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Review

Beyond physical exhaustion: Understanding overtraining syndrome through the lens of molecular mechanisms and clinical manifestation

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ABSTRACT

Background: Overtraining Syndrome (OTS) is a condition resulting from excessive physical activity without adequate recovery, predominantly affecting elite athletes and military personnel. While overreaching can be a temporary state, non-functional overreaching may progress to chronic OTS. This review explores various hypotheses regarding the pathogenesis of OTS, including glycogen depletion, dysregulated cytokine response, oxidative stress, and alterations in the autonomic nervous system function. It also highlights the systemic impact of OTS on multiple organ systems, immune function, and overall health, linking the condition to chronic inflammation and an increased disease susceptibility. Additionally, it addresses the role of the gut microbiome in health modulation through physical activity.

Methods: This narrative review was conducted through a structured search of peer-reviewed journal articles in databases such as PubMed, Web of Science, and Google Scholar, focusing on studies involving human participants and published in English.

Results: OTS has systemic effects on multiple organ systems, immune function, and overall health, leading to chronic inflammation and increased disease susceptibility. Athletes with OTS exhibit higher morbidity rates, influenced by factors such as sleep deprivation and stress. The review also emphasizes the role of the gut microbiome as a significant modulator of health through physical activity.

Conclusion: Balanced training and recovery are crucial for preventing OTS and maintaining optimal health and quality of life in physically active individuals. Understanding the complex pathophysiology of OTS is essential for developing effective prevention and treatment strategies.

1. Introduction

It is generally known that hypoactivity and inactivity negatively impact human health. They elevate the risk of cardiovascular and metabolic diseases, increase muscle weakness and likelihood of depression, and decrease bone tissue density.[1,2] Awareness of the importance of increasing physical activity is the focus of many prevention programs aimed at improving population health. However, for physical activity to be beneficial, it must be balanced with adequate rest and recovery.[3]

When cumulative training and non-training stressors overwhelm an individual's adaptive mechanisms, it results in prolonged fatigue, decreased physical and cognitive performance, neuroendocrine alterations, and potential immunological dysregulation: overtraining syndrome (OTS). OTS is a complex, multisystemic physiological and psychological condition. Unlike normal training-induced fatigue, OTS involves a sustained reduction in performance that persists despite extended rest periods and can significantly impair an athlete's competitive capacity and overall well-being.[4,5]

It is important to communicate about OTS to address its impact on athletes and regular people who are exposed to excessive physical stress. Understanding this complex condition can provide insight into the intricate relationship between physical exertion and the body's adaptive capabilities, challenging traditional narratives about endurance, strength, and personal resilience. Especially for athletes or professional soldiers, OTS can be a career-ending phenomenon.[6]

Beyond professional domains, OTS increasingly resonates with broader public health concerns. Many people engage in fitness programs, visit gyms, or participate in activities that exceed their physical abilities and their understanding of physiological limits, which makes them particularly vulnerable to systemic breakdown and increases the risk of developing various pathological conditions that require medical care. It should not be forgotten that OTS is not just a physical fitness problem, but it also affects the whole body, including the functioning of the

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Abbreviation list

ACTH	Adrenocorticotropic Hormone
ALP	Alkaline Phosphatase
AVP	Arginine Vasopressin
BCAAs	Branched-Chain Amino Acids
BDNF	Brain-Derived Neurotropic Factor
BMR	Basal Metabolic Rate
CRH	Corticotropin-Releasing Hormone
CRP	C-Reactive Protein
EROS	The Endocrine and Metabolic Responses on Overtraining Syndrome
GLUT4	Glucose Transporter Type 4
HDL	High-Density Lipoprotein
HPA	Hypothalamus-Pituitary-Adrenal Axis
IRS-1	Insulin Receptor Substrate 1
ITT	Insulin Tolerance Test
LDL	Low-Density Lipoprotein
NOR	Nonfunctional Overreaching
OR	Overreaching
OTS	Overtraining Syndrome
POMS	Profile of Mood States
RESTQ-Sport	Recovery Stress Questionnaire for Athletes
ROS	Reactive Oxygen Species
SAM	Sympathetic-Adrenal Medullary Axis
SCFAs	Short-Chain Fatty Acids
SCR	Salivary Cortisol Rhythm
TNF-α	Tumour Necrosis Factor-alpha
$\dot{V}O_2max$	Maximal Oxygen Uptake

hormonal and immune system, and has a negative effect on the mental health.[7]

This review article aims to provide a deeper insight into OTS, the pathological mechanisms involved, its diagnosis, and other aspects of OTS related to excessive training/exercise. The article precedes our study, in which we want to focus on the evaluation of the organism's biological and psychological response to intense physical and psychological stress during demanding military course.

2. Method

This study was conducted as a narrative review to comprehensively synthesize existing evidence on the potential health effects of OTS. A structured search of online databases, including PubMed, Web of Science, and Google Scholar, was performed to identify relevant peer-reviewed studies. The search strategy utilized predefined and structured keyword combinations with Boolean operators, specifically: (“overtraining syndrome” OR “overtraining” OR “overreaching”) AND (“fatigue” OR “muscle damage” OR “hormonal imbalance” OR “immune system” OR “immune system dysfunction”), (“overtraining syndrome” OR “overtraining”) AND (“oxidative stress” OR “DNA damage” OR “mitochondrial dysfunction”), (“overtraining syndrome” OR “overtraining”) AND (“microbiome” OR “gut health”) AND (“neuroendocrine system” OR “HPA axis”), (“overtraining syndrome” OR “overtraining”) AND (“hemorheological parameters” OR “blood viscosity”) AND (“metabolism” OR “energy metabolism”), (“overtraining syndrome” OR “overtraining” OR “overreaching”) AND (“diagnostics” OR “diagnosis” OR “biomarkers”), (“overtraining syndrome” OR “overtraining” OR “overreaching”) AND (“athletes” OR “military” OR “soldier” OR “excessive training”). The search was restricted to peer-reviewed journal articles published in English, and additional filters were applied to limit the results to articles involving human participants.

To ensure the rigor and relevance of the review, predefined inclusion and exclusion criteria were employed. Studies were included if they explicitly investigated OTS or related physiological and psychological effects, provided quantitative or qualitative data on OTS symptoms, causes, diagnosis, or management, and involved athletes of any sport or level of competition or military personnel. Studies were excluded if they focused solely on general fatigue unrelated to training, were non-original research articles such as editorials, commentaries, or letters, or if the full text was unavailable.

3. Overtraining characteristics, causes, and prevention

Elite athletes, professional soldiers, and others engaged in excessive physical activity are particularly susceptible to OTS. This chronic condition arises from training regimens that consistently exceed the body's recovery capacity, leading to persistent declines in performance and systemic physiological and psychological disturbances that defy standard recovery interventions. OTS is characterized by sustained, unexplained performance degradation despite maintained or increased training volume and inability to complete previously manageable training loads, decreased power output, and reduced work capacity. It is also associated with a higher risk of injuries, permanent fatigue, prolonged recovery periods between training sessions and competitions, mood disturbance, emotional lability, reduction in motivation, sleep problems, burnout, and disruption in multiple body systems (immune, endocrine, and nervous), and injuries such as stress fractures,[8–10] which will be discussed in more detail in the following text.

