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Overtraining syndrome in bodybuilding and the difficulty of searching for informative biomarkers for disadaptation diagnostics

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Abstract

Purpose. To study the characteristic features of overtraining syndrome (OTS) in bodybuilding in athletes with different resistance levels and to identify informative blood biomarkers to diagnose disadaptation.

Material & Methods. 90 people aged 22±1.3 years, with experience in strength training of 3.2±0.5 years, were examined. 3 groups (30 people each) were created. Group A – bodybuilders who, according to the medical examination results, had no contraindications (HBM). Group B – bodybuilders with symptoms of functional exhaustion (BFE). Group C – healthy men engaged in power fitness (HMF). By analyzing biochemical blood markers (CPK, LDH, cortisol, and testosterone), the characteristics of adaptive and compensatory responses to test exertions were identified (R_a=0.64 and R_a=0.74). Control was carried out at the beginning of the study and after 14 days of high-intensity strength training (HIRT).

Results. The study showed that the baseline levels of the monitored biochemical blood parameters in HBM, BFE, and HMF participants were within the normal reference range. Despite the growth of the baseline level of CPK, LDH, and cortisol in the blood in bodybuilders of the HBM and BFE groups after two weeks of using HIRT, the values remained within the reference range. The determination of functional overreaching (FOR) in the HBM and HMF groups was made possible through the analysis of results showing a 50-53% increase in CPK and a 23-26% rise in cortisol levels in response to high-intensity loads (R_a=0.74). Based on the analysis of changes in LDH and cortisol levels in the blood in response to strength loads, it was possible to detect non-functional overreaching (NFOR) in BFE, HBM, and HMF groups. In individuals diagnosed with NFOR, even in response to loads under conditions of the creatine phosphokinase energy supply mechanism, there is a significant increase in LDH (30–47%) and cortisol (75–107%). In response to test loads, regardless of their intensity or type of anaerobic energy supply, there was a simultaneous increase in CPK (143-173%), LDH (66–92%), and a decrease in cortisol (46–54%) in BFI individuals suspected of having OTS.

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Conclusions. The proposed mechanism for identifying FOR, NFOR, and OTS during bodybuilding training, based on evaluating the nature of changes in the body's adaptive-compensatory responses to a stress stimulus, is a tool for monitoring disadaptation. The results indicate that, for diagnosing OTS in HBM, BFE, and HMF athletes, the baseline levels of CPK, LDH, cortisol, and testosterone in the blood are not informative biomarkers. Using test loads (R_a=0.64 and R_a=0.74) as stress stimuli for the HBM, BFE, and HMF groups allows for identifying short-term adaptation or compensatory response manifestations based on the acute response of blood biomarkers.

Keywords: overtraining syndrome, bodybuilding, disadaptation, biochemical blood parameters, energy supply, load.

Introduction

In modern sports, issues related to disadaptation and overtraining resulting from excessive training loads combined with insufficient recovery periods are among the most debated scientific topics (Grandou et al., 2020; Carrard et al., 2022). However, the main challenge in practical implementation lies in the absence of an optimal assessment system for identifying overtraining syndrome (OTS) using modern physiological and biochemical diagnostic methods (Kajaia et al., 2021; Soler-López et al., 2024). Most researchers (Carfagno et al., 2014; Symons et al., 2023) consider that OTS, as well as functional (FOR) and non-functional overreaching (NFOR), are manifestations of reduced performance capacity due to a breakdown in adaptation, associated with metabolic, immune, and hormonal dysfunctions. However, it is important to consider that the development of OTS is not only related to the mismatch between training loads and the body's adaptive reserves, but also the lack of an effective training management system (Olkhovyi et al., 2016; Cadegiani et al., 2019; Annunziata et al., 2024).

Considerable attention is devoted to studying the feasibility of using biomarkers as indicators for assessing manifestations of disadaptation processes in various sports disciplines (Cadegiani et al., 2021; Weakley et al., 2022). However, the complexity of using these biomarkers as unified markers for evaluating OTS is justified by the specifics of the sport, the training period, and the athletes' level of resistance to training loads (Olkhovyi et al., 2020; Cheng et al., 2020; Chernozub et al., 2020). In investigating the nature of adaptive-compensatory responses under conditions of high-intensity resistance training (HIRT) and low-intensity blood flow restriction training (LI-BFR), a significant number of researchers employ biochemical blood markers (Grandou et al., 2020; Chernozub et al., 2023). In most cases, they monitor enzyme activity indicators (CPK, LDH, AST, ALT) and hormone concentrations (cortisol, testosterone, insulin, somatotropin) during training and competition activities (Silva et al., 2022). However, the majority of studies (Tian et al., 2015; Klymovych et al., 2020; Younger, 2025) focus on short-term and long-term adaptation processes in the context of improving training systems. At the same time, insufficient attention is given to diagnosing disadaptation processes, studying their main mechanisms, searching for informative control markers, and developing effective re-adaptation models.

Purpose of the Study. To study the characteristic features of overtraining syndrome (OTS) in bodybuilding in athletes with different resistance levels and to identify informative blood biomarkers to diagnose disadaptation.

Material and Methods

Participants

The study involved 60 bodybuilders and 30 men engaged in power fitness. The participants had almost identical ages (22±1.3 years), training experience (3.2±0.5 years), body weight (85.5±2.2 kg), body fat percentage (15.7±1.1%), and strength capabilities (1RM) in basic exercises. The participants were divided into three groups (30 individuals in each group). Group A consisted of bodybuilders who had no contraindications for participation in the study according to the results of a preliminary medical examination (HBM – healthy bodybuilders-men). Group B included athletes who, during a series of previous training sessions, exhibited symptoms of functional exhaustion, but after a comprehensive medical examination, it was determined that there were no deviations from reference values (BFE – bodybuilders with functional exhaustion). Group C was composed of physically healthy men engaged in strength fitness (HMF – healthy men engaged in fitness). The study was conducted in 2024 at the KINEZUS Modern Kinesiology Research Center and its branches (Uzhhorod, Odesa, Chernivtsi, Mykolaiv, Rivne, Ukraine). The ethics committee of the Lesya Ukrainka Volyn National University, Ukraine, approved the study design. After explaining the risks and benefits of the study, participants signed an informed consent form prepared

following the ethical standards of the Declaration of Helsinki.

