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Comparative Biochemical Analysis of Blood Parameters in Elite Athletes for Monitoring Overtraining Syndrome: A Pilot Study

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Abstract

Introduction. Elite athletes undergo regular high-intensity training to maintain fitness and improve performance. Training overload is necessary to achieve significant results. However, failure to balance training and recovery can lead to overtraining syndrome. To prevent this, regular monitoring of biochemical markers is required to assess the body's adaptive processes.

Aim of the study. Comparative analysis of blood biochemical parameters in Olympic athletes of Kazakhstan to assess training load effects and prevent overtraining syndrome

Methods. A biochemical analysis was conducted to measure levels of creatinine, creatine kinase (CK), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in Olympic athletes.

Results. Significant differences in biochemical markers were observed among sports disciplines. Creatinine ($p<0.001$), alkaline phosphatase ($p<0.001$), AST ($p<0.001$), CK ($p=0.008$), and LDH ($p<0.001$) levels differed significantly between groups, whereas ALT showed no significant variation ($p=0.191$). Post hoc analyses revealed higher creatinine levels in weightlifters and freestyle wrestlers, elevated alkaline phosphatase levels in combat sport athletes, and significant sport-specific differences

in AST, CK, and LDH, reflecting variations in muscle metabolism and training-related physiological adaptation.
Conclusion. These findings underscore the importance of monitoring biochemical markers in elite athletes to prevent overtraining and optimize the training process.

Keywords: biochemical markers, sports medicine, creatine kinase, lactate dehydrogenase, athletes of Kazakhstan.

1. Introduction

Athletes are considered among the healthiest individuals, given that regular physical activity is one of the most effective ways to improve health and reduce cardiovascular disease (CVD) risk factors [1,2]. Nevertheless, preparing elite athletes requires long-term, systematic training. While training overload is considered essential for achieving performance enhancement, its effectiveness depends critically on adequate recovery. While an adequate increase in training intensity balanced with proper recovery can lead to significant improvements, an imbalance between training and recovery may result in decreased performance. Consequently, athletes are frequently exposed to states of overreaching, overtraining, and various fatigue-related syndromes [3].

The pathogenesis of fatigue in athletes remains insufficiently understood and shares many mechanisms with overtraining. Both conditions are characterized by reduced performance capacity, premature fatigue, and increased muscle soreness. However, in cases of fatigue, recovery typically requires several days or weeks, whereas overtraining—associated with more profound physiological and pathological alterations—may require months for full restoration [4]. The key indicators of overtraining include an athlete's inability to tolerate high-intensity physical loads and a persistent decline in performance even when training intensity is markedly reduced.

The body's response and adaptation to increased muscular activity are accompanied by biochemical changes that affect all physiological systems. Biomarkers involved in muscle contraction and neural signal transmission, such as calcium, urea, and creatinine, also undergo significant biochemical alterations in response to training load [5]. Therefore, monitoring these adaptive changes is essential for coaches, sports physicians, and athletes. Biochemical monitoring using blood-based biomarkers is widely employed to assess training responses and to aid in the detection and diagnosis of overtraining in athletes [6,7].

Particular attention is given to biomarkers such as creatine kinase (CK), creatinine, lactate dehydrogenase (LDH), alkaline phosphatase, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST). During high-intensity training, elevated CK activity may result from mechanical or metabolic stress imposed on muscle tissue [8–10]. LDH plays a key role in energy metabolism by converting lactate to pyruvate and is therefore associated with cellular disruption caused by muscular overload and tissue damage [11]. ALT and AST levels serve as indicators of increased stress on the liver and on cardiac and skeletal muscle [12,13]. Monitoring these biomarkers enables coaches and sports physicians to optimize training programs and reduce the risk of overtraining in athletes.

The aim of this study was to compare blood biochemical parameters among elite athletes across different sports to monitor for overtraining syndrome.