OTS can be divided into two types. Parasympathetic OTS is commonly seen in endurance athletes (such as cyclists or long-distance runners) and is associated with fatigue, bradycardia, decreased sympathetic activity and reduced performance. Sympathetic OTS occurs in power athletes (for example sprinters or weightlifters) and is characterized by increased sympathetic activity and psychological stress, such as insomnia, irritability, restlessness, agitation, tachycardia, and hypertension. Other symptoms of OTS may include anorexia, weight loss, lack of mental concentration, stiff, sore, and/or heavy muscles.[4,11] Studies on the changes in the body that occur in the context of OTS show that OTS is a risk factor for a variety of conditions, including metabolic and cardiovascular diseases.

3.1. Factors contributing to OTS

It is important to mention that not only excessive physical load is involved in the development of OTS. One of the other important factors is monotonous training regimes. Repeated identical movement patterns create consistent mechanical stress on specific muscle groups, joints, and connective tissues which can lead to localized tissue microtrauma and potential structural adaptations that exceed tissue tolerance as well as potential compensatory movement patterns. It also results in repetitive neuromuscular patterns and therefore leads to reduced neural plasticity, diminished motor unit engagement, decreased neuromuscular adaptive response, and chronic stress response.[12,13] Another factor is inadequate recovery. Insufficient and/or improper rest and regeneration processes may fail to counterbalance the physical and psychological stress associated with intense training. This includes insufficient rest or relaxation, insufficient sleep, and inadequate active recovery techniques such as low-intensity activities that promote blood flow and tissue repair (e.g., light exercise, stretching).[14,15]

Suitable nutritional habits represent a very important group of factors contributing to the emergence and development of OTS. Inadequate nutritional intake hinders the ability of the body to recover. The OTS is often associated with carbohydrates, proteins, micronutrients, and

caloric deficiency. Carbohydrates are the primary fuel source for high-intensity exercise, and their deficiency leads to the glycogen depletion, fatigue, and reduced performance. Proteins are crucial for muscle growth, repair, and maintenance, and their deficiency can impair muscle recovery, increase muscle damage, and delay healing. Micronutrients such as vitamins and minerals (vitamin B, D, magnesium, iron, and zinc) are broadly involved in muscle functions and repair. Electrolyte imbalances and hydration also play important role, as they are critical for muscle function, nerve conduction, temperature regulation, nutrient transport and waste removal.[16,17]

Psychological stressors play an important role in the process of emergence and development of OTS. Among psychological stressors, we can list personal, work-related, and emotional challenges that negatively influence not only mental health but also physical performance.[18]

Finally, it is necessary to mention the influence of environmental factors in which physical activity is carried out and that may have a negative impact on performance and recovery. These include, for example, extreme temperature, humidity, altitude, and air contamination.

3.2. Prevention

Based on the knowledge of the above factors, it is possible to propose preventive measures to reduce the risk of the occurrence and development of OTS. Key preventive measures include a controlled increase in the intensity and volume of physical activity. In this process, it is necessary to pay close attention to early warning signs such as fatigue and muscle soreness.

Cross-training and introducing variety into exercise routines help reduce repetitive strain and allows different muscle groups to recover. Proper recovery strategies are critical, including adequate sleep, active recovery techniques (light stretching and low-intensity activities) and incorporating rest days into training plans.

Mental and psychological recovery should include stress management techniques and is just as important as physical recovery. Nutritional support with a focus on eating a balanced diet rich in the necessary nutrients and maintaining proper hydration, also plays a key role.

It follows that a comprehensive approach to training load, regeneration, mental health and nutrition can significantly reduce the risk of developing OTS while maintaining optimal performance and well-being.

At this juncture, it is essential to introduce another term: overreaching syndrome. Overtraining differs from overreaching (OR). There are two types of OR. Functional OR is characterized by a short-term decrease in performance due to accumulated training and stress, and recovery from this state can take days or weeks. Nonfunctional overreaching (NOR) is a prolonged process of intense training leading to stagnation and a permanent decline in performance that can last for weeks or months. Overreaching, especially nonfunctional, can progress to OTS if adequate recovery is not achieved.

4. Hypotheses of overtraining

There are many factors responsible for the development of OTS. Over the years of research into the pathogenesis of overtraining, several hypotheses have been developed, each focusing on specific pathologies accompanying OTS. Among the best-known are the following hypotheses. However, inconsistencies have been identified in each, meaning they cannot be considered unambiguous, and the OTS most likely arises as a combination of the aforementioned hypotheses.[4,19]

4.1. Glycogen depletion hypothesis

Glycogen serves as the main energy substrate during exercise intensities exceeding 70% of maximal oxygen uptake ($\dot{V}O_2max$). When glycogen depletion occurs in active muscles, fatigue occurs. After exercise, the rate of glycogen synthesis from blood glucose increases to replenish glycogen stores. Prolonged, excessive physical exertion can lead to depletion of glycogen stores, which may not be fully replenished. However, some studies challenge this hypothesis, as even athletes with normal levels of glycogen levels can suffer from OTS.

4.2. Cytokine hypothesis

Chronic, excessive physical activity is associated with tissue damage, immune system activation, and increased production of various cytokines. Cytokines affect not only muscle function but also the nervous and cardiovascular systems, liver, and other tissues. Therefore, they can regulate physical performance and efficiency, as well as fatigue and mood changes.[20,21]

4.3. Oxidative stress hypothesis

High volumes and intensities of exercise are associated with inflammation, endoplasmic reticulum stress, and mitochondrial stress. The production of free radicals and reactive intermediates increases peroxidation of biomacromolecules like lipids, proteins, DNA and RNA, potentially leading to significant tissue damage if antioxidant mechanisms are overloaded.[4,22]

4.4. Glutamine hypothesis

Excessive physical activity results in an increased rate of glutamine oxidation. Its depletion leads to dysregulation of immune system functions and increased susceptibility to infections.[23]

4.5. Central fatigue/branched-chain amino acid hypothesis

Reduces the levels of branched-chain amino acids (BCAAs; leucine, isoleucine, valine) through oxidation. BCAAs compete with tryptophan in the blood-brain barrier. Lower BCAA levels allow more tryptophan to enter the brain, increasing serotonin concentrations, as tryptophan is a serotonin precursor. Higher serotonin levels contribute to fatigue, sleep dysregulation, and mood swings.[24] This sedative effect of serotonin occurs because serotonin is a precursor of melatonin, which regulates wakefulness and sleep. Two enzymes are involved in the transformation of serotonin into melatonin, serotonin N-acetyltransferase and hydroxyindole-O-methyltransferase.[25]

4.6. Autonomic nervous system hypothesis

Exercise is accompanied by reduced adrenal reactivity to ACTH (adrenocorticotropic hormone), which is initially compensated by an increase in ACTH production. Later, however, ACTH production declines. In addition, reduced intrinsic sympathetic activity and target organ sensitivity to catecholamines is demonstrated at this stage.[26]

5. Complications of extensive exercise and OTS

Excessive physical activity and OTS can be associated with various health complications. Many organs and systems can be affected, and acute symptoms can develop into chronic conditions or permanent health damage (Figs. 1 and 2).