Measurements

Biochemical blood markers

The activity of the lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) in the blood serum of study participants was determined using the kinetic method on equipment from High Technology Inc. (USA) with the PRESTIGE 24i LQ LDH reagent kit (Poland). The concentration of steroid hormones, cortisol and testosterone, in the blood serum of the study participants was measured by enzyme-linked immunosorbent assay (ELISA) using the SteroidIFA-testosterone reagent kit on equipment from Alkor Bio. The reference values for the studied biochemical markers in blood serum are as follows: CPK (40-270 U/L), LDH (195-462 U/L), cortisol (150-660 nmol/L), and testosterone (8.64-29.01 nmol/L). Blood sampling was performed by a medical professional following the internationally accepted standards for medical and biological research (Tietz, Finley & Pruden, 1995) in designated areas of sports clubs and gyms. The results obtained after test loads were sent to medical laboratories in special refrigerated boxes to preserve the blood serum. The study determined the baseline level (before load) of the controlled biochemical blood markers and monitored their changes after completing the proposed test load. The total number of biochemical samples during the study was 720 units.

Research Design

The research was conducted in several stages during 2024.

At the first stage, the medical records of the examined contingent were analyzed. The available protocols of biochemical blood tests conducted in medical institutions during the previous examinations of the participants were analyzed. The results allowed us to assess the initial adaptation reserves level of the examined people and possible manifestations of disadaptation. After analyzing the research results of leading scientists (Carrard et al., 2022; Weakley et al., 2022), key issues in the diagnostic system for overtraining against the background of pronounced maladaptive processes were identified. One of the approaches to addressing this issue is the identification of the main structural characteristics of the diagnostic system for states of overreaching and overtraining in bodybuilding (Fig. 1).

It is crucial to identify reliable biomarkers for evaluating disadaptation and recovery processes during functional overreaching (FOR), non-functional overreaching (NFOR), and overtraining syndrome (OTS).

To implement this approach in practice, a research algorithm was developed to identify potential states of overreaching and overtraining in bodybuilding. This algorithm is based on analyzing the dynamics of the body's adaptive-compensatory responses to stress stimuli (Fig. 2).

At the second stage, two test loads were developed to achieve the set goal. Each test load had a classic bodybuilding training model based on the variable combination of appropriate methods, principles, and tools.

During test task 1, a high-intensity strength training mode (R_a = 0.74) was used in combination with the creatine-phosphate mechanism for ATP resynthesis (Chernozub et al., 2020). The training lasted 24-25 minutes. In test task 2, a moderate-intensity strength training mode (R_a=0.65) was used, with anaerobic glycolysis as the primary mechanism for ATP resynthesis (Chernozub et al., 2023; Potop et al., 2023). The training was 28-30 minutes.

Using biochemical research methods, the baseline levels of LDH and CPK activity, and cortisol and testosterone concentrations in the blood serum of study participants from all three groups, were determined at the beginning of the study. The changes in the studied blood biomarkers in response to test tasks 1 and 2 were analyzed to assess the body's resistance to strength loads of varying intensity.

Each group of study participants was divided into subgroups, comparing the baseline biochemical blood indicators with those recorded in response to acute load during the test tasks. The rationale for this distribution was based on the identified nature of adaptive-compensatory responses to acute stress stimuli and their comparison with the findings of other researchers (Kajaia et al., 2021; Soler-López et al., 2024.

Thus, subgroup 1 (1s) included participants whose biochemical blood marker changes in response to the test tasks indicated symptoms of a functional overreaching (FOR) state. Those whose biomarker parameters after acute load pointed to a state of non-functional overreaching (NFOR) formed subgroup 2 (2s). Subgroup 3 (3s) consisted of men whose biochemical responses to the test tasks reflected signs of disadaptation, indicating the presence of overtraining syndrome (OTS).

At the third stage, the features of possible manifestations of short-term adaptation processes or acute compensatory body reactions of the study participants were studied during 14 days of high-intensity resistance training (HIRT). HIRT in structure resembles the classic bodybuilding training model with a high-intensity and low-volume strength training regime (R_a=0.72) (Chernozub et al., 2020). At the same time, the energy supply of muscle activity occurs mainly due to the creatine phosphokinase mechanism of ATP resynthesis and partially anaerobic glycolysis in case of

depletion of creatine phosphate energy reserves (Cadegiani et al., 2021).

After 14 days of using HIRT in combination with a R_a=0.72 load regime, we studied the baseline level of LDH and CPK activity, the concentration of cortisol and testosterone in the blood serum of participants of all three groups. A comparative analysis of changes in the parameters of the baseline level of controlled biomarkers was conducted during all stages of the study. The features of changes in the studied blood biomarkers in response to test tasks 1 and 2 were also determined. Participants in each group were divided into subgroups using the same method as at the start of the study, by analyzing the patterns of their adaptive-compensatory responses to an acute stress stimulus. A comparative analysis of the dynamics of blood biomarkers of the participants was carried out during all stages of the study. The results were processed.

