratio (anabolism index) can be used as an actual physiological load indicator during exercise, and can also act as anabolic status indicator. In a relative muscle rest state on an empty stomach, the anabolism index was 0.037 ± 0.003, it indicates a lack of recovery and the development of fatigue. Carbohydrate food intake led to a slight increase in the anabolism index to values of 0.057 ± 0.005 at the 105th minute of the postprandial period.

Performing a 60-minute cycle ergometric loading caused multidirectional changes in the studied hormones' content. Thus, immediately after exercise before meals, the cortisol concentration was 757.5 ±43.5 nmol/l and continued to increase ($p < 0.05$) during meals to 981.0 ± 71.7 nmol/l. And only at the 105th minute there was a decrease to 645.4 ± 57.4 nmol/l.

Thus, in contrast to non-loading conditions, when food intake significantly reduced cortisol concentration to the level of normal values, this did not happen under load conditions. This is consistent with studies where high-intensity endurance exercise has been shown to promote a catabolic response, and the level of response is highly dependent on the previous training level (Popovic et al., 2019).

Carbohydrate intake did not cause significant changes in cortisol concentration after 60 minutes of muscle tension during the one-and-a-half-hour postprandial period. And only in 105 minutes after the loading end and the carbohydrates intake, cortisol content in blood serum decreased. This fact, in our opinion, may be due to both natural (temporary) restitution and the delayed nature of food intake effect on hormonal and metabolic processes. Both the first and second provisions require clarification.

Changes in the opposite direction are established in relation to testosterone. Performing a muscle loading led to a decrease in testosterone concentration in the subjects' blood serum. The combined effect of physical activity and carbohydrate intake during the entire postprandial period caused a decrease in testosterone concentration from 33.0 ± 2.5 from fasting conditions to 25.4 ± .1 nmol/l at 75th minute of the inter-digestive period ($p < 0.05$). In the profile state, the food component had a pronounced character of a stimulator of anabolic processes and was characterized by an increase in the anabolism index from 0.037±0.003 nmol/l in lean conditions to 0.057 ± 0.005 nmol/l at the 105th minute of the postprandial period. The increase was 54 %.

It is important that the anabolism index in the fasting profile state is only 0.037 ± 0.003 nmol/l, which is characterized as a lack of recovery and the development of fatigue after the previous muscle loading. At the same time, carbohydrate food intake led to an increase in the anabolism index to the reference values of the index - 5-8%. Muscle load had a significant impact on the examined athletes' hormonal pool, which affected both the anabolic/catabolic hormones content in blood serum and the anabolic index. Human studies have shown that cortisol introduction into the bloodstream at rest leads to a decrease in testosterone level in blood. Many researchers have used these results to suggest that exercise-induced increases in cortisol may lead to a subsequent decrease in circulating testosterone level.

The analysis of the degree and direction of the studied hormones' mutual influence and the anabolism index in relative muscle rest conditions and the action of 60 minutes of muscle load in the examined athletes showed the presence of negative statistical associative relationships (Table 4).

Table 4. The relationship of the studied hormones and the anabolism index in the conditions of relative muscle rest and the action of 60 minutes of muscle load in the examined athletes (only significant changes are indicated)

rest loading		Cortisol					Testosterone				
		T	15	45	75	105	T	15	45	75	105
T/C	T	______ - 0,714**					0,678* 0,821***				
	15		______ - 0,836***	______- 0,705 *				0,896*** 0,872***	0,711 * 0,692 *	0,908*** 0,618 *	
	45		_____ -0,71*	-0,653* - 0,788*** 				_____ 0,803***	0,664* 0,925***		
	75				-0,635* - 0,818***					0,634* 0,768***	
	105					___-___ - 0,908***				0,644 * 0,644*	0,844*** 0,782***

Note: According to the ranking table: - weak relationship - correlation coefficient from 0 to ± 0.299, - medium relationship - ± 0.3 to ± 0.699, - strong relationship from ± 0.7 to ± 1.
* - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$

In our study, the anabolism index in the fasting post-exercise period was 0.046 ± 0.005 and during the entire postprandial period it decreased to 0.028 ± 0.003 at 75th minutes. A decrease in AI below 3% indicates serious disorders of endocrine regulation and overexertion, overtraining development. And only at the 105th minute, the anabolism index is restored to 0.048 ± 0.005. In connection with the detected changes, it can be assumed that the possible reason for such a significant decrease in the anabolism index in the examined athletes-wrestlers under muscle loadings conditions is: a developing state of acute or chronic overstrain (overtraining), which occurred under the action of previous loads. A marker of this condition is high cortisol values in profile conditions. And if carbohydrates intake at rest led to a decrease in cortisol, and, accordingly, an increase in the anabolism index, then after the action of muscle loading, this was not observed until the 105th minute of the postprandial period. This may indicate both a certain excess of the previous load, and incomplete recovery, while a more likely option may be the combined effect of these factors.

Presumably, the intake of different composition (protein, fat, carbohydrate, mixed) food and the time of its intake will allow adjusting hormonal response, thus strengthening the anabolic component - muscle glycogen and maintain the maximum daily rate of muscle protein synthesis (Moore, 2015). This fact, as we assume, is able to intensify the recovery processes (anabolism) after the performed muscle loading and ensure readiness for subsequent stress. Carbohydrate intake after exercise has proven to be an important factor in determining muscle glycogen synthesis and recovery, and carbohydrate intake during high-intensity exercise can improve performance (Beelen, 2015). Natural biological recovery stimuli use, in the form of consumed nutrients, is a powerful stimulus and accelerator of restitution processes. The study is in this direction: the physiological (nutritive) mechanisms of recovery processes after muscle tension action seem to us the most promising (da Silva et al., 2021).

Conclusions

Studying the effect of carbohydrate intake in muscle rest state and under muscle load action on the catabolic/anabolic hormones content in highly qualified athletes' blood serum, we found: 1. carbohydrate food helps to reduce the high fasting concentration of cortisol. This effect becomes significant in 75 minutes after eating. 2. Carbohydrates intake did not cause a significant change in cortisol concentration in blood serum after the 60-minute muscle cycling exercise. The cortisol content remained high throughout the entire inter-digestive period (105 minutes). 3. there were no significant changes in testosterone concentration when taking carbohydrate food, both in a relative muscle rest state, and under muscle dynamic load action. 4. the anabolism index was reduced in the background conditions of 0.037 ± 0.003, the carbohydrate food intake led to a slight increase in this indicator after 105 minutes of the inter-digestive period.

We believe that carbohydrate food can have a protective effect, being a metabolic mechanism, affecting hormonal processes of tension/recovery of the athletes' body.

We also believe that additional research is needed to find out the hormonal and metabolic mechanisms of the athletes' body recovery processes after muscle tension and the influence of food of different chemical composition on these processes – as an irritant for restitution processes. Further investigation of the response of specific nutrient/learning strategies to metabolic and cellular signaling pathways is needed to determine the main mechanisms by which such interventions have an impact. However, such studies should be accompanied by studies assessing the impact of these responses on the «real» athletes' training adaptation.

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