OVERTRAINING SYNDROME: PECULIARITIES AND PERSPECTIVES OF DIAGNOSTICS

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Abstract. Overtraining syndrome (OTS) is a condition associated with prolonged dysfunctional adaptation to physical exercise and a long-term imbalance between training and recovery that results in decreased sports performance lasting from several weeks to months with serious consequences for the health of athletes. The problem of diagnosis and prevention of OTS remains relevant, as the diagnosis is often made retrospectively. Currently, no clear reliable biochemical or functional markers for early detection of OTS are described, and the features of pathogenesis of this syndrome remain unclear. The present overview describes the basic theories of OTS development, the main biomarkers and their diagnostic significance, as well as some novel parameters and methods that may be possibly perspective for early detection of OTS.

Keywords: overtraining syndrome, athletes, diagnosis, biomarkers, metabolism, hormones, athletic performance.

List of Abbreviations

ACTH – adrenocorticotropic hormone
ANS – autonomic nervous system
BAP – biological antioxidant potential
BCAA – branched-chain amino acids
d-ROMs – diacron-reactive oxygen metabolites
EEG – electroencephalography
EROS – Endocrine and Metabolic Responses on Overtraining Syndrome
GSH – reduced glutathione
GSSG – oxidized glutathione
HAMD – Hamilton Depression Scale
HF – high frequency
HRV– heart rate variability
IgA – immunoglobulin A
IGF-1– insulin-like growth factor-1
IL – interleukin
ITT – insulin tolerance test
LF – low frequency
MADRS – Montgomery-Asberg Depression Rating Scale
OTS – overtraining syndrome
POMS – Profile of Mood States
RESTQ – Recovery Stress Questionnaire
SFMS– French Society of Sport Medicine
TBARS – thiobarbituric acid reactive substances
TDS – Training Distress Scale
TNF-α – tumor necrosis factor alpha
TP – total power of spectrum
VLF – very low frequency

Introduction

Overtraining syndrome (OTS) is characterized by a decrease in performance after periods of intense training, which can have serious consequences for the health of athletes. As it is known, the athletic performance can be improved by increasing training loads. Successful training is often accompanied by overload, and some athletes can periodically experience short-term performance decrement without severe negative symptoms. At the same time, a persistent imbalance between training and recovery may lead to prolonged decreased performance, known as overtraining syndrome.

There is currently no consensus on the mechanisms of pathogenesis of overtraining syndrome or a single diagnostic marker of overtraining (Weakley *et al.,* 2022). A highly qualified athlete is constantly balancing on the edge of an optimal level of training and overtraining. According to literature data, up to 20% of highly skilled athletes can experience overtraining during their athletic careers (Cadegiani *et al.*, 2019; Kreher, 2016).

Certain terminology is usually used in the literature to describe the conditions associated with an imbalance between training and recovery. According to some authors, there is a need to distinguish between «overreaching» and «overtraining» (Bell *et al.*, 2020; Meeusen *et al.*, 2010). Several international literature sources refer to the work of R. Kreider (Kreider *et al.*, 1998), according to which:

– overreaching is the accumulation of training and/or non-training stress, leading to a short-term decrease in performance in the presence or absence of appropriate physiological and psychological signs and symptoms of poor adaptation; it can take from a few days to a few weeks to restore performance capacity;

– overtraining is the accumulation of training and/or non-training stress, leading to a long-term decrease in performance in the presence or absence of appropriate physiological and psychological signs and symptoms of poor adaptation, which may take several weeks or months to restore performance capacity.

Thus, the difference between overreaching and overtraining is basically in the time needed to recover performance, but not in the type or duration of exercise-related stress or in the degree of decrease in performance (Halson & Jeukendrup, 2004).

Some authors also distinguish such a concept as fatigue, which is a typical impairment of performance after more or less prolonged work (Wan *et al.*, 2017; Dikunets *et al.*, 2019). It can be expressed in the difficulty of controlling new movement skills and the presence of technical errors in the performance of the athlete's usual exercises, sleep disorders and unstable mood (Wan *et al.*, 2017).

In the Joint Consensus Statement of the European College of Sports Science and the American College of Sports Medicine «Prevention, Diagnosis and Treatment of Overtraining Syndrome» (Meeusen *et al.*, 2013), it was pointed out that several concepts should be distinguished: functional overreaching, non-functional overreaching and overtraining syndrome. The main difference between these conditions is the recovery time: in the case of functional overreaching, recovery takes several days, less often weeks; in the case of non-functional overreaching, recovery takes weeks, less often months; in the case of overtraining syndrome, athletes need from several months to several years to completely recover (Buyse *et al.*, 2019; Carrard *et al.*, 2022). By using the term «syndrome», the authors point to its multifactorial etiology and acknowledge that exercise is not necessarily the only etiological factor of the syndrome. The key to recognizing overtraining syndrome may be a long-term poor adaptation of not only the whole organism of an athlete, but also of individual biological, neurochemical and hormonal regulatory mechanisms. Overtraining is regarded as a pathological condition manifested in violation of the level of functional readiness achieved during training and in the change of regulation mechanisms functioning, which leads to disorder of the optimal relationship between the cerebral cortex and other parts of the nervous system, the muscular system and the internal organs.

Pathogenesis of the overtraining syndrome

Glycogen hypothesis. The reason for overtraining and declining performance can be partially explained by the negative effect of decreased muscle glycogen on performance capacity. Athletes do not always follow a balanced diet and do not maintain optimal calorie intake, which results in reduced muscle glycogen. Thus, the repetitive process of glycogen depletion can cause changes in metabolic pathways responsible for the energy supply of skeletal muscles (Dikunets *et al., 2019).* Additionally, low levels of muscle glycogen lead to increased oxidation and decreased concentrations of branched-chain amino acids, which may affect the synthesis of major neurotransmitters involved in fatigue (Kreher & Schwartz, 2012). This hypothesis has not been widely accepted in the literature (Armstrong *et al.*, 2022; Cheng *et al.*,2020). Many athletes who consume increased amounts of carbohydrates and maintain normal levels of glycogen experience the symptoms of overtraining syndrome.