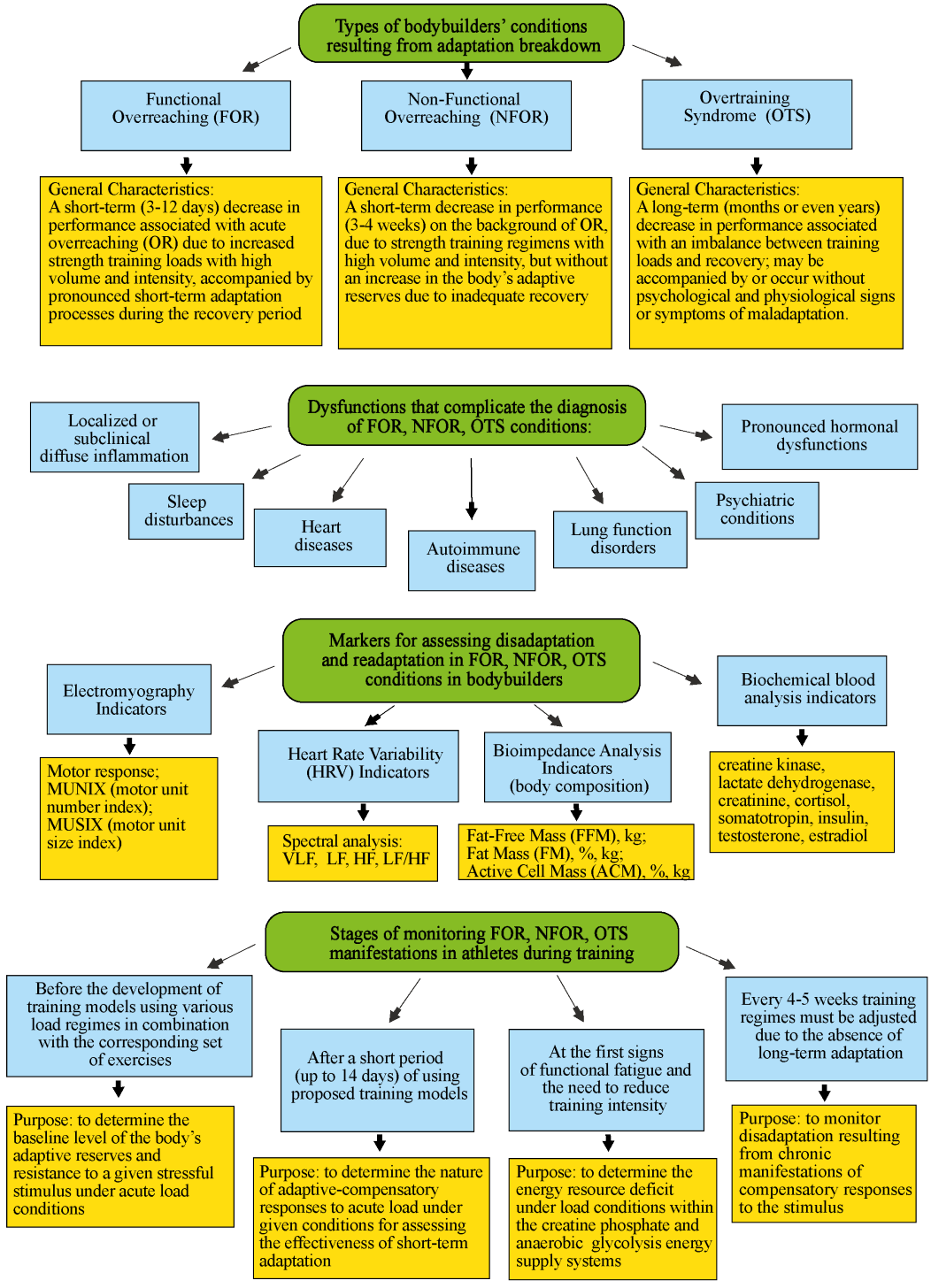

Figure 1. Key structural components of the diagnostic system for identifying overreaching states (FOR, NFOR) and overtraining syndrome (OTS) in bodybuilders during disadaptation.

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Statistical Analysis

Statistical analysis of the research data was conducted using the IBM *SPSS* Statistics 26 software package (StatSoft Inc., USA). The G-Power 3.1.96 program (Germany) was used to calculate the minimum required sample size by assessing statistical power. Nonparametric statistical methods were applied to determine the median (Me) and interquartile range (IQR). The Kruskal-Wallis H test was employed to compare baseline parameters across the three subject groups, while the Wilcoxon signed-rank test was used to analyze differences between two related samples.

Results

Fig. 1 presents the scheme we developed, which reveals the main structural characteristics of the system for diagnosing overreaching and overtraining in bodybuilding athletes during dis-