5.1. Immune system

Several factors contribute to increased morbidity in athletes and professional soldiers. These include prolonged close contact in a group of people, frequent social interactions, extensive and prolonged travel, previously mentioned poor sleep quality, and changes in the immune system, which will be focus of this chapter. Both innate and adaptive immunity, i.e., cellular and humoral components, are affected. The

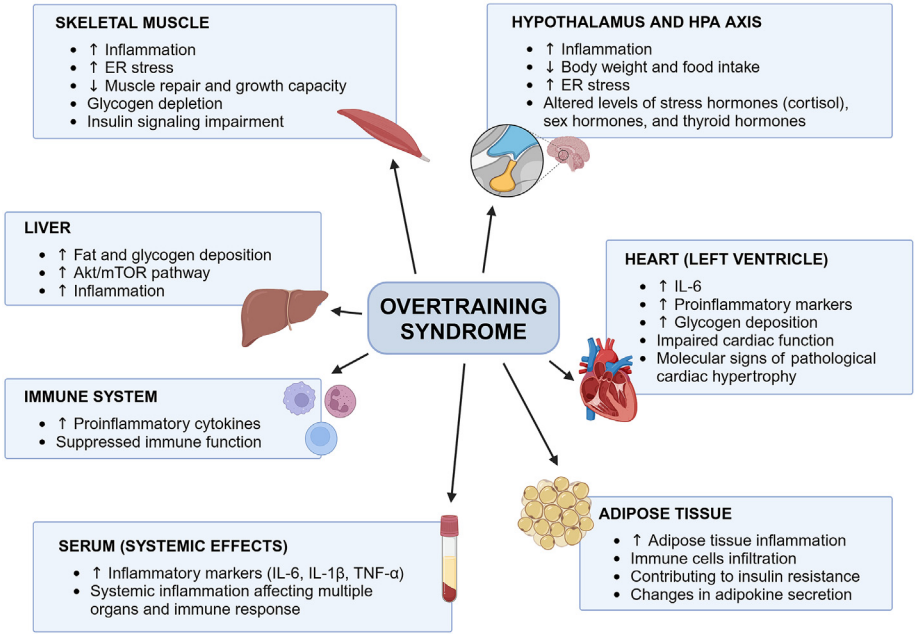

Fig. 1. Organs and systems influenced by OTS. OTS triggers increased inflammation and affects the function of various organs and systems, including skeletal muscle, hypothalamus and HPA axis, heart, adipose tissue, immune system, and liver. These impairments are linked to changes in bioactive molecule levels, disruptions in physiological processes, and tissue damage, which can lead to systemic effects such as systemic inflammation. Akt/mTOR Pathway, Protein kinase B/Mammalian Target of Rapamycin Pathway; ER, Endoplasmic Reticulum; HPA Axis, Hypothalamic-Pituitary-Adrenal Axis; IL-1β, Interleukin-1 beta; IL-6, Interleukin-6; TNF-α, Tumour Necrosis Factor-alpha. Created with BioRender.com.

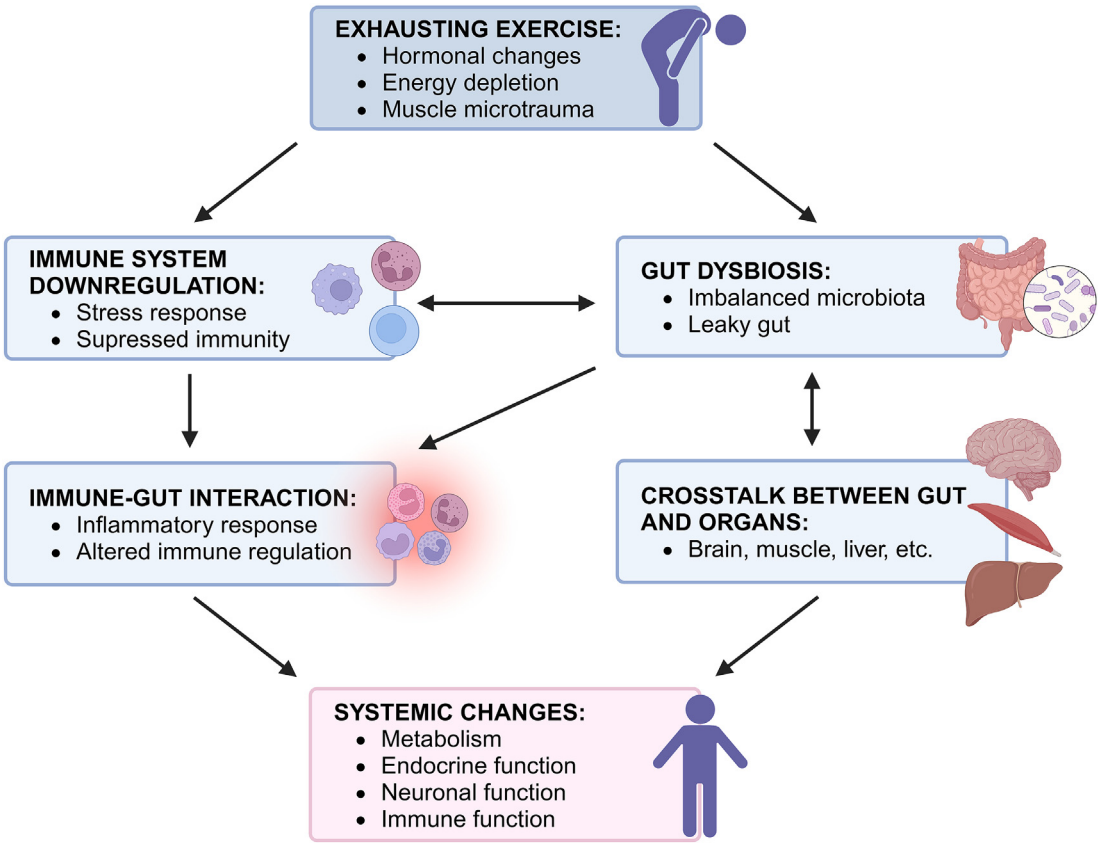

Fig. 2. Exhausting exercise, immune system downregulation, and gut dysbiosis. Exhausting exercise causes immune system downregulation and gut dysbiosis, leading to altered immune-gut interaction. In addition, gut dysbiosis impacts various organs, including brain, muscle, and liver. These effects together contribute to systemic changes affecting metabolism, endocrine and neuronal function, and the immune system. Created with BioRender.com.

presence of chronic, even mild, inflammation negatively affects overall health, increasing the risk of many serious diseases, including cardiovascular, metabolic, neurological and immune disorders.