Central nervous system fatigue hypothesis. Overtraining syndrome is always accompanied by the disorders of the nervous system: exhaustion, weakness and sleep disorders. The neurotransmitter serotonin is involved in regulating these functions, and its precursor tryptophan is competing with branched-chain amino acids (BCAA) to penetrate the blood-brain barrier (Barik, 2020; Kreher, 2016). High-intensity workouts reduce BCAA due to their increased oxidation, which facilitates the penetration of tryptophan into the brain. The increase of tryptophan in the central nervous system stimulates serotonin synthesis. High serotonin levels are thought to cause mood and sleep changes, reduced motor neuronal excitability, loss of appetite, and inhibition of hormones secreted by the hypothalamus (Anish, 2005; Kreher & Schwartz, 2012). However, mood swings and fatigue are subjective traits that are difficult to identify, and they can be affected by more than just the concentration of neurotransmitters.

Glutamine hypothesis. Decreased glutamine levels can be responsible for immune dysfunction during overtraining. Glutamine is the main source of energy for immune system cells, playing an important role in nucleic acid synthesis, nitrogen transport, gluconeogenesis and acid-alkali balance (Kreher & Schwartz, 2012). Intense long-term exercises cause two-phase responses to blood glutamine levels; its concentration increases during the exercise, but then it drops significantly during rest periods, especially during the first few hours before reaching the initial level (Dikunets *et al., 2019)*. It can be assumed that insufficient rest between intense workouts may limit glutamine emissions by skeletal muscles, and therefore immune cells experience nutritional deficiency. This can cause increased susceptibility to infection in overtrained athletes. Some authors claim that serum glutamine levels do not correspond to the bioavailability of glutamine; thus, glutamine depletion may not be responsible for the number of other symptoms described in athletes with OTS (Shah *et al.*, 2020).

Autonomic nervous system hypothesis. Imbalance in ANS functions, in particular, decreased sympathetic activation and the predominance of parasympathetic department activity, may lead to reduced productivity, fatigue, depression and bradycardia. According to some literature data, two forms (phases) of overtraining can be distinguished: sympathetic and parasympathetic. The sympathetic form appears to be the first phase of overtraining and is identical to the acute stress response. This phase progresses to exhaustion of the ANS when sympathetic activity decreases, presumably due to the decrease in the secretion of catecholamines, which usually modulate metabolic reactions and adaptation to physical and psychological work. Also, decreased organ sensitivity to catecholamines may be responsible for the symptoms of decreased sympathetic activation (Kreher & Schwartz, 2012; Batdieva *et al.*, 2018).

Decreased activity of the sympathetic nervous system in athletes with OTS is often observed. But not all studies confirm the data about decreased catecholamine secretion in athletes with OTS and the information about HRV changes in OTS-affected athletes is also controversial (Meeusen R. *et al.*, 2013; Carrard J. *et al.*, 2022). However, the balance between the sympathetic and parasympathetic parts of the ANS can usually be restored after a week of rest.

Hypothalamus hypothesis. Changes in the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes may be the cause of OTS. Overtrained athletes may have changes in levels of cortisol, ACTH, testosterone and other hormones. Also, it was revealed that the response of pituitary hormone secretion (ACTH, prolactin and growth hormone) to the action of stress factors and physical exercise in overtrained athletes can be lower than in healthy athletes. It can also be evidence of the central (hypothalamus or pituitary gland) disorders of hormonal response in athletes with OTS (Cadegiani & Kater, 2017; Carrard *et al*., 2022). Unfortunately, the available literature data on the nature of hormonal changes in the development of OTS are very contradictory. In many studies of athletes' training and OTS, no significant changes in the levels of testosterone and cortisol were observed (Ambroży *et al.*, 2021; Kreher & Schwartz, 2012). Alterations of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes are individualized and depend on other factors, including physical activity and the athlete's own susceptibility to stress factors.

The hypothesis of oxidative stress. In the process of oxidation and reduction, highly active oxygen forms are continuously formed. During the performance of the physical load, oxygen consumption increases by 40 times. As a result, the formation of highly active oxygen forms also increases significantly. Reducing the negative effects of these forms of oxygen on skeletal muscle cells is carried out with the help of the protective antioxidant system, which can be enhanced by training to develop endurance (Powers *et al.*, 2020). Some oxidative stress is desirable during exercise, since reactive oxygen species released from damaged muscles regulate cell repair. However, when oxidative stress becomes pathological, reactive oxygen species (for example, peroxide, hydrogen peroxide and hydroxyl radical) can cause inflammation, muscle fatigue and soreness, which leads to a decrease in athletic performance. Markers of oxidative stress at rest are higher and increase with physical exertion in overtrained athletes compared to the control group. Thus, athletes with overtraining syndrome may be more susceptible to oxidative damage (Kreher & Schwartz, 2012; Dikunets *et al*., 2019).

It is not known whether the state of increased oxidative stress is a trigger or the result of overtraining. Clinically relevant studies are limited. Moreover, the influence of factors such as the menstrual cycle and the antioxidant properties of estrogen is unknown.

Cytokine hypothesis. It is the complex hypothesis that most fully answers the questions about the causes of overtraining and the development of its main symptoms. According to this theory, during physical exercise muscles are damaged, and microtraumas occur not only in the muscles, but also in the tendons and joints. Muscle microtrauma causes a decrease in strength and range of motion due to swelling in the area of injury, as well as a local inflammatory reaction with activation of circulating monocytes and systemic inflammation (Carfagno & Hendrix, 2014; Kreher & Schwartz, 2012). These microdamages trigger local inflammatory processes that stimulate the formation of interleukins, mainly interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) (Smith, 2004). These substances affect a number of processes in the body, namely the utilization of glucose by the muscles (Suzuki, 2019). In cases of disturbance of muscle nutrition, BCAA (leucine, isoleucine, valine) are oxidized to replenish glucose stores. A decrease in these amino acids leads to the fact that the muscles do not recover well enough.