2. Materials and Methods

This study was designed as a cross-sectional pilot study. The study population consisted of active athletes from the Republic of Kazakhstan's Olympic and national teams, all of whom engaged in regular training. Recruitment was conducted at the training camps "Zhaksy-2" (Borovoe, Northern Kazakhstan) and "AIBA" (Almaty region, Southeastern Kazakhstan), where athletes from different sports disciplines were present simultaneously. Athletes were enrolled in the study only after signing informed consent. All participants underwent an interview and completed a questionnaire that included anthropometric measurements such as

height, weight, and body mass index (BMI). Medical information was also collected and entered into the project's database.

Medical data and biochemical analysis results were obtained from the medical records of the National Center for Sports Medicine and Rehabilitation (Almaty, Kazakhstan) and the "ProSport" Center for Sports Medicine and Rehabilitation (Astana, Kazakhstan).

The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the local ethics committee of the Private Institution "National Laboratory Astana" (Protocol No. 05-2022, November 21, 2022).

Blood samples were collected between 8:00 and 10:00 a.m. after a 12-hour overnight fast. To minimize variability in biochemical parameters, athletes were instructed to refrain from morning training prior to blood collection. Blood samples were drawn into tubes coated with microionized silica particles to accelerate clotting and containing a polymer gel for serum separation (BD, NJ, USA). The collection tubes were centrifuged for 15 minutes at 2,000 rpm to separate serum, after which serum aliquots were stored at −80 °C until further analysis.

Serum samples were used to determine biochemical parameters. All serum samples underwent a single freeze–thaw cycle prior to measurement to ensure analytical consistency. Biochemical marker analysis was performed upon request of the National Center for Sports Medicine and Rehabilitation, and the results were provided to the research team for study purposes. All biochemical analyses were conducted at the "Olymp" Clinical Diagnostic Laboratory (CDL).

Statistical analysis of the data was performed using RStudio (version 2024.09.1+394). All variables were assessed for plausibility and the presence of outliers. Normality assumptions were evaluated using the Shapiro–Wilk test. Normally distributed variables were compared between sport groups using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for pairwise comparisons. For variables that did not meet the assumption of normality, the Kruskal–Wallis test was applied, followed by Dunn's post hoc test with Bonferroni correction.

3. Results

Study group

A total of 48 active male athletes from Kazakhstan's main and reserve National and Olympic teams across various sports disciplines were recruited for the study. Recruitment was conducted between November 2023 and February 2024, following the completion of 2-week intensive training camps. For most athletes, this period corresponded to the end of a training cycle, which may reflect peak physiological load rather than baseline conditions. An exception was observed in speed skaters, who were in a competitive season phase during the time of sampling. The athletes represented seven different sports: speed skating, boxing, judo, weightlifting, Greco-Roman wrestling, freestyle wrestling, and taekwondo. The mean age of the participants was 22.9±2.7 years. The average height was 168.3±5.7 cm, the mean body weight was 72.5±24.5 kg, and the mean body mass index (BMI) was 25±7.7 kg/m². All the participants were male and predominantly Asian (81.25%). The mean duration of athletic training was 12±5.7 years. Demographic characteristics of the recruited athletes are presented in Table 1.

Biochemical analysis

Biochemical marker data were available for all 48 athletes. The assessed parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, creatine kinase (CK), lactate dehydrogenase (LDH), and alkaline phosphatase (AP) levels. The mean values, ranges, and reference values for these biochemical parameters are presented in Table 2.

Table 1 – Demographic data (n = 48)

Age (years, mean ± SD)	22.9 ± 2.7
Sex, n (%)	
Male	48 (100)
Nationality, n (%)	39 (81.25)
Asian	9 (18.75)
Caucasian	
Experience (years, mean ± SD)	12 ± 5.7
Weight (kg, mean ± SD)	72.5 ± 24.5
Height (cm, mean ± SD)	168.3 ± 5.7

BMI (kg/m2, mean ± SD)	25 ± 7.7
Sport type	
Boxing	7
Speed skating	7
Freestyle wrestling	6
Greco-Roman wrestling	7
Taekwondo	7
Judo	7
Weightlifting	7

BMI: body mass index; SD: standard deviation

Statistical analysis

Data were assessed for normality using the Shapiro–Wilk test. AST (W=0.778, p<0.001), ALT (W=0.886, p<0.001), CK (W=0.727, p<0.001), and LDH (W=0.900, p<0.001) were not normally distributed, whereas creatinine (W=0.964, p= 0.149) and alkaline phosphatase (W=0.965, p=0.163) were normally distributed.