There are several studies investigating the effect of OTS on the immune system (Fig. 2). Some have analysed markers of immune system activity, and others have examined the incidence of infectious and inflammatory diseases in elite athletes or professional soldiers. Many studies describe the occurrence of recurrent infections, mainly of the upper respiratory tract.[27]

Reid et al. demonstrated that competitive athletes often suffer from

humoral immune deficiency and unresolved viral infection, allergic diseases, asthma, and impaired asthma control; furthermore, there was a higher risk of Epstein-Barr virus (EBV) reactivation and autoantibodies production. The study also revealed the prevalence of non-fasting hypoglycaemia, sleep disorders and iron depletion.[28]

The review article by Gleeson et al. focused on recurrent and/or persistent upper respiratory disease in athletes. The most significant pathogens causing upper respiratory tract diseases were Rnihovirus, Influenzae A and B, Parainfluenzae, EBV reactivation, Cytomegalovirus, *Streptococcus pneumoniae*, and *Chlamydia pneumoniae*. The authors also addressed the non-infectious inflammatory causes of upper respiratory diseases, such as allergic reactions to aeroallergens, asthma, and injuries to airway epithelial membranes. These conditions were particularly prevalent among athletes exposed to airway drying caused by increased ventilation or cold air.[29] Valtonen et al. designed small but interesting study that included athletes, staff members and controls. Respiratory viruses were detected more often in athletes compared to staff and controls, with athletes exhibiting a seven-time higher risk of respiratory diseases than normal exercising controls.[30]

The same problem of increased morbidity as seen in athletes can also be observed in professional soldiers who undergo intensive training. The study of Tiollier et al. showed that French commando training (three weeks of training and five-day combat course) adversely affected mucosal immunity and was associated with higher incidence of respiratory tract diseases, including rhinopharyngitis, bronchitis, tonsilitis.[31]

Korzeniewski et al. also mentioned the prevalence of infections in association with military deployment. Factors contributing to respiratory illnesses in soldiers include exposure to extreme air temperatures, desert dust, emissions from burn sites, and industrial pollutants or airborne contaminants. They further pointed out that acute respiratory illnesses are the leading cause of outpatient treatment and hospitalization among soldiers, with an incidence up to three times higher than in the adult civilian population. The primary pathogens responsible for these infections were Adenoviruses, Influenza A and B viruses, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, coronaviruses and rhinoviruses.[32]

Athletes and professional soldiers frequently experience poor quality of sleep. Hamlin et al. analysed the frequency of illness and injury in elite athletes as a result of sleep duration finding that athletes who slept more than 8 hours (h) or had higher levels of sleep quality were less likely to suffer injuries or illnesses.[33,34] Wentz et al. conducted a study involving 651 British Army recruits and found that sleeping less than 6 h per night increased the risk of respiratory disease four times.[35]

The studies confirmed that chronic excessive exercise has a pro-inflammatory effect and can impair protective immune functions, which is associated with increased incidence of infectious diseases. “Open window” theory suggests that after intense exercise, the immune system's defences decrease for up to 72 h after exercise. Inadequate recovery after intense exercise leads to an accumulation of the negative effects on the immune system and altered immune function.[36]

The increase in inflammatory activity in the body is reflected by elevated levels of IL-6, TNF-α, and IL-1β. Inflammation arises from tissue damage (e.g., muscles and connective/bone tissues), which releases DAMPs (damage-associated molecular patterns), enhances oxidative stress, and activates the immune system. The presence of inflammation and pro-inflammatory cytokines further modulates the activity of other organs and systems. High levels of IL-6, TNFα and IL-1β affect the hypothalamus, reducing food intake. In skeletal muscles, they drive insulin resistance and muscle atrophy; however, in cardiac muscle, pro-inflammatory cytokines induce cardiac muscle hypertrophy.[19,37]

Physical activity also impacts antibody levels, especially IgA. In their review article and meta-analysis, Drummond et al. examined the effect of excessive acute and chronic exercise on IgA levels. While acute excessive exercise increased IgA levels, chronic excessive exercise resulted in a decrease in IgA. IgG levels, however, remained unchanged.[38]

Physical activity influences the number of immune cells. Most overtrained athletes have abnormally low blood leukocyte counts. An important sign of overtraining is the neutrophil/lymphocyte ratio. The number of neutrophils increases during acute activity as they are mobilised from the bone marrow into the circulation. However, in the case of chronic stress, bone marrow stores are depleted. A lower ratio is associated with OTS. With exercise, not only the number of neutrophils changes, but also the number of lymphocytes and their subsets. It has been documented that CD45RO expression on $CD4^+$ T cells is significantly enhanced in OTS.[39]

Hatch-McChesney et al. conducted a study with soldiers who underwent an initial 22-week military training and measured immune parameters and reactivation of persistent viruses. They observed increased number of terminally differentiated T cells ($CD4^+$ and $CD8^+$); however, the granulocyte, monocyte, and memory $CD4^+$ and $CD8^+$ levels decreased. They also confirmed altered cytokine response to lipopolysaccharide stimulation.[40]

Peak et al. discussed the impact of exercise on the immune system and the importance of regeneration time needed to restore immune function. They described that OR suppressed neutrophil degranulation, lymphocyte proliferation, and antibody production. Non-functional OR is associated with decreased IgA production and cytokine production by monocytes, neutrophils, and dendritic cells.[41]

5.2. Microbiome

The gut microbiome, especially its composition, plays a crucial role in human health. Its composition is modulated by many host and microbiome factors, including diet, physical activity, drugs, chronic and acute diseases, and the presence of certain bacterial species, and bacteriophages.

Studies have shown that mild to moderate training can serve as an important intervention to diversify the gut microbiome independently of diet. Notably, an increase in the number of bacteria producing short-chain fatty acids (SCFAs), especially butyrate, has been observed. SCFAs have an anti-inflammatory and regenerative effect on the intestinal mucosa.[5,42,43]

Excessive exercise can adversely alter the microbiome and modulation of its composition can influence performance (Fig. 2). The study by Akazawa et al. observed significant differences in the abundance of the genera *Prevotella*, *Bifidobacterium*, *Parabacteroides,* and *Alistipes*, as well as in the distribution of enterotypes between the transitional and preparatory phases of the season. In the longitudinal study, periodization of the preparatory phase altered the levels of *Bacteroides*, *Blautia,* and *Bifidobacterium.* Interestingly, these microbiome changes were significantly correlated with variations in aerobic capacity and tended to correlate with the anaerobic capacity.[44,45]

Alterations in the gut microbiome can result in increased intestinal permeability, commonly known as leaky gut syndrome, as well as increased production of inflammatory mediators and reduced intestinal barrier function. It is not rare that athletes undergoing high-intensity training often suffer from gastrointestinal symptoms, including nausea, cramps, diarrhoea or constipation, bloating, and even bleeding.[46] There is some scientific evidence that vigorous endurance training ($\geq$ 60 min at $\geq$ 70% of maximum work capacity; $\geq$ 70% $\dot{V}O_2$max) may increase the intestinal permeability; however, determining an exact exercise threshold to avoid leaky gut is challenging due to the variability in individual responses and the complex interaction of factors within the intestinal environment. In general, it seems obvious that the physical stress and hypoxia in the gut during intense exercise contribute to the increased intestinal permeability, weakening the tight junctions between intestinal cells and promoting inflammation. Exercise-induced stress hormones like cortisol further exacerbate these effects by disrupting gut barrier function.[47]