The violation of the regulation of the cytokine profile creates the potential for the development of the immunopathological process. In particular, it can lead to increased secretion of glucocorticosteroids, as well as suppression of immune cell function through modulation of intracellular signaling pathways (Ivanchikova & Sherash, 2021; De Sousa Nogueira Freitas, *et al.*, 2020). Studies have established the suppressive effect of some hormones on the production of pro-inflammatory cytokines IL-1β, IL-6, TNF-α under conditions of physical exertion. It should be noted that in these conditions, there is the following hierarchy of sensitivity of cytokine production to the suppressing effect of cortisol: TNF-α is sensitive to physiological doses; IL-1β occupies an intermediate position and begins to decrease under stress conditions; IL-6 remains resistant to the action of cortisol (Luti *et al.,* 2020). These results make it possible to explain the determined stable rise of IL-6 in most cases after physical exertion. It was found that an increase in this cytokine promotes the stimulation of the synthesis of acute-phase proteins, leading to the development of inflammatory processes, including those caused by damage to muscle tissue during exercise.

Pro-inflammatory cytokines also act on the hypothalamus, namely on the hunger center, causing the decreased appetite in athletes (Smith, 2004). As a result, muscle weakness develops from a lack of nutrients. A number of cytokines reduce glutamine reserves and cause activation of T-helpers of the second type, activating humoral immunity, while cellular immunity suffers, which can lead to a higher frequency of infections (Smith, 2000).

In addition, pro-inflammatory cytokines negatively affect the central nervous system; they are involved in the development of depression and mood disorders. As a result, all the symptoms accompanying overtraining syndrome are observed: mental and nervous processes and immune reactions are disrupted, and endocrine disorders can be noted through the hypothalamic-pituitary axis (in particular, prolactin levels increase, the amount of testosterone decreases, the ratio of testosterone to estradiol changes). As a result, the cytokine theory explains the main stimulus initiating many biochemical pathways which can cause the symptoms seen in overtraining athletes.

The markers for the diagnosis of overtraining syndrome

Currently, the diagnosis of OTS is often made retrospectively, since the main criterion for detecting OTS is a decrease in performance capacity, in which recovery takes several months.

The main requirements for biomarkers for the diagnosis of OTS are as follows: the changes in the biomarker should reflect the development of OTS and not depend on the characteristics of the diet or daily and seasonal biorhythms; and the determination of this biomarker should be fast, inexpensive and minimally invasive if possible. According to the literature, the following groups of biomarkers can be diagnostically significant for the detection of OTS.

Metabolic markers

The main metabolic markers of OTS currently include the concentration of glutamine and glutamate in the blood and the assessment of the glutamine/glutamate ratio, lactate levels during submaximal physical exertion, and creatine kinase activity. Most studies describe deviations in these parameters in OTS athletes compared to healthy athletes.

Thus, a decrease in glutamine levels, an increase in glutamate levels and a decrease in the glutamine/glutamate ratio were detected at OTS in athletes of cyclic sports (swimming, rowing, running, cycling, speed skating, cross-country skiing), as well as in athletes of some game sports (football, tennis) (Parry-Billings *et al.*, 1992; Rowbottom *et al.*, 1995; Smith & Norris, 2000; Halson *et al.*, 2003; Coutts *et al.*, 2007).

It should be taken into account that the plasma glutamine concentration depends on the dietary intake: it increases after consumption of a meal containing protein but falls after a low carbohydrate diet (Gleeson, 2002), and some studies have not found a decrease in glutamine during the period of intense physical activity (Halson *et al.*, 2003, Coutts *et al.*, 2007). In this regard, it is recommended to use the glutamine/glutamate ratio as an indicator of overtraining (Coutts *et al.*, 2007; Smith & Norris, 2000; Walsh *et al.*, 1998; Halson *et al.*, 2003).

Most studies of athletes with OTS reported elevated levels of creatine kinase activity in the blood. Cadegiani *et al.* conducted the Endocrine and Metabolic Responses on Overtraining (EROS) study and examined OTS-affected male athletes who performed both endurance and resistance activities. In the EROS-BASAL study (Cadegiani *et al.*, 2019), the authors noted high levels of plasma creatine kinase activity in the examined athletes with OTS in comparison with healthy athletes. Similar results were determined in cyclists with overreaching (Halson *et al.*, 2003) and in athletes of game sports (Coutts *et al.*, 2007).

However, some authors in the studies of OTS-affected athletes of strength sports noted that the level of creatine kinase was not a useful marker of non-functional overreaching or OTS. So, during the 8-year longitudinal study of female wrestlers, it was demonstrated that for this group of athletes, creatine kinase had low diagnostic sensitivity (Tian *et. al.*, 2015). Another study of the athletes of strength sports with OTS also did not reveal significant changes in creatine kinase (Afanasyeva & Tajmazov, 2011).

The problem of using creatine kinase as a marker of overtraining is mainly due to the fact that the levels of creatine kinase can vary greatly among individuals and are associated with the level of training, muscle size, and fiber type (Tian *et al.*, 2015). And since this enzyme reflects an acute impairment in exercise tolerance, controlling its activity is important primarily for the prevention of OTS development.

Lower blood lactate responses during submaximal exercise were revealed in OTS-affected athletes of endurance sports (cycling, running) (Gleeson, 2002; Jeukendrup *et al.*, 1992; Bosquet *et al.*, 2001). Also, sportsmen with OTS did not reach maximal lactate concentrations above 8 mmol/l after the performing of the two-bout exercise protocol (Meeusen *et al.*, 2010), and a maximal lactate value < 8 mmol/l after both bouts was proposed as the marker with high sensitivity for OTS. But it is necessary to mention that lactate level differences depend on the type of the exercise test, and that the changes in lactate concentrations were basically revealed in athletes of endurance sports (Meeusen *et al*, 2013).