Subsequently, an analysis was conducted to evaluate differences in biochemical parameters among sports groups. Biochemical profile indicators stratified by sport are presented in Table 3.

Table 2 – General indicators of athletes' biochemical profile

Indicators	Athletes		
	Mean ± SD(n)	Range	Reference value
AST	19.7 ± 11.8	6.5-59.1	0-40 units/l
ALT	8.5 ± 5	2.2-23.9	0–41 units/l
Creatinine	93.8 ± 18,8	18-135	62–132 μmol/L
CK	356 ± 331.5	58.8-1666	0–192 units/l
LDH	185.7 ± 50.6	50-349	0–232 units/l
AP	62.8 ± 20.4	20-126	0–129 units/l

ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; CK: creatine kinase; LDH: lactate dehydrogenase

Table 3 – Biochemical profile indicators of athletes by sport type

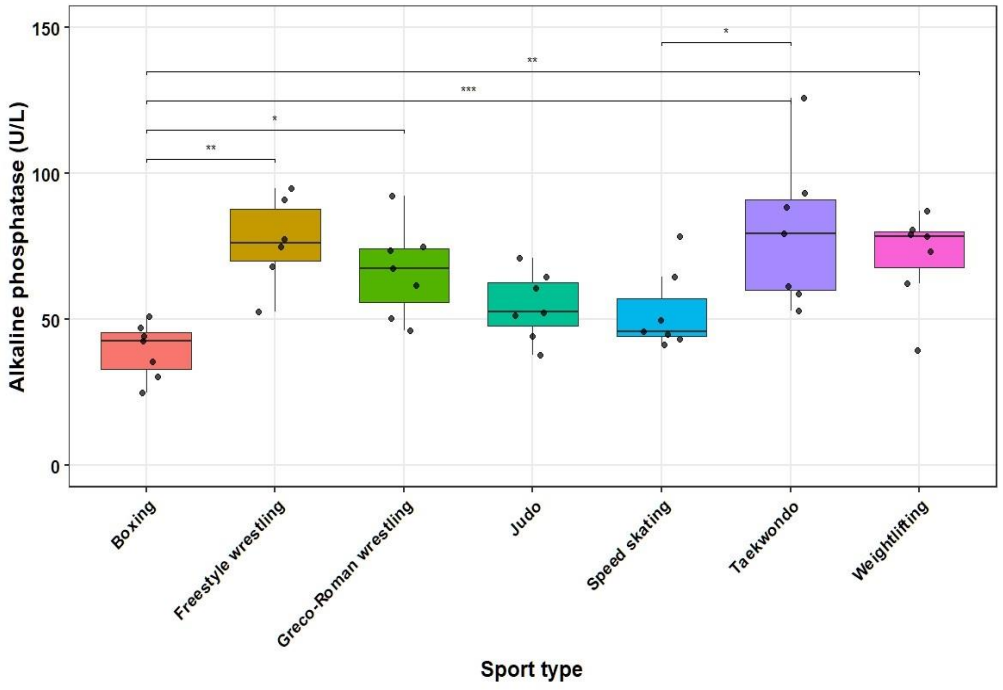
Indicators	Sport types						
	Boxing	Freestyle wrestling	Greco-Roman wrestling	Judo	Speed skating	Taekwondo	Weightlifting
AST units/l	10 ± 1.8	39.9 ± 19.1	18.1 ± 4.7	15.2 ± 8	15.5 ± 2.4	16 ± 2.8	26.3 ± 6.6
ALT uits/l	7.7 ± 4.2	13.8 ± 6.3	9.5 ± 7.1	8.5 ± 6.2	7.1 ± 2.3	5.4 ± 2.9	8.5 ± 2.1
Creatinine μmol/L	77.3 ± 23.5	104.2 ± 18.8	88.1 ± 12	96.6 ± 8.1	97.6 ± 16.6	79.4 ± 6.8	114.6 ± 13
CK units/l	237.2 ± 205.4	798.5 ± 464	218.7 ± 125.9	251.8 ± 192	191 ± 86	329 ± 198.5	5217.4 ± 475
LDH units/l	175.1 ± 41.9	217.3 ± 40.3	178 ± 17.5	158.4 ± 19	151.9 ± 24	160.6 ± 22.1	263 ± 64
AP units/l	39.4 ± 9.4	76.6 ± 15.5	66.6 ± 15.7	54.6 ± 11.6	52.7 ± 13.8	80.1 ± 25.4	71.5 ± 16.1