On the other hand, Przewłócka et al. found that supplementation with probiotics and vitamin D increased β-diversity and prolonged the time to exhaustion in MMA athletes.[48] Similar results were reported in a study by

Mazur-Kurach et al. Probiotics were administered to 26 competitive road cyclists, resulting in an increase in the relative maximal oxygen uptake (65.28 mL·kg^{-1}·min^{-1} *vs.* 69.18 mL·kg^{-1}·min^{-1}) and training duration to failure.[49]

5.3. Adipose tissue

Adipose tissue is metabolically and hormonally active tissue involved not only in fat metabolism and storage but also serving as a source of numerous cytokines that exert significant effects on various organs throughout the body. In their study, Mallardo et al. focused on the role of adipose tissue in the context of OTS (Fig. 3). Physical activity induces changes in adipokines levels; however, the final biological effects are related with its duration and intensity. Adequate and regular physical activity is beneficial, as it increases the production of anti-inflammatory adipokines and decreases the production of pro-inflammatory adipokine TNF-α. Additionally, it helps to remodulate adipose tissue and improve metabolic function. Conversely, OTS is associated with a decrease in the production of leptin, adiponectin, resistin and irisin and elevated production of TNF-α. This imbalance results in metabolic and neurological dysfunction and activation of the immune system.[50]

5.4. Muscles

Exercise is a powerful stimulus that induces adaptations in skeletal muscles. Depending on the intensity, type, and duration of exercise, these adaptations can include increased endurance due to mitochondrial biogenesis and angiogenesis, as well as increased strength due to skeletal muscle hypertrophy.[51] However, the exercise is also accompanied by exhaustion (fatigue) and varying degrees of impairment, which are accompanied by changes in metabolism and activation of the immune system.

Recovery and regeneration are as critical for muscle adaptation as exercise itself. Sufficient recovery time is necessary for metabolic and structural adaptations in muscles. Excessive exercise, however, is associated with range of intramuscular changes, including glycogen depletion, insulin resistance, muscle (myocyte) damage, inflammation, oxidative stress, decreased mitochondrial capacity, deterioration of the capillary network, decreased synthesis and increased degradation rate of contractile proteins. Such changes result in decreased performance, exercise intolerance and muscle atrophy with increased myocyte apoptosis.[52,53]

At the cellular level, OTS is associated with damage to muscle fibre ultrastructure, including loss of Z-disc integrity), regional disorganization of myofilaments, overextended sarcomeres with loss of sarcomere boundaries, and tearing of sarcolemma membrane with release of creatine kinase into blood circulation. These structural disruptions also lead to electrophysiological and ionic imbalances. In pathological conditions such as OR and OTS, the homeostasis of intracellular Ca^{2+} and its changes during resting and activity are altered. In OTS, the release of Ca^{2+} from the sarcoplasmic reticulum is reduced during performance, while the resting concentration of Ca^{2+} in the cytoplasm is elevated. These alterations can impair muscle contractility, relaxation, and mitochondrial function. Elevated Ca^{2+} levels also activate calpains, which are responsible for degradation of cytoskeletal, myofibrillar and membrane proteins (titin, desmin, and vimentin) and thus increasing sarcomere vulnerability to exercise-induced damage and changes in muscle structures.[54] Additionally, the Na^{+} gradient is also disrupted, leading to increased Na^{+} concentrations in myocytes, which result in impaired membrane polarization and depolarization.

In normal muscle tissue, muscle contraction stimulates glucose uptake via the glucose transporter GLUT4 (Glucose Transporter Type 4), the expression of which is both insulin-dependent and insulin-independent. Physical activity is therefore crucial for the maintenance of glycemia. However, excessive exercise and OTS are associated with reduced insulin sensitivity and reduced GLUT4 expression. In addition, glycogen depletion occurs, and glycogen replenishment is markedly slowed due to the observed reduction in insulin sensitivity. Furthermore, glucose metabolism shifts toward decreased oxidative and increased non-oxidative glucose degradation, which is accompanied by an elevated lactate production during exercise.

Oxidative stress induced by exercise, resulting from cell damage and immune system activation, may contribute to impaired insulin-stimulated glucose uptake and glucose utilization after exercise. Increased levels of reactive oxygen species (ROS) lead to lipid peroxidation, producing compounds that accumulate in cells and that can modify insulin signal transduction by non-enzymatic and irreversible changes in the amino acid sequence of target proteins, including insulin receptor substrate 1 (IRS-1). Oxidative stress also decreases GLUT4 mRNA levels and GLUT4 expression.[55]

The study by Kajaia et al. focused on NOR and OTS and their effect on markers of myocyte and cardiomyocyte injury. They enrolled athletes with NOR/OTS and control athletes, measuring season levels of creatin kinase (CK-NAC; Creatine Kinase N-Acetylcysteine, and CK-MB; Creatine Kinase Myocardial Band) and cardiac troponin I. After exercise, the levels of these markers were elevated in all athletes but significantly higher in those with NOR/OTS. In these athletes, the parameters remained elevated relative to baseline even 48 h after exercise.[56]

Maskhulia et al. evaluated the dynamics of cardiac biomarkers in athletes with OTS and non-OTS during the season. They confirmed that CK-MB and troponin I levels were higher in OTS athletes immediately after exercise and after 48 h of recovery. They also observed slight differences in cardiac functions (minor diastolic changes) during 6 h after exercise.[57]

Ross et al. investigated musculoskeletal stress and injury associated with military overtraining. Sustained operations over several weeks or rapid succession, with limited time for sleep, recovery, and inadequate nutrition, result in undernutrition, hypercatabolism, and systemic inflammation. These factors contribute to decreased skeletal muscle mass and reduced performance.[58]

5.5. Bone tissue and soft tissue

Bone is not an inert tissue but is constantly undergoing production,

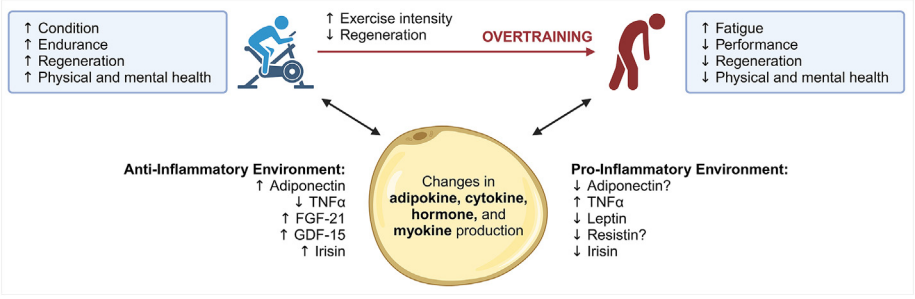

Fig. 3. Adipose tissue, training, and overtraining. Both moderate exercise and overtraining induce changes in the production of adipokines, cytokines, hormones, and myokines. The changes resulting from moderate exercise foster an anti-inflammatory environment that enhances both physical and mental health. Conversely, overtraining, characterized by increased exercise intensity and non-adequate recovery, creates a pro-inflammatory environment, leading to fatigue, decreased performance, impaired recovery, and an overall decline in physical and mental health. FGF-21, Fibroblast Growth Factor-21; GDF-15, Growth Differentiation Factor-15; TNFα, Tumour Necrosis Factor-alpha. Created with BioRender.com.

breakdown, and remodelling. These processes must remain in balance to maintain bone tissue health. Excessive exercise without proper recovery can negatively impact bone quality.