Biochemical parameters such as blood levels of glucose, urea and uric acid do not fulfill the requirements for a reliable indicator of the overtraining, mainly due to temporary changes and the influence of the diet. But these parameters are helpful in evaluating the actual health status of the athlete for the exclusion of other pathological states that can cause a decrease in performance (Meeusen *et al.*, 2013; Gleeson, 2002). In addition, there is no information that a high concentration of myoglobin in the blood is a sensitive marker for OTS detection (Dikunets *et al.*, 2019).

The plasma concentration ratio of free tryptophan (FT) to BCAA was also proposed as a diagnostic tool for detecting OTS. Some studies of exercise supplementation (Blomstrand *et al.*, 1997) found a positive effect of BCAA feeding, in particular on performance. However, in studies, this parameter did not demonstrate its diagnostic significance in athletes with OTS. Thus, the study of athletes who trained in endurance and had signs of unexplained underperformance syndrome did not show a significant difference in plasma levels of free tryptophan, BCAA, or the free tryptophan /BCAA ratio (Budgett *et al.*, 2010).

Also, in the study of athletes of strength sports, the parameters of the total and effective concentration of serum albumin were evaluated using the standard fluorescent method. A significant increase in the total and effective concentration of serum albumin was determined in the group of athletes with overtraining syndrome compared to healthy athletes (Afanasyeva & Tajmazov, 2011). The parameter of the effective concentration of albumin is sensitive to metabolic disorders, since endogenous intoxication blocks part of the binding centers of the albumin molecule, and the results received in the study presumably can be explained by the adaptation to the increased formation of metabolic products in athletes with overtraining.

There is literature data about using the method of Fourier transform infrared spectroscopy to assess metabolic changes in athletes with OTS. In the longitudinal study of rowers (Petibois *et al.*, 2000) this method was applied to assess the difference in the spectrum of blood serum in order to determine metabolic responses to exercise. In athletes who were subsequently diagnosed with OTS, reduced saccharide absorption spectrum disorders were revealed during training, followed after several weeks by reduced lipid and peptide absorption spectrum. Also, with the help of this method in OTS-affected rowers the disorders of the plasma lipid profile were revealed: an increase in glycerol concentrations and a decrease in the levels of triglycerides, very low-density lipoprotein and apolipoprotein C3 (Petibois & Deleris, 2004).

The level of hormones

Studies evaluating hormone levels in OTS-affected athletes include both the determination of basal hormone levels and hormonal responses to exercise and the insulin tolerance test (ITT).

Basal hormone levels. The findings of Cadegiani *et al.* from the EROS study of athletes of endurance and resistance sports revealed the changes in serum basal hormone levels in OTS-affected athletes compared to healthy athletes: decreased testosterone, increased estradiol, decreased serum testosterone-estradiol ratio, and decreased salivary cortisol in the morning after awakening (Cadegiani *et al.*, 2019; Cadegiani & Kater, 2017). The authors proposed using the level of salivary cortisol 30 minutes after awakening > 530 ng/dL as a criterion highly predictable for the exclusion of OTS.

Also, according to the results of the EROS study, diagnostic scores were developed, that could help distinguish OTS-affected from healthy male athletes. Hormonal parameters included in these scores as possible risk factors of

OTS are basal growth hormone <0.1 μg/L, basal prolactin <7.1 ng/mL, total testosterone <400 ng/dL, testosterone-estradiol ratio <13.

It should be noted that some authors have revealed controversial results regarding basal levels of pituitary hormones and testosterone in OTS-affected athletes. Thus, athletes of cyclic sports showed an increase in the basal levels of ACTH, growth hormone, prolactin (Meeusen *et al.*, 2004; 2010), cortisol and testosterone (Meeusen *et al.*, 2010; Gustafsson *et al.*, 2008). Concerning the athletes of strength sports, in the study of female wrestlers it was demonstrated that cortisol and testosterone are poor markers of overreaching or overtraining in this group of sportsmen, since no mean differences in the levels of these hormones were detected between when wrestlers were and were not diagnosed with non-functional overreaching (Tian *et al.*, 2015).

The resting plasma testosterone/cortisol ratio is widely used in sports medicine as it is considered a useful marker of the anabolic/catabolic state of an athlete (Cadegiani & Kater, 2017; Czuba et al., 2022). This parameter reflects the physiological strain of training and decreases in relation to the intensity and duration of training (Meeusen *et al.*, 2013). The testosterone/cortisol ratio has been used as a marker of OTS when reduced by at least 30% (Cadegiani *et al.*, 2019). Currently, some authors suggest that the testosterone/cortisol ratio only indicates an actual physiological state and may not be selective enough to diagnose an overtraining state if it is not used in combination with other biological markers (Dikunetz *et al.*, 2019; Cadegiani *et al.*, 2019; Orzhenikidze *et al.*, 2018). Thus, according to the study of Cadegiani et al., the testosterone/cortisol ratio did not differ between the groups of OTS-affected and healthy athletes, and the testosterone/estradiol ratio is the better parameter for evaluation of the anabolic/catabolic state. During OTS, increased estradiol in the absence of increased testosterone or even with a decrease in testosterone was observed, and it suggests an abnormally increased level of aromatase enzyme and increased conversion to estradiol. This leads to a decreased testosterone/estradiol ratio, which may reflect an antianabolic state (Cadegiani *et al.*, 2019).

Literature data on the levels of night urinary catecholamines are contradictory (Duclos, 2008; Meeusen *et al.*, 2013). Some studies report an increase (Cadegiani *et al.*, 2019), a decrease (Lehmann *et al.*, 1992; Lehmann *et al.*, 1998), or no change in urinary catecholamine excretion (Urhausen *et al.*, 1998) at overreaching or overtraining. As a rule, a decrease in sympathetic activation in overtrained athletes is accompanied by a decrease in nocturnal excretion of catecholamines in urine (Halson & Jeukendrup, 2004).