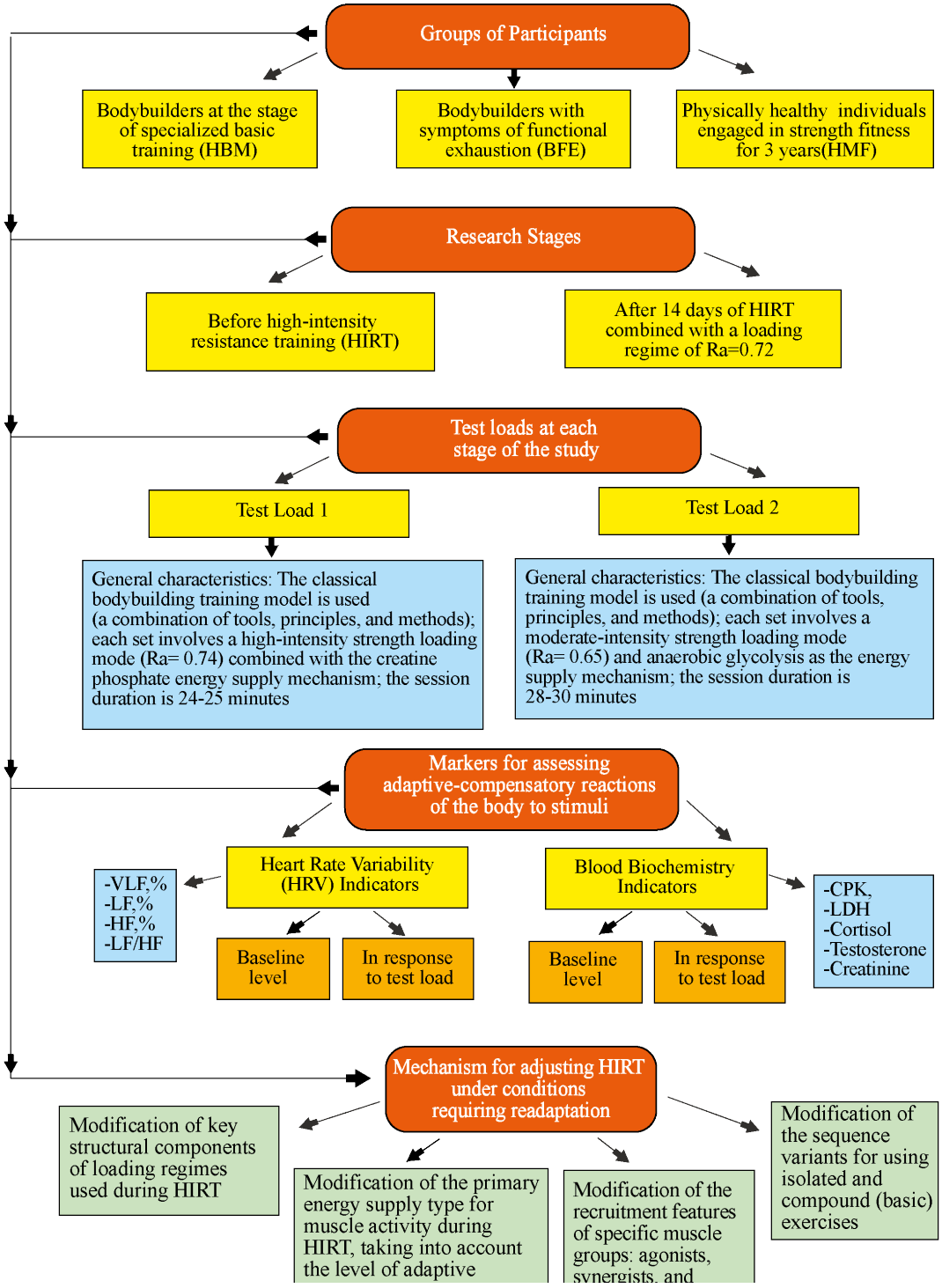

Figure 2. Organization of research on the determination of overreaching states (FOR, NFOR) and overtraining syndrome (OTS) in bodybuilding based on the assessment of changes in the adaptive and compensatory reactions of the body to stressful stimuli.

adaptation.

One of the most controversial and at the same time unresolved issues in the system of training athletes is the lack of clear boundaries of the transition from the state of overexertion to overtraining, due to the failure of adaptation during training. (Cadegiani et al., 2019). It is known that the consequences of the failure of adaptation are the manifestations of functional (FOR) and non-functional overreaching (NFOR), and overtraining syndrome (OTS) (Carfagno et al., 2014; Symons et al., 2023). However, despite the detailed characteristics of the manifestation of the FOR state and its possible consequences (Kajaia et al., 2021), the views of leading scientists regarding the clear criteria for the difference between the NFOR and OTS states have a large discrepancy. The problem is that in a state of non-functional overreaching, even under conditions of stabilization of the body's systems during the recovery period of 3-4 weeks, the activity of increasing reserves for short-term adaptation is not observed (Silva et al., 2022). At the same time, against the background of overtraining syndrome, we can continue to record disadaptation processes even under conditions of reducing the parameters of the volume and intensity of the load (Chernozub et al., 2024). A short-term decrease in working capacity, which is one of the consequences of the failure of adaptation, is not the key factor in the difference between the FOR and NFOR states. Only the possibility of a pronounced increase in adaptive reserves (supercompensation) during the recovery period is a reflection of the state of functional overstrain (positive overtraining) (Weakley et al., 2022).

The described manifestations of disadaptation can be a consequence of the imbalance between training loads and recovery, or the influence of other stressful stimuli on the body. Pronounced hormonal dysfunctions, autoimmune diseases, psychiatric conditions, heart and lung diseases are among the wide range of key dysfunctions that complicate the diagnosis of FOR, NFOR, and OTS (Cadegiani et al., 2019). Accordingly, these circumstances complicate the solution of the problem associated with the definition of clear markers for assessing disadaptation and readaptation of athletes in such states of overstrain and overtraining. It is known that in sports activities, a wide range of physiological and biochemical markers are used to assess the adaptive and compensatory reactions of the body in response to physical loads of various nature (Chernozub et al., 2020). In conditions of pronounced disadaptation and during the period of readaptation of athletes, in most cases, indicators of biochemical blood analysis (creatine kinase, lactate dehydrogenase, testosterone, cortisol), heart rate variability (VLF, LF, HF, LF/HF), electromyography (M-response, Munix, Musix), and bioimpedancemetry (BJM, JM, AKM) are the most informative markers for assessing FOR, NFOR, and OTS states. However, a substantiated system, based on fundamental research of using physiological and biochemical markers that characterize a particular state of stress or overtraining in bodybuilding, does not currently exist. This problem is relevant both in conditions of disadaptation and during control of readaptation processes, which requires scientists to conduct in-depth research.

Fig. 2 presents the organization of research on the determination of overreaching states (FOR, NFOR) and overtraining syndrome (OTS) in bodybuilding based on the assessment of the nature of changes in the adaptive and compensatory reactions of the body to a stressful stimulus. One of the key features of the organization of this research was the use of clear patterns between the selection of the contingent, test loads, and a complex of biomarkers for monitoring adaptation or possible disadaptation. It was assumed that the results obtained may allow a more in-depth study of the problem of finding informative biomarkers for diagnosing disadaptation in bodybuilding and manifestations of FOR, NFOR, and OTS states.

Table 1 presents the changes in the studied biochemical blood parameters of the participants of the examined groups under test loads before high-intensity resistance training (HIRT).

The results of the baseline level of controlled blood biomarkers, recorded in representatives of all three groups and detected at the beginning of the study, correspond to the reference values. It was established that groups A and C detected no significant difference in the baseline level of the studied blood parameters. At the same time, in representatives of group B, the initial parameters of creatine phosphokinase, lactate dehydrogenase, and cortisol in blood serum exceeded the results recorded in the examined groups A and C by 31.6%.

After test load 1, the studied blood biomarkers demonstrate different changes in each group. Depending on the characteristics of the adaptive and compensatory reactions to the stimulus, based on the analysis of changes in the studied blood parameters, the groups of participants were divided into subgroups (1s, 2s, 3s). We observed an increase in CPK (+57.4%) and cortisol (+26.5%) in the absence of changes in LDH in the blood in representatives of subgroups A^{1s} and C^{1s} in response to test load. In B^{1s} athletes, LDH activity in the blood increased (+10.2%) against the background of a less noticeable increase in CPK (+21.5%) and cortisol (+11.2%) in blood serum. In participants of subgroups A^{2s} and C^{2s}, there was a minimal increase in CPK activity (+4.8%), but LDH (+30.9%) and cortisol (+67.9%) parameters

Table 1. Results of changes in the studied biochemical blood parameters of the participants of the examined groups under test load conditions before using high-intensity strength training (median, IQR), n=90