ALT: alanine aminotransferase; AP: alkaline phosphatase; AST: aspartate aminotransferase; CK: creatine kinase; LDH: lactate dehydrogenase

Accordingly, one-way ANOVA was applied to creatinine and alkaline phosphatase, and the Kruskal–Wallis test to AST, ALT, CK, and LDH. Significant differences among sport types were observed for creatinine ($F(6,41)=5.44$, $p<0.001$) and alkaline phosphatase ($F(6,41)=5.74$, $p<0.001$). Post hoc Tukey analysis revealed significantly higher creatinine levels in freestyle wrestlers compared with boxers ($p=0.038$), in weightlifters compared with boxers ($p< 0.001$), and in weightlifters compared with Greco-Roman wrestlers ($p=0.032$). Alkaline phosphatase levels were significantly higher in freestyle wrestlers ($p=0.003$), Greco-Roman wrestlers ($p=0.042$), taekwondo athletes ($p<0.001$), and weightlifters ($p=0.009$) compared with boxers. In addition, taekwondo athletes demonstrated higher alkaline phosphatase levels than speed skaters ($p=0.039$) (Figure 1).

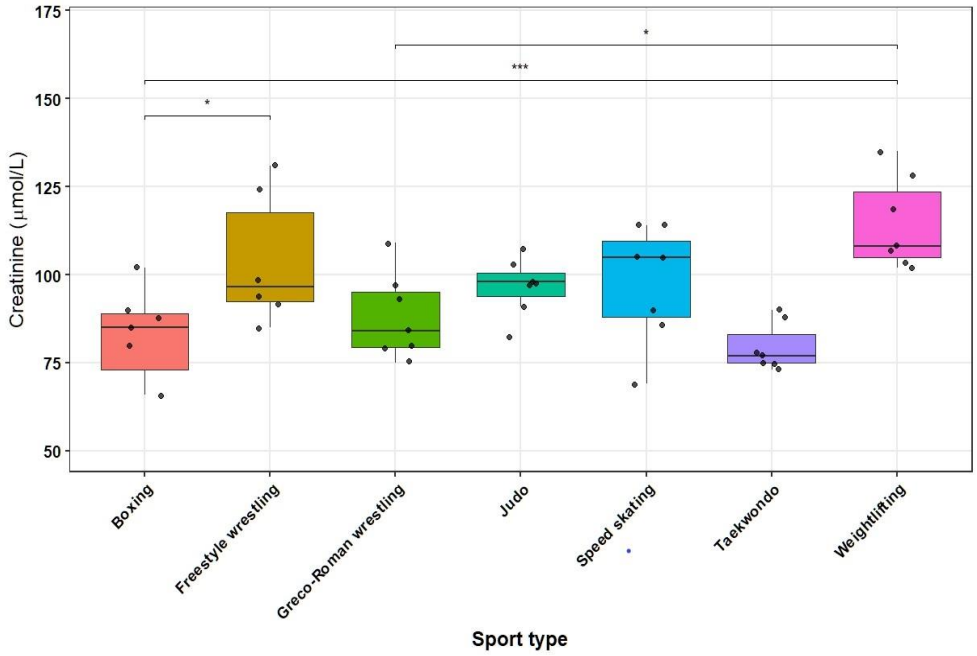
For non-normally distributed variables, the Kruskal–Wallis test identified significant differences in AST ($\chi^2(6)=29.20$, $p<0.001$), CK ($\chi^2(6)=17.50$, $p=0.008$), and LDH ($\chi^2(6)=23.72$, $p<0.001$), whereas no significant differences were observed for ALT ($\chi^2(6)=8.70$, $p=0.191$).