Other hormones, such as leptin, adiponectin, and ghrelin, have been recently investigated as possibilities for the monitoring of training (Jürimäe *et al.*, 2011; Meeusen *et al.*, 2013). But there are few studies of them as markers of OTS. Thus, the decrease in plasma leptin levels was revealed in OST-affected athletes compared to healthy athletes (Joro *et al.*, 2017).

Hence, the large number of studies of basal levels of hormones in athletes with OTS is controversial, demonstrating that these parameters could not be reliable markers for OTS detection. Conversely, stimulated hormone levels, particularly acute responses to stressful conditions, were proposed as good predictors of underperformance syndromes (Cadegiani & Kater., 2017; Carrard *et al.*, 2022).

Hormonal responses to stimulation. The insulin tolerance test (ITT), various single exercise tests and the test with two consecutive maximal exercises were used as stress stimuli to study hormonal response in athletes with overtraining.

In OTS-affected athletes, a decreased rise in pituitary hormones (ACTH, growth hormone and prolactin) in response to a stressful stimulus is reported (Urhausen *et al.*, 1998; Meeusen *et al.*, 2013; Cadegiani & Kater, 2017; Cadegiani & Kater, 2018; Cadegiani *et al.*, 2019).

In the EROS study carried out by Cadegiani *et al.*, the hormonal response to the insulin tolerance test was evaluated. It was found that OTS-affected athletes in response to ITT showed blunted responses of ACTH, cortisol, growth hormone and prolactin secretion (hormone secretion in response to ITT was less than in healthy athletes). The following hormone changes in response to ITT have been proposed as diagnostically significant for OTS detection: ACTH 30 minutes after hypoglycemia < 35 pg/mL, growth hormone 30 minutes after hypoglycemia < 1.0 μg/L, prolactin during ITT < 12 ng/mL, prolactin 30 minutes after hypoglycemia < 10 ng/mL, cortisol response to ITT < 19.1 μg/dL (Cadegiani *et al*., 2020).

The study of hormonal response to a short-term exhaustive endurance test on a cycle ergometer in endurance athletes with OTS (cyclists and triathletes) also showed a significantly lower maximal exercise-induced increase of ACTH and growth hormone (Urhausen *et al*., 1998).

The test protocol with two consecutive maximal exercise tests separated by 4 hours of rest (the two-bout protocol exercise test) was proposed by Meeusen *et al.* for the estimation of stress-induced hormonal reactions. The use of the two-bout protocol exercise test as a stimulus to study hormonal response allows evaluating the recovery capacity of athletes (Meeusen *et al.*, 2004). In normal healthy subjects, the test reveals an increase in the circulating concentrations of the hormones after both the first and the second exercises. Athletes with OTS have an extremely large increase in circulating pituitary hormones' concentration after the first exercise bout, followed by a complete suppression of the levels of these hormones in the second exercise bout. This could indicate a hypersensitivity of the pituitary followed by its insensitivity or exhaustion (Meeusen *et al.*, 2013; Anisimov *et al.*, 2018; Orzhenikidze *et al.*, 2018).

Hormone secretion in response to the two-bout protocol exercise test at OTS has been investigated in athletes of various sports. Studies of athletes of cyclic sports (running) and motocross revealed that plasma ACTH, growth hormone and prolactin responses to the second bout were reduced in OTS-affected athletes (Meeusen *et al*. 2004; Meeusen *et al*. 2010). Also, the study of football players using a modified version of the two-bout exercises test was performed, and the results of the second hormone level measurement confirmed the high significance of the change in ACTH, growth hormone and prolactin levels with an unchanged or reduced cortisol level in the diagnosis of OTS and its differentiation with non-functional overreaching (Anisimov *et al.*, 2018). According to the study of athletes of various sports using discriminant analysis, the conclusion was made that reduced ACTH and prolactin responses to the second exercise test were most sensitive to non-functional overreaching and OTS (Buyse *et al*., 2019).

Therefore, for the diagnosis of OTS the use of hormone secretion responses to stress factors is more accurate. In OST-affected athletes, ACTH, prolactin and growth hormone were reduced in response to both physical exercise and the insulin tolerance test, and only cortisol showed different responses. Presumably, these disorders of the hormonal response are central (hypothalamus or pituitary gland), while the adrenal glands respond normally to ACTH stimulation in athletes with OTS (Cadegiani & Kater, 2017; Carrard *et al.,* 2022). Currently, the assessment of ACTH, growth hormone and prolactin using the two-bout protocol exercise test is probably one of the most effective methods for diagnosing OTS, but verification of this diagnostic approach requires more research to be conducted (Ordzhonikidze *et al.,* 2018).

The assessment of psychological status

Even after several days of intense training, reduced positive emotions (for example, the sensation of performance capacity) and increased negative emotions (tension, depression, anger and fatigue) may be observed (Batdiyeva *et al.,* 2018). Dose-response relationships between training load and mood state have been revealed in studies involving athletes of various sports. The changes in scores on psychological scales often precede changes in commonly used biochemical markers of training stress, such as cortisol (Meeusen *et al.,* 2013). This fact points to the perspective of using psychological questionnaires as a tool for the early detection of OTS.

The largest amount of literature data about the psychological questionnaires for the detection of OTS refers to the use of the Profile of

Mood States (POMS). Thus, according to the EROS study, the increase in total POMS score, reduction of vigor score, increase in tension score and fatigue score were revealed in OTS-affected athletes. The following criteria for OTS detection included in the EROS-CLINICAL score were proposed: POMS anger score > 14, POMS fatigue score > 8. POMS tension score > 13, POMS vigor score < 18 (Cadegiani *et al.*, 2020).

The results of other studies have also demonstrated changes in various POMS subscales in athletes with OTS. So, the scores of anger, vigor, and fatigue showed significant differences between the groups of endurance athletes with OTS and nonfunctional overreaching, and only tension was similar in both groups (Grant *et al.*, 2012). In athletes-skiers, an increase in total POMS score, fatigue score, confusion score (Gustafsson *et al.*, 2008), tension, anger and depression score (Hedelin *et al.*, 2000) in OTS-affected athletes in comparison with healthy athletes was revealed. According to some studies, in athletes showing signs of OTS, depression increases the most of all POMS subscales (Meeusen *et al.*, 2013). But the difficulty of interpreting such results lies in the differential diagnosis with primary depression, not associated with overtraining (Batdiyeva *et al.*, 2018).