Groups of participants		Biochemical blood parameters			
		CPK (U/L)	LDH (U/L)	Cortisol (nmol/L)	Testosterone (nmol/L)
Baseline (before load)					
A (HBM)		56.83 (3.04)	233.14 (12.64)	193.21 (14.07)	13.67 (1.23)
B (BFE)		73.03 (4.12)	266.09 (17.22)	269.41 (19.32)	15.55 (1.21)
C (HMF)		51.26 (2.99)	241.52 (15.78)	187.94 (15.62)	16.43 (1.06)
After test load 1					
A	A^{1s}	89.41 (4.78)*	239.52 (13.28)	244.56 (16.24)*	18.07 (1.51)*
	A^{2s}	59.12 (4.11)	322.22 (20.24)*	339.81 (24.27)*	13.64 (1.11)
B	B^{1s}	88.75 (4.92)*	293.22 (16.88)	299.66 (19.88)*	16.85 (1.42)*
	B^{2s}	72.95 (5.22)	366.58 (29.91)*	559.41 (48.62)*	15.01 (1.19)
	B^{3s}	199.41 (9.88)*	481.76 (37.44)*	144.25 (10.64)*	15.44 (1.22)
C	C^{1s}	80.86 (4.57)	239.59 (16.08)	237.98 (13.71)*	17.23 (1.26)*
	C^{2s}	54.13 (3.05)	298.82 (18.98)*	301.04 (25.14)*	16.89 (1.42)*
After test load 2					
A	A^{1s}	67.54 (4.33)*	286.11 (20.24)*	242.92 (14.72)*	16.11 (1.31)*
	A^{2s}	55.24 (3.21)	402.09 (30.71)*	176.29 (9.01)*	13.72 (1.08)
B	B^{2s}	74.61 (4.74)	433.42 (33.22)*	234.11 (13.25)*	15.53 (1.22)
	B^{3s}	178.04 (8.56)*	512.86 (36.52)*	143.05 (11.24)*	18.37 (1.46)*
C	C^{1s}	60.87 (3.45)*	341.31 (29.43)*	271.01 (19.35)*	17.59 (1.33)*
	C^{2s}	50.86 (3.05)	433.52 (32.07)*	163.55 (11.02)*	16.51 (1.22)

*Notes: 1s – 1 subgroup; 2s – 2 subgroup; 3s – 3 subgroup; * p< .05 – comparing with the results before the load at rest.*

in the blood after test load increased significantly. The greatest increase in cortisol (+107.6%) and LDH (+37.7 %) in the blood in the absence of CPK changes was found in B^{2s} athletes. The changes in B3s were completely different from the results of other subgroups. Thus, in representatives of B^{3s}, CPK indicators increased by 2.7 times and LDH parameters by 81.0%, and the cortisol concentration in blood serum decreased by 46.4%.

The analysis of results following test load 2 revealed varying shifts in the biochemical blood markers across the examined subgroups. A^{1s} and C^{1s} athletes had an identical increase in CPK (+18.8%) in the blood in response to the test load. However, the increase in cortisol (+44.2%) and LDH (+41.3%) in the blood, detected in subgroup C^{1s}, almost doubled the results of A^{1s} participants. Taking into account the nature of adaptive and compensatory reactions to the stimulus in subgroup B^{1s} participants, they were divided into subgroups B^{2s} and B^{3s}. Participants in the A^{2s}, B^{2s}, and C^{2s} subgroups exhibited a comparable rise in LDH levels (+71.5%) and a reduction in cortisol (–11.5%), while CPK activity remained largely unchanged. B^{3s} athletes showed almost identical changes in the values of blood biomarkers observed in response to test load 1. At the same time, representatives of A^{1s} and B^{3s} increased testosterone concentration by an average of 17.9% in response to test load 2.

Table 2 presents changes in the studied biochemical blood parameters of the participants of the examined groups under test loads after 14 days of high-intensity resistance training (HIRT).

The results of the baseline level of the studied blood biomarkers in the participants of all three groups, detected after 14 days of HIRT use, remained within the reference values. The comparative analysis performed at the initial and final phases of the study revealed a notable rise in CPK levels (group A by 5.8%, group B by 8.4%), LDH levels (group A by 3.6%, group B by 17.5%), and cortisol levels (group A by 14.4%, group B by 17.3%). No significant changes were detected in the representatives of group C. However, the alterations in baseline blood biomarker levels do not allow for a clear determination of FOR, NFOR, or OTS status.

The results presented in Table 2 indicate that in response to test load 1, representatives of subgroups A^{1s} and C^{1s} had almost identical changes in blood biomarkers with those at the beginning

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Table 2. Changes in the studied biochemical blood parameters of the participants of the examined groups under test load after 14 days of high-intensity strength training (median, IQR), n=90

Groups of participants		Biochemical blood parameters			
		CPK (U/L)	LDH (U/L)	Cortisol (nmol/L)	Testosterone (nmol/L)
Baseline level (before load)					
A (HBM)		60.13 (3.56)	241.56 (12.79)	221.17 (17.27)	12.33 (0.87)
B (BFE)		79.23 (5.01)	312.76 (21.25)	316.18 (22.39)	15.43 (1.01)
C (HMF)		52.27 (2.78)	250.28 (18.93)	196.27 (16.92)	16.27 (1.03)
After test load 1					
A	A^{1s}	90.32 (7.11)*	240.14 (14.64)	270.68 (17.79)*	17.69 (1.35)*
	A^{2s}	62.68 (3.41)*	356.17 (23.19)*	369.53 (23.11)*	12.14 (0.78)
B	B^{2s}	78.65 (4.98)	456.12 (34.02)*	587.34 (43.55)*	14.89 (1.02)
	B^{3s}	200.68 (13.93)*	549.22 (41.42)*	169.62 (11.34)*	15.03 (1.16)
C	C^{1s}	62.77 (3.33)*	247.71 (15.23)	235.17 (16.95)*	16.01 (1.13)
	C^{2s}	53.03 (2.95)	350.68 (20.77)*	378.09 (29.52)*	15.77 (1.02)
After test load 2					
A	A^{1s}	75.24 (4.21)*	273.15 (16.67)*	278.64 (18.39)*	16.25 (1.05)*
	A^{2s}	62.19 (3.41)	408.77 (36.09)*	162.49 (12.01)*	12.47 (0.77)
B	B^{2s}	81.04 (5.18)	449.14 (33.32)*	159.78 (11.65)*	15.22 (1.01)
	B^{3s}	193.62 (14.03)*	520.17 (45.49)*	142.66 (12.33)*	14.96 (0.86)*
C	C^{1s}	61.91 (4.11)*	335.14 (22.22)*	285.85 (18.35)*	18.23 (1.33)*
	C^{2s}	51.89 (3.55)	410.64 (35.07)*	356.17 (24.12)*	15.65 (1.12)
	C^{3s}	122.14 (9.95)*	488.27 (33.27)*	160.93 (11.92)*	16.01 (1.14)