Studies focusing on soldiers show that intensive military training may be associated with a higher incidence of fractures.[59] During physical exercise, microfractures occur in the bones, which respond quickly, exhibiting signs of remodelling even in the early stages of training. Bone can be remodelled to become stronger and more resistant to intensive physical stress. However, when physical stress is excessive and prolonged, compensatory reactions in bone tissue become inefficient. This results in weakened bone tissue with a lower mineral density and greater susceptibility to fractures.[58] Kelly et al. conducted a prospective nine-year study involving elite athletes and found that the most common type of injury was gradually recurring bone injuries, specifically stress fractures.[60]

Bone metabolism is assessed through markers such as alkaline phosphatase (ALP), C-terminal cross-linked telopeptide of type I collagen (βCTX), tartrate-resistant acid phosphatase (TRAP5b), and osteocalcin. Excessive prolonged physical activity can decrease ALP, βCTX, and osteocalcin levels, while TRAP5b levels increase. These results suggest that bone formation is suppressed, and bone resorption is increased.[61,62] Bone resorption is also regulated by inflammation and expression of RANK (Receptor Activator of Nuclear Factor Kappa-B) and its ligand RANKL (Receptor Activator of Nuclear Factor Kappa-B Ligand) in osteoclasts, which elevate bone resorption.[8]

Elite athletes also have a higher incidence of connective tissue damage. In repeatedly overloaded ligaments/tendons and joint capsules (associated with OTS), collagen fibres and tissue strength are disrupted, leading to more frequent tearing or rupture of ligaments. Another complication of OTS is inflammation in tendinopathy. Mechanical overload releases extracellular mitochondrial particles from tendon cells, activating macrophages and promoting proinflammatory cytokine production.[63–65]

5.6. Neuroendocrine system, hormones, neurotransmitters, growth factors, and brain functions

External and internal stress trigger a response from the neuroendocrine system. The hypothalamic-pituitary-adrenal (HPA) axis involves the release of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus, which stimulate the pituitary gland to produce ACTH. These factors trigger responses within the adrenal cortex and other peripheral organs/tissues. The sympathetic-adrenal medullary axis (SAM) represents the sympathetic branch of the autonomic nervous system. Together, the HPA and SAM axes regulate the body's response to external and internal stress.[66]

The response to short-term (acute) physical stress differs from the response to long-term (chronic) physical stress without sufficient time for recovery. The immediate response to acute exercise-induced stress involves activation of SAM axis and branches of the sympathetic spinal nerve. Depending on the duration of stress and other stimuli, the HPA axis is subsequently activated. Initially, adrenaline and noradrenaline levels rise, followed by an increase in cortisol levels. These hormones regulate numerous processes in the body. Adrenaline and noradrenaline increase alertness, mobilization of energy substrates, plasma glucose, and cardiovascular responsivity. Cortisol suppresses immune system activity and inflammation, increases catabolism, and regulates blood pressure and glycemia.[67]

The presence of OTS is associated with decreased sensitivity of the HPA axis and adrenal glands and decreased levels of ACTH and cortisol. Steinacker et al. presented an idealized representation of HPA axis responses across months of training. During training with positive adaptations, ACTH and cortisol levels increased in response to training stress. During overreaching, the cortisol response was blunted, whereas the ACTH response was augmented. OTS is characterized by a reduction in both, ACTH and cortisol responses.[5,68]

Cadegiani et al. analysed cortisol, ACTH, and cortisol response levels during insulin tolerance test (ITT), salivary cortisol rhythm (SCR), and performed cosyntropin stimulation test in OTS athletes, non-OTS athletes and non-active individuals. Cortisol and ACTH levels were higher after ITT in athletes without OTS compared to the other groups, with the lowest increase in ACTH levels observed in subjects with OTS. SCR results showed that 30 min after awakening, cortisol levels were highest in athletes without OTS and lowest in those with OTS.[69]

OTS is also associated with alterations in the concentrations of sex hormones, growth hormone, and prolactin. Cadegiani et al. measured the levels of growth hormone and prolactin in athletes with and without OTS and nonactive individuals. Individuals with OTS had significantly lower basal concentration of both analysed hormones.[70] In another study, Cadegiani et al. analysed testosterone, oestradiol, IGF-1 (Insulin-like Growth Factor 1), thyroid stimulating hormone, free thyronine, fractionated catecholamine and metanephrines in athletes with and without OTS, and sedentary individuals. Testosterone levels were lower in OTS athletes compared to non-OTS athletes but similar to levels in sedentary group. Oestradiol, total catecholamines and dopamine levels were higher in OTS athletes.[71] In addition to catecholamines, serotonin levels may also change, and serotonin reuptake can be impaired.[72] Lee et al., in their review article, reported that OTS is associated with decreased testosterone, dehydroepiandrosterone, and IGF-1 levels.[73]

Excessive training also modulates cognitive and executive functions. Beckner et al. analysed the levels of neuropeptide Y (NPY), brain-derived neurotropic factor (BDNF), IGF-1, oxytocin, and α-klotho in soldiers, markers linked to cognitive performance. Five days of simulated military operational stress led to a decrease in brain functions (alertness and working memory), which was associated with a decrease in α-clotho, NPY, IGF-1, and BDNF.[74] In addition, a decrease in cognitive functions has been also reported in endurance athletes with increased training load.[75]

In the context of military operations, overpressure during 50-caliber rifle training and operational training was associated with elevated levels of markers associated with neurotrauma markers, including amyloid β peptides (Aβ-40 and Aβ-42) and ubiquitin carboxyl hydrolase (UCH)–L1.[76,77]

A summary of the mentioned bioactive molecules affecting the HPA axis, the hormonal system, and cognitive and executive functions is presented schematically in Fig. 4.