Hence, according to the Profile of Mood States (POMS), the athletes with OTS have an increased total POMS score with different patterns of mood disturbance compared with healthy athletes and athletes with non-functional overreaching.

Training Distress Scale (TDS) based on discriminant function analyses of POMS data was also proposed for OTS detection (Raglin & Morgan, 1994). It was demonstrated that the seven-item TDS (five depression and two anger items) was accurate in identifying OTS-affected athletes (Meeusen *et al.*, 2013).

For assessment of the psychological status of athletes the overtraining questionnaire of the French Society of Sport Medicine (SFMS) was also used. According to various authors in studies of both endurance and resistance athletes, the SFMS question score ≥ 20 is diagnostically significant for OTS detection (Carrard *et al.*, 2022; Favre-Juvin *et al.*, 2003).

Other questionnaires used to detect OTS were the Hamilton Depression Scale (HAMD), Montgomery-Asberg Depression Rating Scale (MADRS,) and the Recovery Stress Questionnaire for Athletes (RESTQ-Sport.) (Meeusen *et al.*, 2013). Among them, RESTQ-Sport (the questionnaire that assesses both overtraining and recovery responses in athletes) has been the most extensively studied (Kellmann & Kallus, 2001). In athletes with OTS the HAMD and MADRS depression scales, as well as the RESTQ-Sport scales, were higher in comparison with healthy athletes (Carrard *et al.*, 2022).

Hence, according to the majority of studies of OTS-affected athletes, using psychological questionnaires can be an effective tool for early detection of overtraining, especially in combination with other functional and biochemical markers.

Heart Rate Variability

Numerous studies have examined the effects of training on HRV parameters, but only a few studies have investigated HRV in athletes with OTS.

According to the literature data, OTS-affected athletes compared with healthy athletes most often have parasympathetic dominance, which is manifested by an increase in high frequency (HF) oscillations and a decrease in low frequency (HF) oscillations. Thus, in endurance athletes (cross-country skiing) in the overtrained state high frequency (HF) and total powers (TP) in the lying position were higher, and resting heart rate was slightly reduced (Hedelin *et al.*, 2000).

The study of Hynynen et al. of athletes of endurance and game sports revealed, that low frequency power (LF) was lower both in the supine position after awakening and in the orthostatic test during standing in OTS-affected athletes compared to healthy athletes. Also, the authors did not reveal differences in HRV between overtrained and healthy athletes during sleep (Hynynen E. *et al.*, 2006, 2008).

However, some authors have observed increased sympathetic nervous system activity in

OTS-affected athletes. So, in the study of athletes of strength and game sports (wrestling, football), LF and LF/HF ratio were increased in overtrained athletes compared to healthy athletes. This indicates decreased vagal influence along with increased sympathetic cardiovascular control in OST-affected athletes (Kajaia *et al*., 2017). Increased sympathetic activity was also detected in endurance athletes in the test with overtraining induced by the increased training volume, when increased LF power of HRV during the supine test was observed, and HRV in the standing position had a tendency to decrease in overtrained athletes (Uusitalo *et al.,* 2000).

One of the problems of HRV interpretation is that two forms of overtraining can be distinguished: sympathetic and parasympathetic forms (Batdiyeva *et al*., 2018; Bosquet *et al.,* 2008). Therefore, HRV parameters need to be assessed depending on the form of overtraining and in the complex with other biomarkers of OTS.

Also, according to Shlyk N. I., the type of autonomic regulation should be taken into account for the interpretation of HRV parameters. To determine the type of autonomic regulation, two parameters of HRV were taken as a basis: stress index (SI) and very low frequency (VLF) power. The author identifies four types of autonomic regulation; three of them are regarded as normal, and the fourth type of autonomic regulation corresponds to a pronounced predominance of autonomous regulation and is determined when SI is from 10 to 30 units, VLF > 240 ms^2, and total power > 8000 ms^2. In athletes, the fourth type of autonomic regulation can be both physiological and pathological. The physiological type reflects a high level of training. The pathological type indicates a state of overreaching or overtraining and can be determined if the value of SI is sharply reduced and is less than 10, while TP increases dramatically to more than 16000-20000 ms^2 (Shlyk, 2015).

Thus, changes in HRV parameters in athletes with OTS most often reflect the presence of a parasympathetic form of overtraining and are characterized by a predominance of autonomous regulation of cardiac activity.

Psychomotor speed tests

Some symptoms of chronic fatigue syndrome, such as concentration and memory problems and cognitive complaints, are also found in athletes with OTS (Lehmann *et al.*, 1993; Shephard, 2001). The measurement of psychomotor speed is currently considered one of the most promising tools for the early detection of non-functional overreaching and preventing OTS. The advantage of psychomotor speed assessment (attention and reaction time tests) is that these tests are easy to apply in the sports field; they are noninvasive, resistant to conscious manipulation by the athlete, and inexpensive (Meeusen *et al.,* 2013).

Rietjens *et al.* used a reaction time test (finger-precuing test) as a detection tool for non-functional overreaching. The authors found a significant decrease in reaction time in cyclists after they increased their training volume. At the same time, no changes in exercise-induced plasma hormone levels or ITT values were observed, demonstrating that central fatigue precedes peripheral fatigue (Rietjens *et al.,* 2005). The decreased reaction time task was also revealed in speed skaters with nonfunctional overreaching (Nederhof *et al.,* 2008). Additionally, OTS-affected endurance athletes scored a significantly higher number of mistakes during the Stroop Color Word Test compared to healthy athletes (Hynynen *et al.*, 2008).