*Notes: 1s – 1 subgroup; 2s – 2 subgroup; 3s – 3 subgroup; * p< .05 – comparing with the results before the load at rest.*

of the study (Table 1). The only exception is the parameters of CPK activity in blood serum, which, after 14 days of HIRT, demonstrated a twofold smaller increase in response to a physical stimulus. At this stage of the study, no athletes in group B could be attributed to subgroup B^{1s} based on the results of changes in blood biochemical indicators in response to proposed loads. Among participants of subgroups A^{2s}, B^{2s}, and C^{2s}, several discrepancies were found regarding the features of changes in the studied blood biochemical indicators in response to test load 1. Thus, the smallest increase in blood cortisol (+67.1%) in response to a stimulus was observed in A^{2s} athletes, and the largest (+92.6%) in C^{2s} representatives. However, an almost identical increase in LDH activity in the blood by an average of 44.4% was recorded among all representatives of the other subgroups. CPK activity in the blood of A^{2s} athletes increased by 4.2%. B^{2s} and C^{2s} representatives showed no changes. In response to a stressful stimulus, changes in CPK parameters (+153.2%), LDH (+75.6%), and cortisol (–10.8%) in B^{3s} athletes significantly differ from the results of other subgroups.

The results following test load 2 showed that, even within the same subgroup, the monitored indicators varied in their responses. Thus, the increase in cortisol (+25.9%) and LDH (+13.1%) in the blood in response to the load, in A^{1s} athletes, was almost two times less than the results of C^{1s}. The testosterone parameters in the blood of A^{1s} representatives increased (+31.8%), but the results obtained are 2.6 times higher than the changes recorded in C^{1s} participants. In response to a stressful stimulus, A^{2s}, B^{2s}, and C^{2s} representatives significantly increased LDH in the blood (+58.8%). The indicator of cortisol concentration decreased in the blood of A^{2s} athletes (–26.5%), B2p (–49.4%), and increased in the blood of C^{2s} representatives (+87.2%). Athletes of A^{2s} significantly increased CPK activity in the blood (+3.4%) after the test load, compared with the results of B^{2s} and C^{2s}. The changes in the studied blood biomarkers in representatives of the third subgroup (B^{3s} and C^{3s}) demonstrated a similar trend. Thus, some biochemical indicators increased among representatives of C^{3s} (LDH + 95.0%; CPK + 133.6%) and B^{3s} athletes (LDH + 66.3%; CPK + 144.3%). However, the reduction in blood cortisol levels in B^{3s} athletes after the test load was 54.9%, making it 3.5 times greater than the decrease observed in C^{3s} participants.

Discussion

This paper presents a study of overtraining syndrome in bodybuilding and the search for informative biomarkers for diagnosing disadaptation. The features of implementing short-term adaptation and manifestations of compensatory reactions of the body of athletes with different states of overexertion and possible overtraining under conditions of loads of various volumes and intensities were studied (Chernozub et al., 2023). The primary challenge in the study was identifying the optimal set of blood biomarkers that could reliably indicate a state of overreaching in bodybuilders or help diagnose OTS (Annunziata et al., 2024). The need to use the baseline level of biochemical blood indicators as informative criteria for assessing NFOR and OTS remains a rather controversial issue (Grandou et al., 2021). However, the study investigated the baseline level of CPK, LDH, testosterone, and cortisol in blood serum and changes in their parameters in response to an acute stressful stimulus and during HIRT. The practicality of implementing test tasks with varying power load regimes (R_a=0.67–0.74), grounded in the energy supply mechanism, was evaluated to assess body system function under conditions of disadaptation. The main structural characteristics of the diagnostic system for overreaching (FOR, NFOR) and overtraining syndrome (OTS) in bodybuilders during the maladaptation process were modeled.

The results indicate that the baseline level of controlled biochemical blood parameters of the HBM (group A), BFE (group B), and HMF (group C) participants remained within the reference values. Although there was a tendency for elevated baseline levels of CPK, LDH, and cortisol in the blood of bodybuilders from the HBM and BFE groups after two weeks of HIRT, the values remained within reference ranges. The absence of changes in the baseline level of blood biomarkers from the reference, even under the conditions of pronounced symptoms of FOR, NFOR, OTS in athletes, is justified by some scientists in their works by a possible high level of resistance and adaptive reserves (Kajaia et al., 2021). At the same time, many researchers (Silva et al., 2022; Weakley et al., 2022), comparing the baseline level of biomarkers in healthy athletes and with overtraining syndrome, state that such indicators are not informative biochemical markers for assessing the states of FOR, NFOR, and OTS. In the case of a significant change in the baseline level of enzymes and especially steroid hormones in the blood serum of athletes, it makes no sense to diagnose the process of disadaptation and OTS, due to possible hormonal dysfunction (Soler-López et al., 2024).

The study demonstrated that the identification of FOR in the HBM and HMF groups was achievable through the analysis of changes in these biomarkers following acute power loads with an anaerobic energy supply. Following high-intensity loads (R_a=0.74) using the creatine phosphokinase mechanism for ATP resynthesis, CPK levels rose by 50-53%, cortisol increased by 23-26%, and there was a potential rise in testosterone. The validity of such changes in these biomarkers to a stressful stimulus is associated with the involvement of creatine phosphate reserves in the energy supply and a high resistance level (Chernozub et al., 2023). In response to moderate-intensity loads (R_a= 0.65) with ATP resynthesis through anaerobic glycolysis, we observed an increase in LDH (22-35%) and cortisol (25-44%), along with a slight rise in CPK activity (16-18%) in the blood serum. This fact indicates that power loads under anaerobic glycolysis conditions affect the accumulation of lactate, which causes an increase in LDH activity in the blood, and also stimulates a significant increase in cortisol (Cadegiani et al., 2019). However, the similar adaptive and compensatory reactions of HBM and HMF group athletes to power loads (Ra=0.65-0.74) indicated that during the recovery period, the adaptive reserves will increase (Chernozub et al., 2020).