5.7. Hemorheological parameters

Intense exercise is associated with changes in hemorheological parameters. It is documented that exercise increases blood viscosity by 10%–12%, primarily due to decreased plasma volume, which results in higher haematocrit and increased viscosity. Conversely, prolonged exercise decreases the haematocrit and viscosity due to the phenomenon of auto-dilution. Some studies showed that an increase in viscosity induces vasodilatation by induction of nitric oxide synthesis, which may improve athletic performance. Short-term intense exercise with sufficient time for recovery does not have a long-lasting effect on viscosity and does not affect the overall condition of athletes. However, the situation is different in the case of prolonged intense exercise and especially in OTS.[78]

Teległów et al. evaluated changes in blood rheology in elite triathletes undergoing training that included 1 500-m swimming, 36-km cycling, and 10-km mountain running. Immediately after the race, haematocrit, and Na^+ and Cl^- levels decreased. The next day, Na^+ and Cl^- and CRP (C-Reactive Protein) levels increased, while haematocrit remained decreased. However, all values were within physiological limits.[79]

The hemorheology in OTS has been intensively studied by Varlet-Marie, Burn et al. OTS is often associated with the feeling of heavy legs, indicating potential issues with chronic venous insufficiency, or altered hemorheological profile. The results of several studies have shown that athletes with OTS exhibit higher plasma viscosity and higher red cell aggregation compared to subjects without OTS. Changes in

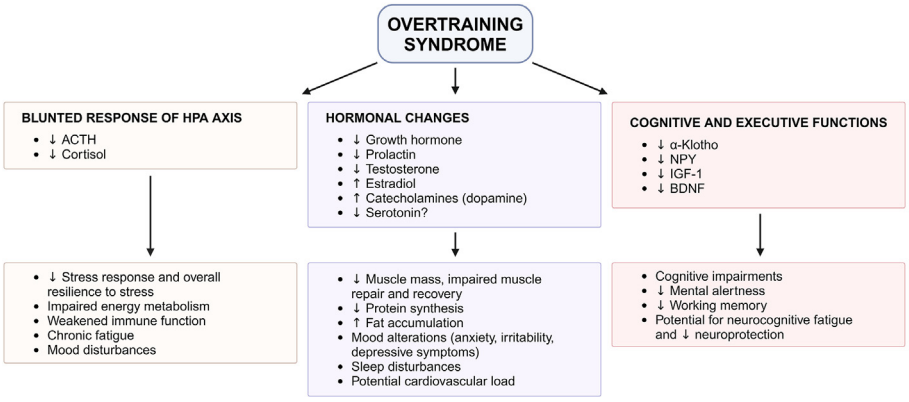

Fig. 4. OTS-induced changes affecting the HPA axis, hormonal balance, and cognitive function. Changes in the levels of various bioactive molecules lead to a range of effects, influencing stress response, overall resilience to stress, energy metabolism, muscle mass and recovery, mood, cognitive function, and sleep patterns. ACTH, Adrenocorticotropic Hormone; BDNF, Brain-Derived Neurotrophic Factor; HPA Axis, Hypothalamic-Pituitary-Adrenal Axis; IGF-1, Insulin-Like Growth Factor 1; NPY, Neuropeptide Y. Created with BioRender.com.

erythrocytes, such as reduced erythrocyte deformability due to increased lactate levels and traumatic damage of erythrocytes, has also been observed. Additional factors affecting hemorheology in OTS include immune cell activation, inflammation, and oxidative stress.[80,81] Blood viscosity predicts fitness. Studies demonstrate that high-fitness subjects tend to have lower blood viscosity. There is a positive correlation between blood fluidity, aerobic working capacity, and endurance time until exhaustion. Blood viscosity and haematocrit also affect oxygen consumption and delivery to tissues during activity and cardiac function.[82]

5.8. Metabolism and nutrition

There are numerous changes associated with OTS, and alteration of metabolism is one of them. The previous text has already mentioned the impact of OTS on insulin resistance in skeletal muscle and brain function, which can also affect food intake. Other studies have reported changes in saccharide, peptide and lipid absorption and metabolism.[83,84]

Coates et al. revealed that carbohydrate oxidation is impaired during exercise in overreached athletes who underwent a five-week training block. They utilized continuous glucose monitoring to detect overreaching status after the ingestion of a beverage containing 50 g of glucose.[85]

The study of Parry-Billings et al. focused on alterations in amino acid concentrations in individuals with OTS, highlighting a significant decline in glutamine levels, which are critical for maintaining proper immune system function.[86] Later, Ikonen et al. investigated plasma amino acid levels in soldiers before and after military training and their association with overreaching. In overreached soldiers compared to non-overreached, the glutamine/glutamate ratio, along with alanine and arginine levels, were significantly higher. The concentration of tyrosine increased in non-overreached soldiers but remained unchanged in those who were overreached.[87]

A meta-analysis and review article by Stellingwerff et al. identified similarities between OTS and Relative Energy Deficiency in Sport (RED-S). This deficiency is associated with low carbohydrate and energy availability.[88] Similarly, Kuikman et al. and described markers of low energy availability in athletes with OTS focusing on fat mass, resting metabolism rate, leptin, body mass, cortisol, insulin, and testosterone. The authors confirmed that while OTS tends to be associated with low energy availability, some OTS athletes exhibit no link to this condition.[89]

It must be noted that while many studies show the negative effects of OTS on nutrient metabolism, other studies highlight the need for proper nutrition to prevent or significantly reduce the impact of OTS.[16]

5.9. Oxidative stress, DNA damage, and mitochondrial damage

Excessive physical load without adequate recovery period is associated with tissue damage, inflammation, metabolic changes and increased oxidative stress. It is hypothesized that oxidative stress plays a central role in OTS. In this context, more studies have been conducted in rodents rather than humans. Overtraining leads to increased lipid peroxidation along with compensatory activation and increased activity of superoxide dismutase, catalase, and glutathione reductase in muscle and blood. Oxidative stress has also been detected in immune cells and the liver. Human studies have shown that overtraining leads to higher levels of oxidative stress markers at rest, and these elevated levels increase further during subsequent exercise.[90]

Margonis et al. measured levels of oxidative stress markers in subjects who underwent 12-week resistance training. Performance metrics (strength, power, jump ability) began to decline from the second week onward, indicating overtraining. Subsequent exercise periods were associated with leukocytosis, increased levels of urinary isoprostanes, thiobarbituric acid, protein carbonyls, catalase, glutathione peroxidase, and oxidized glutathione. A decrease was detected in the glutathione levels and the glutathione/oxidized glutathione ratio, which correlated with performance decline.[91]

Tanskanen et al. also studied oxidative stress and antioxidant status in athletes with OTS compared to control athletes. They analysed biomarkers at baseline and after six months (both at rest and immediately after exercise) and their findings indicated that OTS is associated with increased oxidative stress, even at rest, and a reduced capacity to reduce it, resulting in the inability to perform exercise effectively and impaired adaptation to physical exercise.[92]

An important factor that plays a major role in oxidative stress and have not been mentioned is mitochondrial dysfunction. While exercise usually enhances mitochondrial function and increases the number of mitochondria, OTS can cause mitochondrial damage, exacerbating oxidative stress. Mitochondrial dysfunction in OTS is further linked to impaired glucose tolerance and reduced ATP production.[93–95]

Mitochondria can easily adapt to exercise and the presence of ROS through processes such as increased mitochondria biogenesis and mitophagy, which maintain proper mitochondria turnover. Importantly, long-term exposure to high doses of stress can lead to a hormetic adaptive stress response known as mitohormesis. Mitohormesis involves improvements in mitochondrial function, oxidative capacity, antioxidant response, proteostasis, and reduction in ROS production. However, when mitohormesis capacity is exceeded, mitochondrial dysfunction occurs.[96]

Fang et al. confirmed that an eight-week training with acute exhaustive exercise in rats increased ROS production while reducing the activity of respiratory complex V and ATP levels.[97] Cardinale et al. revealed that even short-term intensified training can temporarily alter mitochondrial respiratory capacity and mitochondrial turnover in endurance athletes. After training, mitochondrial respiration was 20% lower, although mitochondrial density increased by 5%–50%, suggesting a decrease in mitochondrial quality.[98]