Post hoc Dunn's tests with Bonferroni correction demonstrated significant differences in AST between boxers and freestyle wrestlers (adjusted $p<0.001$), and between boxers and weightlifters (adjusted $p<0.001$). Significant differences in CK were observed between boxers and freestyle wrestlers (adjusted $p=0.033$) and between freestyle wrestlers and speed skaters (adjusted $p=0.034$) (Figure 2). For LDH, significant differences were identified between weightlifters and judo athletes (adjusted $p=0.014$), speed skaters (adjusted $p=0.004$), and taekwondo athletes (adjusted $p=0.030$). The differences among athletes in LDH are shown in Figure 3.



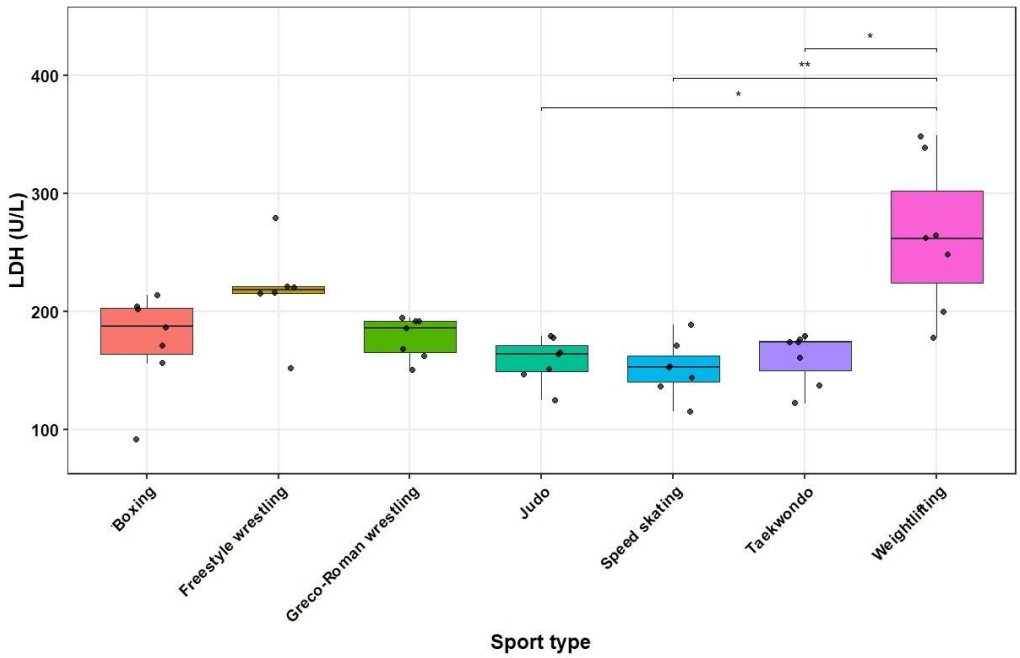
Data are presented as boxplots showing the median and interquartile range with individual observations overlaid. Significant pairwise differences identified by Tukey's post hoc test are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001)

Figure 1 - Sport-specific differences in alkaline phosphatase levels among elite athletes



Data are presented as boxplots showing the median and interquartile range with individual observations overlaid. Significant pairwise differences identified by Tukey's post hoc test are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001)

Figure 2 - Sport-specific differences in creatinine levels among elite athletes



Data are presented as boxplots showing the median and interquartile range with individual observations overlaid. Significant pairwise differences identified by Dunn's post hoc test with Bonferroni correction are indicated by asterisks (*p<0.05, **p<0.01, ***p<0.001)

Figure 3 - Sport-specific differences in LDH levels among elite athletes

4. Discussion

In this study, we compared blood levels of CK, creatinine, LDH, AP, AST, and ALT among elite athletes from different sports disciplines. Biochemical analysis data from 48 athletes were obtained and analyzed.