Based on the analysis of changes in LDH and cortisol levels in the blood in response to strength loads (R_a=0.65-0.74), NFOR can be detected for BFE, HBM, and HMF groups. In athletes with NFOR, even in response to loads under the creatine phosphokinase mechanism of energy supply, there was a significant increase in LDH (30-47%) and cortisol (75-107%). These changes indicate insufficient creatine phosphate reserves or low resistance to high-intensity loads due to disadaptation (Carfagno et al., 2019; Silva et al., 2022). However, in response to loads in anaerobic glycolysis energy supply, which are more prolonged, a more pronounced increase in LDH (62-79%) and a slight decrease in cortisol (13-49%) occurred. The characteristic manifestations of compensatory reactions in response to loads (R_a=0.65), where muscle glycogen is the primary source of ATP resynthesis, suggest its depletion due to the inefficiency of recovery processes and the need to engage auxiliary resources (gluconeogenesis) (Soler-López et al., 2024).

In response to test loads, regardless of their intensity or type of anaerobic energy supply, a simultaneous increase in CPK (143-173%), LDH (66-92%), and a decrease in cortisol (46-54%) was observed in the BFE group with suspected OTS. A characteristic feature is that similar changes in controlled biomarkers were recorded during all stages of the study, indicating the manifestation of pronounced disadaptation processes. An excessively high increase in CPK in the blood af-

ter strength training was primarily due to muscle damage against the background of fatigue due to insufficient recovery after the systematic use of eccentric or non-standard exercises in the HIRT (Watanabe et al., 2025). However, the activity of creatine phosphokinase in blood serum reflects the ATP resynthesis during muscle tension and characterizes the features of load regimes (volume and intensity parameters) (Chernozub et al., 2023). Several researchers, in their studies on OTS, proposed that the activity levels of CPK and LDH in athletes' blood could serve as sensitive indicators for assessing muscle damage, particularly in the context of chronic overtraining (Younger et al., 2025).

Conclusions

The proposed approach for identifying FOR, NFOR, and OTS in bodybuilding, through evaluating changes in the body's adaptive and compensatory responses to stress, serves as an effective tool for monitoring disadaptation. The results indicate that for OTS diagnostics in HBM, BFE, and HMF athletes, the parameters of the baseline level of CPK, LDH, cortisol, and testosterone in the blood are not informative biomarkers. Using test loads (R_a=0.64 and R_a=0.74) as stressful stimuli for the bodies of HBM, BFI, and HMF athletes allows for identifying manifestations of short-term adaptation, or compensatory reaction, based on the results of the acute response of blood biomarkers. Analyzing changes in LDH and cortisol levels in response to strength loads (Ra = 0.65–0.74) enabled the identification of NFOR in the BFE, HBM, and HMF groups. A marked increase in CPK (143–173%) and LDH (66–92%), along with a decrease in cortisol levels (46–54%) in response to strength training, may serve as an indicator of OTS in BFE athletes experiencing disadaptation.

References

Annunziata, G., Paoli, A., Frias-Toral, E., Marra, S., Campa, F., Verde, L., Colao, A., Lukaski, H., Simancas-Racines, D., Muscogiuri, G., & Barrea, L. (2024). Use of phase angle as an indicator of overtraining in sport and physical training. *J Transl Med*, 22(1), 1084. https://doi.org/10.1186/s12967-024-05918-w

Cadegiani, F., & Kater, C. (2019). Basal Hormones and Biochemical Markers as Predictors of Overtraining Syndrome in Male Athletes: The EROS-BASAL Study. *J Athl Train*, 54(8), 906-914. https://doi.org/10.4085/1062-6050-148-18

Cadegiani, F., Silva, P., Abrao, T., & Kater, C. (2021). Novel Markers of Recovery From Overtraining Syndrome: The EROS-LONGITUDINAL Study. *Int J Sports Physiol Perform*, 16(8), 1175-1184. https://doi.org/10.1123/ijspp.2020-0248

Carfagno, D., & Hendrix, J. (2014). Overtraining syndrome in the athlete: current clinical practice. *Curr Sports Med Rep*, 13(1), 45-51. https://doi.org/10.1249/JSR.0000000000000027

Carrard, J., Rigort, A., Appenzeller-Herzog, C., Colledge, F., Königstein, K., Hinrichs, T., & Schmidt-Trucksäss, A. (2022). Diagnosing Overtraining Syndrome: A Scoping Review. *Sports Health*, 14(5), 665-673. https://doi.org/10.1177/19417381211044739

Cheng, A., Jude, B., & Lanner, J. (2020). Intramuscular mechanisms of overtraining. *Redox Biol*, 35, 101480. https://doi.org/10.1016/j.redox.2020.101480

Chernozub, A., Potop, V, Korobeynikov, G, Timnea, O.C., Dubachinskiy, O., Ikkert, O., Briskin, Y., Boretsky, Y., & Korobeynikova, L. (2020). Creatinine is a biochemical marker for assessing how untrained people adapt to fitness training loads. *PeerJ,* (5), e9137. https://doi.org/10.7717/peerj.9137

Chernozub, A., Manolachi, V., Tsos, A., Potop, V., Korobeynikov, G., Manolachi, V., Sherstiuk, L., Zhao, J., & Mihaila, I. (2023). Adaptive changes in bodybuilders in conditions of different energy supply modes and intensity of training load regimes using machine and free weight exercises. *PeerJ,* 11, e14878. https://doi.org/10.7717/peerj.14878

Chernozub, A., Tsos, A., Olkhovyi, O., Ikkert, O., Koval, V., & Kirychuk, Y. (2024) Comparative study of adaptive reserves of servicemen and servicemen-athletes with neuromuscular injuries after the post-acute rehabilitation. *Physical rehabilitation and recreational health technologies*, 9(6), 468-475. https://doi.org/10.15391/prrht.2024-9(6).02