Ostojic, in his review article, also mentioned the impaired expression and activity of mitochondrial enzymes such as citrate synthase, and increased production of malondialdehyde and growth factors involved in

mitochondria biogenesis, activity and dysfunction, as well as amplification of mitochondrial DNA deletion. In addition to functional changes, long-term excessive exercise can also lead to uneven distribution of mitochondria within the cell and morphological changes such as larger, swollen mitochondria with abnormal cristae.[99]

Rasmussen et al. showed that exhaustive exercise reduced NAD-linked pathways (involving pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, glutamate dehydrogenase and fatty acid β-oxidation) while increasing α-glycerophosphate dehydrogenase and exo-NADH oxidase activity. These enzymes can catalyse the oxidation of sarcoplasmic NADH.[100]

Oxidative stress, mitochondrial damage, and inflammation create an environment in which DNA damage can occur. The results of animal studies have shown that overtraining can cause DNA damage in blood, skeletal muscle, and liver.[101,102] Tryfidou et al., in a review and meta-analysis, focused on DNA damage following acute aerobic exercise. They concluded that long-term high-intensity exercise increased DNA damage, as confirmed by comet assay results.[103]

6. Diagnostics

It must be emphasized that there are still no clear diagnostic criteria for OTS. Current diagnostics of OTS is largely based on the parameters tested in the EROS system (The Endocrine and Metabolic Responses on Overtraining Syndrome). There are several variants of EROS system, including EROS-HPA, EROS-stress, EROS-basal, and EROS-profile.

EROS-HPA describes the response of peripheral and central components of the HPA axis. This involves measuring baseline ACTH and cortisol levels, their response to the insulin tolerance test, as well as cortisol response to the cosyntropin stimulation test and salivatory cortisol rhythm.

EROS-Stress assesses the basal secretion of growth hormone, prolactin, glucose, and their response to the insulin tolerance test.

EROS-Basal examines (1) changes in hormonal markers such as total testosterone and oestradiol, IGF1, TSH, free triiodothyronine, total catecholamines, noradrenaline, epinephrine, dopamine, total metanephrines, and normetanephrines; (2) changes of blood and biochemical markers including erythrocyte sedimentation rate, CRP, ferritin, neutrophils, lymphocytes, eosinophils, platelets, LDL (Low-Density Lipoprotein) cholesterol, HDL (High-Density Lipoprotein) cholesterol, triglycerides, vitamin B12, lactate, creatine kinase, creatinine; (3) changes in ratios: testosterone/oestradiol, testosterone/cortisol, neutrophils/lymphocytes, and platelets/lymphocytes.

EROS-Profile describes (1) general patterns (numbers of hours of activities, duration of night sleep, self-reported sleep quality, and self-reported libido); (2) eating patterns (calorie, protein carbohydrate and fat intake); (3) psychological patterns (Profile of Mood State, POMS); (4) body metabolism (Body Metabolism Rate, BMR and percentage of fat burned compared with total BMR); (5) body composition (chest-to-waist circumference, visceral fat, body fat percentage, muscle mass weight, body water percentage, and extracellular water vs. body water percentage).

Cadegiani et al. performed modifications and parameter combinations of individual EROS variants, creating EROS-COMPLETE. In their article, they introduced EROS-COMPLETE/DIAGNOSIS evaluation table of risk parameters (scores with ranges) which includes interpretation of results. According to the authors, this approach is currently the most effective method for diagnosing OTS.[104]

Finally, it is important to mention other methods (approaches) to OTS diagnosis evaluation. These methods include, for example, the "Response to one- or two-bout exercise test" (measuring ACTH, cortisol, growth hormone, lactate, fatigue, vigour, and tension score), the POMS questionnaire (assessing fatigue, vigour, depression, anger, tension, and confusion score), the "Recovery Stress Questionnaire for Athletes (RESTQ-Sport)", and the "Overtraining Questionnaire of the French Society of Sport Medicine". OTS is also associated with an elevated resting heart rate, indicating the presence of chronic stress and inadequate recovery, as well as an altered heart response to exercise, including higher heart rate during submaximal workload or a blunted heart rate at maximum effort.[83]

7. Discussion

The findings of our review illuminate the complex and multifaceted nature of OTS, revealing significant challenges in understanding this sophisticated physiological and psychological phenomenon. While appropriate, regular, and adequate physical activity is beneficial for health, physical exertion must be carefully balanced with sufficient rest and recovery.

Without proper recovery, individuals may progress through a spectrum of physiological stress responses: from OR to NOR, and potentially to OTS. OTS represents a chronic maladaptive response to unbalanced training regimes, characterized by profound fatigue and systemic damage across multiple body organs and systems.

Although OTS theoretically affects anyone who does not follow appropriate guidelines for physical activity and recovery periods, professional athletes and soldiers with inadequate training programs are most susceptible to this condition. As shown by the literature, the onset and development of OTS are not determined solely by physical exertion but are influenced by a complex interplay of factors including insufficient sleep, work or study stress, and suboptimal nutrient intake. Over the years, researchers have proposed several hypotheses to explain the origin and development of OTS. However, inconsistencies in these theories have prevented the establishment of a definitive understanding, making OTS diagnosis particularly challenging. This diagnostic complexity underscores the need for continued research into the intricate mechanisms underlying heavy physical activity and its systemic impacts.

Our review not only provides significant insights but also exposes critical gaps in current scientific knowledge. The limited number of longitudinal studies and diagnostic difficulties in the field of OTS represent significant research challenges. Particularly promising are potential future investigations into molecular and genetic markers, which could offer earlier and more precise detection mechanisms.

By further studying the influence of OR, NOR, and OTS, researchers can develop a more comprehensive understanding of how heavy physical activity affects individual organs and systems. Such research will be crucial in designing training programs that minimize the risk of adverse physiological responses, ultimately promoting safer and more effective athletic and military training strategies.

8. Conclusion

OTS is a complex and multifactorial condition that significantly impacts the health and performance of physically active individuals, particularly athletes and military personnel. The interplay of metabolic, immune, and psychological factors in OTS highlights the need for comprehensive prevention and management strategies. Despite several hypotheses proposed to explain the origin and development of OTS, the precise mechanisms behind OTS remain unclear, and its diagnosis remains challenging. To mitigate the risk of heavy physical load on the body, early recognition of symptoms and implementation of appropriate recovery strategies are critical. Future studies should aim to refine diagnostic criteria and explore targeted interventions that enhance recovery while optimizing performance. Raising awareness among athletes, coaches, and healthcare professionals about the dangers of overtraining and the importance of balanced training and recovery can foster healthier and more sustainable training practices, improving performance, long-term health, and well-being in physically active populations.

CRediT authorship contribution statement

Ondrej Fiala: Writing – review & editing, Writing – original draft, Conceptualization. **Michaela Hanzlova:** Writing – review & editing, Visualization. **Lenka Borska:** Supervision, Funding acquisition. **Zdenek Fiala:** Writing – review & editing. **Drahomira Holmannova:** Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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