The assessment of biochemical parameters in athletes enables monitoring of fatigue-related markers and evaluation of training load, thereby allowing timely adjustments to training programs and an optimal balance between exercise and recovery [14,15]. Increased training load and cellular stress in muscle tissue are accompanied by measurable biochemical alterations. Levels of LDH, CK, creatinine, alkaline phosphatase, AST, and ALT are important indicators of overtraining status in athletes.

Creatinine, alkaline phosphatase, AST, CK, and LDH levels differed significantly across sport disciplines, whereas ALT levels remained comparable between groups. The highest creatinine values were observed in weightlifters and freestyle wrestlers, which likely reflects greater skeletal muscle mass and increased muscle turnover associated with strength-oriented training. Similarly, elevated CK levels in freestyle wrestlers are consistent with previous reports linking intensive anaerobic exercise and muscle fibre microdamage to increased circulating CK activity [16,17].

Differences in LDH levels were particularly evident between weightlifters and athletes participating in judo, speed skating, and taekwondo. LDH, an important enzyme for anaerobic energy production, can indicate metabolic stress and tissue adaptation from training [17]. These differences across sports disciplines likely reflect differences in physiological demands, training intensity, and recovery patterns.

Interestingly, AST levels also differed significantly between sport groups, particularly between boxers and both freestyle wrestlers and weightlifters. Because AST is present in skeletal muscle as well as the liver, elevated AST levels might indicate increased muscle stress rather than hepatic dysfunction. In contrast, ALT levels did not differ significantly across sports, suggesting that sport-specific variation primarily affects markers associated with muscular adaptation rather than liver-specific enzymes.

Increased alkaline phosphatase levels in freestyle wrestlers, taekwondo athletes, and weightlifters might suggest enhanced bone remodelling and musculoskeletal adjustments resulting from high-impact and strength-focused training. Since alkaline phosphatase is closely associated with bone metabolism, the observed differences may indicate sport-specific adaptations to repetitive mechanical loading. However, further studies are required to determine whether these changes are associated with training intensity, recovery status, or long-term skeletal adaptation.

Overtraining syndrome is a condition that develops as a consequence of excessive physical load combined with inadequate recovery processes and chronic muscular fatigue. Research suggests that high training loads are ultimately necessary to achieve athletic performance gains. However, they must be accompanied by adequate recovery strategies and the inclusion of several “light” training days [18]. The consequences of overtraining include impaired sleep quality, mood disturbances, increased injury risk, and immune suppression [19–21].

It is important to note that overtraining syndrome involves not only muscular exhaustion but also alterations in the immune system, nervous system dysfunction, and disturbances in hormonal balance, which may further exacerbate its negative consequences.

There are several limitations in this pilot study. One limitation of the present study is its relatively small, small sample size across sports disciplines, which may reduce the statistical power of the analysis. Second, the absence of a control group limits the ability to compare the findings with baseline physiological values. Furthermore, biochemical parameters were assessed at a single time point following intensive training periods, which may reflect peak physiological load rather than baseline conditions. Finally, individual training programs and dietary intake were not accounted for, both of which may influence biochemical parameters. These limitations will be addressed in future studies.

5. Conclusion

Taken together, these findings support the use of creatinine, CK, LDH, AST, and AP as potentially informative biomarkers for monitoring physiological adaptation and excessive training load in elite athletes.

Conflict of interest: The authors declare no conflict of interest.

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All authors read and approved the final version of the manuscript and signed the copyright transfer form.

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Түйіндеме

Кіріспе. Элиталық спортшылар үздік нәтижелерге қол жеткізу және дене дайындығын сақтау үшін үнемі жоғары қарқындылықтағы жаттығулардан өтеді. Жаттығу жүктемесі маңызды нәтижелерге жету үшін қажет, алайда жаттығу мен демалыстың дұрыс үйлестірілмеуі шамадан тыс жүктелу синдромының дамуына әкелуі мүмкін. Бұған жол бермеу үшін ағзаның адаптациялық процестерін бағалау мақсатында биохимиялық көрсеткіштерді тұрақты бақылау қажет.

Зерттеу мақсаты. Қазақстандық олимпиадалық спортшылардың қанындағы биохимиялық көрсеткіштердің салыстырмалы талдауы: жаттығу жүктемесінің әсерін бағалау және шамадан тыс жүктелу синдромын алдын алу.