Grandou, C., Wallace, L., Impellizzeri, F., Allen, N., & Coutts, A. (2020). Overtraining in Resistance Exercise: An Exploratory Systematic Review and Methodological Appraisal of the Literature. *Sports Med*, 50(4), 815-828. https://doi.org/10.1007/s40279-019-01242-2

Grandou, C., Wallace, L., Coutts, A., Bell, L., & Impellizzeri, F. (2021). Symptoms of Overtraining in Resistance Exercise: International Cross-Sectional Survey. *Int J Sports Physiol Perform*, 16(1), 80-89. https://doi.org/10.1123/ijspp.2019-0825

Kajaia, T., Maskhulia, L., Chelidze, K., Akhalkatsi, V., & Kakhabrishvili, Z. (2021). Assessment of effects of non-functional overreaching and overtraining on responses of skeletal muscle and cardiac biomarkers for monitoring of overtraining syndrome in athletes. *Georgian Med News*, (311), 79-84.

Klymovych, V., Oderov, A., Romanchuk, S., Korchagin, M., Chernozub, A., Olkhovyi, O., & Zolochevskyi, V. (2020). The Influence of Professionally Oriented Physical Training Means on the Operator's Physical Ability Level. *Sport Mont,* 18(1), 93-97. https://doi.org/10.26773/smj.190616

Mallardo, M., Daniele, A., Musumeci, G., & Nigro, E. (2024). A Narrative Review on Adipose Tissue and Overtraining: Shedding Light on the Interplay

among Adipokines, Exercise and Overtraining. *Int J Mol Sci*, 25(7), 4089. https://doi.org/10.3390/ijms25074089

Meeusen, R., Duclos, M., Foster, C., Fry, A., Gleeson, M., Nieman, D., Raglin, J., Rietjens, G., Steinacker, J., & Urhausen, A. (2013). Prevention, diagnosis, and treatment of the overtraining syndrome: joint consensus statement of the European College of Sport Science and the American College of Sports Medicine. *Med Sci Sports Exerc*, 45(1), 186-205. https://doi.org/10.1249/MSS.0b013e318279a10a

Olkhovyi, O., Klymovych, V., & Romanchuk, S. (2016). Adoption of youth`s bodies to educational conditions in higher educational institutions. J*ournal of Physical Education and Sport*, 16 Supplement issue (1), Art 98, 620-622. https://doi.org/10.7752/jpes.2016.s1098

Olkhovyi, O., Chernozub, A., Danylchenko, S., Korchagin, M., Romanchuk, S., Oderov, A., Klymovych, V., & Zhembrovskyi, S. (2020). Experience of determining the priority of complex process or system (on the example of physical education and sport). *Journal of Physical Education and Sport*, 20 (Supplement issue 6), Art 451. 3330-3335. https://doi.org/10.7752/jpes.2020.s6451

Potop, V., Manolachi, V., Chernozub, A., Kozin, V., Syvokhop, E., Spivak, A., Sharodi, V., & Jie, Z. (2023). Changes in circumference sizes of bodybuilders using machine and free weight exercises in combination with different load regimes. *Health, Sport, Rehabilitation*, 9(2), 74-85. https://doi.org/10.34142/HSR.2023.09.02.06

Potop, V., Mihailescu, L., Mahaila, I., Zawadka-Kunikowska, M., Jagiello, W., Chernozub, A., Baican, S., Timnea, C., Ene-Voiculescu, C., & Ascinte, A. (2024). Applied biomechanics within the Kinesiology discipline in higher education. *Physical Education of Students*, 28(2), 106-19. https://doi.org/10.15561/20755279.2024.0208

Silva, A., Aghidemand, M., Kharatzadeh, M., Ahmadi, V., Oliveira, R., Clemente, F., Badicu, G., & Murawska-Ciałowicz, E. (2022). Effects of High-Intensity Resistance Training on Physical Fitness, Hormonal and Antioxidant Factors: A Randomized Controlled Study Conducted on Young Adult Male Soccer Players. *Biology (Basel)*, 11(6), 909. https://doi.org/10.3390/biology11060909

Symons, I., Bruce, L., & Main, L. (2023). Impact of Overtraining on Cognitive Function in Endurance Athletes: A Systematic Review. *Sports Med Open*, 9(1), 69. https://doi.org/10.1186/s40798-023-00614-3

Soler-López, A., Moreno-Villanueva, A., Gómez-Carmona, C., & Pino-Ortega, J. (2024). The Role of Biomarkers in Monitoring Chronic Fatigue Among Male Professional Team Athletes: A Systematic Review. *Sensors (Basel)*. 24(21), 6862. https://doi.org/10.3390/s24216862

Tian, Y., He, Z., Zhao, J., Tao, D., Xu, K., Midgley, A., & McNaughton, L. (2015). An 8-year longitudinal study of overreaching in 114 elite female Chinese wrestlers. *J Athl Train*, 50(2), 217-23. https://doi.org/10.4085/1062-6050-49.3.57

Torre, M., Monda, A., Messina, A., Stefano, M., Monda, V., Moscatelli, F., Tafuri, F., Saraiello, E., Latino, F., Monda, M., Messina, G., Polito, R., & Tafuri, D. (2023). The Potential Role of Nutrition in Overtraining Syndrome: A Narrative Review. *Nutrients*, 15(23), 4916. https://doi.org/10.3390/nu15234916

Watanabe, D., & Wada, M. (2025). Cellular mechanisms underlying overreaching in skeletal muscle following excessive high-intensity interval training. Am *J Physiol Cell Physiol*, 328(3), C921-C938. https://doi.org/10.1152/ajpcell.00623.2024

Weakley, J., Halson, S., & Mujika, I. (2022). Overtraining Syndrome Symptoms and Diagnosis in Athletes: Where Is the Research? A Systematic Review. *Int J Sports Physiol Perform*, 17(5), 675-681. https://doi.org/10.1123/ijspp.2021-0448

Younger, D. (2025). Prescribed Fitness for Overtraining Syndrome in an Amateur Bodybuilder With Obsessive Compulsive Disorder and Athletic Heart. *Can J Cardiol*, 41(3), 558. https://doi.org/10.1016/j.cjca.2025.01.004

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