All the above-mentioned data suggest that central fatigue is an early manifestation of overreaching and overtraining, and that reaction and attention tests are promising tools in the early detection of OTS. But further studies are needed in athletes with the confirmed diagnosis of OTS.

Immune parameters

In studies of athletes with OTS, a change in some immunological parameters is observed. The majority of overtrained athletes have abnormally low blood leukocyte counts (Gleeson, 2002). The decreased neutrophil-lymphocyte ratio (Cadegiani *et al.*, 2019) and low levels of salivary IgA have also been reported in overtrained athletes. Regular monitoring of salivary IgA levels may be useful for detecting overtraining, but it is essential to determine the individual's normal baseline value of this parameter (Gleeson, 2002).

In the study of athletes of strength sports the state of overtraining was accompanied by the decrease in lysozyme activity (both in saliva and serum) and in the phagocytic index (% number of white blood cells capable of phagocytosis), as well as the decrease in the spontaneous enzymatic activity of neutrophils (nitroblue tetrazolium test) in comparison with athletes without signs of overtraining (Afanasyeva & Tajmazov, 2011).

In the study of Joro *et al.* plasma IL-6, IL-10, TNF-α, IL-1β, leptin, and insulin-like growth factor-1 (IGF-1) concentrations were measured in overtrained and healthy control athletes before and after exercise to exhaustion. At rest, the levels of inflammatory cytokines did not differ between groups of athletes, and leptin concentrations were lower in OTS-affected athletes. With exercise, IL-6 and TNF-α concentrations increased in both groups, but pro-inflammatory IL-1β levels increased only in overtrained athletes. Also, the decrease of IGF-1 with exercise was revealed in OTS-affected athletes. The authors conclude that a low leptin level at rest and a pro-inflammatory cytokine response to acute exercise may reflect a chronic maladaptation state during overtraining (Joro *et al.*, 2017).

Immunophenotyping analysis of leukocyte membrane antigens in most of the studies has failed to find any differences in athletes diagnosed as suffering from overtraining syndrome compared with healthy athletes (Gleeson, 2002; Mackinnon *et al.*, 1997; Rowbottom *et al.*, 1995). It was only revealed that activated T cells (CD3+HLA/DR+) showed slight increases during OTS without reaching pathological ranges (Gabriel *et al.*, 1998).

In the recent research performed by Leal *et al.*, changes in several immunological parameters were evaluated after the intensified training period. The authors did not reveal changes in total leukocyte counts or in most leukocyte subsets occurred pre-training or post-training. Yet, a 194% acute elevation in γδ T-lymphocyte number occurred pre-training, and average resting concentrations of these cells were 174% higher post-training. Also, baseline polymorphonuclear leukocyte phagocytic activity was 47% lower post-training. Thus, the γδ T-lymphocytes sensitivity to exercise was noted. It is currently suggested that these cells act as stress sensors, and these warrants further studies in the context of intense exercise and OTS development (Leal *et al.*, 2021).

Additionally, the case study has been reported demonstrating blood protein changes in an endurance cyclist who exhibited non-functional overreaching symptoms. The increase of immune-related proteins involved with complement activation and the acute phase response was revealed. The blood proteins with the largest increase included complement component C7, complement C4-B, serum amyloid A-4 protein, inter-alpha-trypsin inhibitor heavy chain H4, and alpha-1-antitrypsin (Merritt *et al.*, 2019). These parameters can be considered possible biomarkers that could be useful for non-functional overreaching and overtraining detection.

Thus, the most informative immunological parameters for OTS detection are decreased circulating numbers of leukocytes, decreased neutrophil-lymphocyte ratio, levels of salivary IgA, plasma and salivary levels of lyzocyme and phagocytic activity of blood neutrophils. Possible markers that can be diagnostically important are IL-1β levels, proteins involved with complement activation and γδ T-lymphocyte number.

One of the problems of interpretation for immunological parameters at OTS is that infection might be one of the triggering factors that can lead to the induction of OTS, and it is necessary to distinguish OTS from infection or postviral fatigue states (Meeusen *et al.*, 2013).

Parameters of oxidative and antioxidant status

According to recent data, the imbalance between oxidant production and antioxidant capacity can be a cause of overtraining, which can be detected with the help of several redox-related biomarkers.

Thus, Margonis *et al.* examined the responses of oxidative stress biomarkers to a resistance training protocol. The overtraining state in athletes was characterized by the increasing of urinary isoprostanes, thiobarbituric acid, reactive substances (TBARS), protein carbonyls, catalase, glutathione peroxidase, and oxidized glutathione (GSSG) and the decreasing of reduced glutathione (GSH), GSH/GSSG and total antioxidant capacity. The high correlation between the levels of isoprostanes and GSH/GSSG and the decline in performance and increase in training volume was detected. These results demonstrate that overtraining induces a marked response of oxidative stress biomarkers (Margonis *et al.* 2007).

In the study of Tanskanen *et al.* the indicators of oxidative stress (plasma protein carbonyls, nitrotyrosine, and malondialdehyde) and antioxidant status (oxygen radical absorbance capacity) were measured in overtrained and healthy control endurance athletes at baseline and after 6 months of recovery, both at rest and after exercise. At baseline, overtrained athletes had higher plasma protein carbonyls at rest than controls. Both at baseline and after recovery, exercise to exhaustion caused the increase in oxygen radical absorbance capacity and malondialdehyde in the control group but not in the overtrained athletes. These results also confirm increased oxidative stress in overtraining syndrome (Tanskanen *et al.*, 2010).

Kajaia *et al.* evaluated the serum oxidative and antioxidant status of overtrained wrestling and football athletes. Diacron-reactive oxygen metabolites (d-ROMs) and biological antioxidant potential (BAP) in serum were assessed at baseline and after rest. Comparing the results of the examined groups of athletes, it was revealed that at baseline d-ROMs were higher in OTS-affected athletes, whereas antioxidant potential was significantly higher in healthy athletes. After 3 months of rest there was an improvement in the oxidative status of athletes with OTS, reaching normal values, but the antioxidant status remained without significant improvement, showing subnormal BAP values and a decreased BAP/d-ROM ratio. The results demonstrate increased oxidative stress in the overtraining state and the imbalance between d-ROM production and antioxidant capacity (Kajaia *et al.*, 2018).