Әдістер. Олимпиадалық спортшылардың қанында креатинин, креатинкиназа (СК), лактатдегидрогеназа (ЛДГ), сілтілік фосфатаза (ЩФ), аспартатаминотрансфераза (АСТ) және аланинаминотрансфераза (АЛТ) деңгейлері бойынша биохимиялық талдау жүргізілді.

Нәтижелер. Биохимиялық маркерлердің деңгейлері спорт түрлері арасында статистикалық тұрғыдан маңызды айырмашылықтар көрсетті. Креатининнің (p<0,001), сілтілі фосфатазаның (p<0,001), АСТ-ның (p<0,001), креатинкиназаның (КК) (p=0,008) және лактатдегидрогеназаның (ЛДГ) (p<0,001) деңгейлері топтар арасында айтарлықтай ерекшеленді, ал АЛТ деңгейінде статистикалық маңызды айырмашылық анықталған жоқ (p=0,191). Пост-хок талдау ауыр атлеттер мен еркін күрес спортшыларында креатинин деңгейінің жоғары екенін, жекпе-жек спорт түрлерімен айналысатын спортшыларда сілтілі фосфатаза деңгейінің жоғарылағанын, сондай-ақ АСТ, КК және ЛДГ көрсеткіштері бойынша спорт түрлеріне тән елеулі айырмашылықтардың бар екенін көрсетті. Бұл айырмашылықтар бұлшықет метаболизмінің және жаттығу жүктемелеріне физиологиялық бейімделудің ерекшеліктерін көрсетеді.

Қорытынды. Аталған деректер элиталық спортшыларда биохимиялық көрсеткіштерді бақылаудың шамадан тыс жүктелуді алдын алу және жаттығу процесін оңтайландыру үшін маңызды екенін растайды.

Түйін сөздер: биохимиялық маркерлер, спорттық медицина, креатинкиназа, лактатдегидрогеназа, Қазақстан спортшылары.

Сравнительный биохимический анализ показателей крови элитных спортсменов для контроля синдрома перетренированности: Пилотное исследование

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Резюме

Введение. Элитные спортсмены подвергаются регулярным высокоинтенсивным тренировкам для поддержания формы и улучшения спортивных результатов. Перегрузка на тренировке необходима для достижения значимых успехов. Однако несоблюдение режима чередования тренировок и отдыха может привести к развитию синдрома переутомления. Во избежание этого требуется регулярный контроль биохимических показателей для оценки адаптационных процессов организма.

Цель исследования. Сравнительный анализ биохимических показателей крови у олимпийских спортсменов Казахстана для оценки влияния тренировочных нагрузок и предотвращения синдрома перетренированности.

Методы. Проведен биохимический анализ уровней креатинина, креатинкиназы (СК), лактатдегидрогеназы (ЛДГ), щелочной фосфатазы (ЩФ), аспартатаминотрансферазы (АСТ) и аланинаминотрансферазы (АЛТ) у олимпийских спортсменов.

Результаты. Были выявлены статистически значимые различия биохимических показателей между спортивными дисциплинами. Уровни креатинина (p<0,001), щелочной фосфатазы (p<0,001), АСТ (p<0,001), креатинкиназы (КК) (p=0,008) и лактатдегидрогеназы (ЛДГ) (p<0,001) достоверно различались между группами, тогда как для АЛТ значимых различий не обнаружено (p=0,191). Пост-хок анализ показал более высокие уровни креатинина у тяжелоатлетов и спортсменов по вольной борьбе, повышенные уровни щелочной фосфатазы у

представителей единоборств, а также значимые различия по АСТ, КК и ЛДГ между спортивными дисциплинами, отражающие особенности мышечного метаболизма и физиологической адаптации к тренировочным нагрузкам.

Выводы. Данные подтверждают значимость контроля биохимических показателей у элитных спортсменов для предотвращения перетренированности и оптимизации тренировочного процесса.

Ключевые слова: биохимические маркеры, спортивная медицина, креатинкиназа, лактатдегидрогеназа, спортсмены Казахстана.