Additionally, the case study of an elite athlete (rowing) with alterations in redox homeostasis in combination with a diagnosis of unexplained underperformance syndrome was reported. The increasing of hydroperoxides, superoxide dismutase, α-tocopherol and the decreasing of antioxidant capacity, red blood cell glutathione, coenzyme q10, γ-tocopherol, carotenoids were revealed. These results also provided evidence of altered redox homeostasis (Lewis *et al.*, 2018).

Thus, according to the literature, athletes with OTS have increased oxidant and reduced antioxidant capacities (Carrard *et al.*, 2022). Moreover, markers of oxidative stress are higher in overtrained athletes in comparison with controls both at rest and at physical activity (Tanskanen *et al*, 2010; Margonis *et al*, 2007), and the decreased antioxidant capacity can remain even after a long period of rest (Kajaia *et al.*, 2018). Currently, total plasma protein carbonylation and lipid oxidation (TBARS and malondialdehyde) are considered the main redox-related parameters, which could be useful markers of estimation of the imbalance between the oxidative and antioxidant status of athletes after strenuous and prolonged training without an adequate rest period (Luti *et al.*, 2020).

The problem of using the parameters of oxidative and antioxidant status for assessment of the functional state of athletes and for detection of overreaching and OTS is the lack of standardized values for these biomarkers and the technical difficulties in their determination.

Body composition parameters

Only a few studies evaluated body composition parameters in athletes with OTS, and the main methods used were bioelectrical impedance analysis and air displacement plethysmography. In the study of Cadegiani *et al.*, lower muscle mass and body water were revealed in OST-affected athletes of endurance and resistance sports compared to healthy athletes. Diagnostically significant changes included in the EROS-COMPLETE score are muscle mass < 46 % and body water < 61 % (Cadegiani *et al.*, 2019, 2020). In the other study, increased fat mass in OTS-affected athletes was revealed (Joro *et al.*, 2017). Body composition parameters can be used as additional markers of the functional state of athletes because of individual differences depending on gender and sports specialization.

Electroencephalography

The possible diagnostic tool for the evaluation of overreaching and overtraining states that has not yet received much attention is electroencephalography (EEG). Recently, one study was performed by Bian F., in which EEG parameters were evaluated in athletes of various sports. According to the study results, several EEG features were revealed in athletes with OTS compared to healthy athletes.

At rest, increased amplitude of β-waves, slow wave occurrence (mainly θ-waves) and decreased α-index were detected in OST-affected athletes. Hyperventilation-induced EEG-responses in athletes with OTS included an increased number of slow waves, an increased slow wave index and a blunted increase of α-wave amplitude. During exercise, α-wave amplitude increased in healthy athletes, but no such changes were detected in the group of OTS-affected athletes (Bian, 2018). This study highlights the need for more research to be conducted regarding the study of EEG features in overtrained athletes.

Conclusion

In modern sports, in the conditions of the increased training and competitive loads, effective monitoring of the functional state of athletes should be provided for maintaining their health and sports performance. In this regard, the challenge of finding and developing biomarkers for early detection of signs of overtraining development currently remains relevant. The main problems and peculiarities concerning the diagnostics of OTS are as follows:

1) literature data on a large amount of the biomarkers proposed for the diagnosis of OTS are controversial; this can be partially explained by the fact that in some of the studies of diagnostic markers the tests and procedures were performed with the use of natural-occurring OTS, while other authors examined athletes with the induced OTS caused by temporary intentional increasing of training loads, which tends to manifest as overreaching;

2) the OTS markers described in the literature were mainly determined in male sportsmen; hormonal and biochemical changes in OTS in female athletes have been less studied, partly because of the complexity of data interpretation, as it is necessary to take into account the influence of the menstrual cycle phase and the use of hormonal contraceptives;

3) most of the studies describing changes in physiological or biochemical parameters in OTS were carried out in athletes training in endurance, while data on athletes of strength sports are few;

4) many of the proposed biomarkers, although demonstrating sufficient diagnostic significance in OTS, are at the same time expensive, invasive and time-consuming, and their use as a means of screening and early detection of OTS seems difficult.

Taking into account the literature data, it is possible to point out the main biomarkers of overtraining, which have practically no controversial data and which demonstrate similar changes in OTS-affected athletes of different sport types. Such group of biomarkers include the decreased responses of hormone secretion (ACTH and prolactin) to the two-bout exercise test and IIT, the decreased glutamine/glutamate ratio, lower blood lactate responses during submaximal exercise and the increased scores of psychological questionnaires (in particular, the total POMS score, RESTQ-Sport and SFMS question scores). Also concerning the evaluation of basal hormone levels, it is proposed to use the decreased testosterone/estradiol ratio instead of the cortisol/testosterone ratio as a marker of OTS. The most informative immunological biomarkers for OTS detection are the levels of salivary IgA, plasma and salivary levels of lyzocyme and phagocytic activity of blood neutrophils.

There are several perspective methods and biomarkers for the detection of OTS that need to be more widely tested in the groups of overtrained athletes for research of their diagnostic significance. First of all, these are psychomotor speed tests (finger-precuing test, Stroop Color Word Test and others), which can be effective in early detection of OTS, and, additionally, the parameters of electroencephalography and the redox-related parameters (in particular, plasma protein carbonyls, malondialdehyde and TBARS).

Currently, the most accepted hypotheses of OST development is cytokine hypotheses, and this fact determines the prospects for the search for immunological biomarkers of OTS. Possible immunological markers that can be diagnostically important for OTS are IL-1β levels, proteins involved with complement activation and the amount of γδ T-lymphocytes.

Nevertheless, recently no single test or method is able to identify the exact point at which overreaching becomes OTS, and more research of OTS in athletes needs to be conducted.

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