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Stress and anxiety disorders – prognostic significance of various biochemical indicators in combat sports athletes

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Abstract

Anxiety and stress are surprisingly common, both in the context of general population, and in relation to elite athletes, especially in combat sports. The aim of this review paper is the usefulness of changes in physiological indicators during diagnostic procedures of anxiety disorders and stress in combat sports athletes, on the basis of the available literature reports. Particular attention was paid to variability in hormonal balance, which undergoes dynamic fluctuations under conditions of increased mental stress as well as depending on the competition results (victory, defeat). Additionally, limitations of the conducted analyzes as well as the type of applied biological material were characterized. Both advantages and disadvantages of using different types of samples were taken into account.

Articles qualified for analysis have been carefully selected from the available current Polish and English-language scientific original or review publications. They were acquired primarily from online databases (PubMed, EBSCO) and from the available library resources. The basic criterion for inclusion of the articles to a detailed analysis were a minimum of two measurements of a specific laboratory indicator (at rest and in relation to the competition or intensive training) in combat sports practitioners.

The reported differences in available data, including the correlation of laboratory indicators with the assessment of anxiety and stress, may suggest that physical exercises and participation in important events produce very strong psychological burden and activate the various stress-related mechanisms. Despite quite controversial results, researchers support a theory that the outcome of the competition depends on the level of psychophysiological arousal.

Key words: combat sport ● cortisol ● martial arts ● testosterone ● secretory IgA ● α- amylase

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Combat sport – *noun* a sport in which one person fights another, e.g. wrestling, boxing and the martial arts [52].

Martial arts – ancient forms of combat, defined as offensive and defensive combat systems, modified for modern sport and exercise [1]. Every combat sports is both a martial art but not vice versa [53].

ADHD – attention deficit hyperactivity disorder.

Anxiety – persistent readiness to experience unreasonable negative emotional states [9].

Stress – "an imbalance (perceived or actual) between what is expected of an athlete and his or her ability to respond to these expectations" [47].

Cortisol – "catabolic hormone, secreted by the supra-adrenal cortex in response to physical and psychological stress, which allows indirect deduction of physical stress resulting from the fights" [10].

Testosterone – the main androgenic hormone that promotes development and maintenance of masculine characteristics, probably involved in reduction of anxiety in athletes [31].

sIgA – dominant antibody in saliva, used as a marker of stress [38].

α-Amylase – "one of the major salivary enzymes in humans, secreted from the salivary glands in response to sympathetic stimuli", increased by psychological stress [28].

2-Heptanone – ketone released in the preputial gland, which is able to produce anxiety by acting on the conspecific receptor [14].

Kata – predetermined and choreographed physical exercises, which together with free exercises (randori), lectures (kōgi) and discussions (mondō) form the four critical pillars of Kōdōkan jūdō educatinn [54]

Kumite – is a semi-contact karate competitive concurrence, where two athletes perform various kicking, punching and blocking techniques towards each other with maximum control in order to gain points and win the match. Destruction is fictive.

Introduction

Martial arts have been practiced for thousands of years. Also at the present time, they are a very common and important form of exercise for many people, regardless of age or gender. Styles of martial arts vary (classified as soft or hard) and should be selected in a manner suitable to the participants' abilities [1, 2]. Literature sources suggest that they are both recommended for patients with reduced mobility and the elderly in order to improve their physical fitness and motor coordination. They can be used as an adjuvant therapy in children with diagnosed ADHD, in some patients with depression or sleep disorders as well as in fighting against obesity and physical inactivity. Additionally, they increase the sense of security, develop the ability to control anger, self-control, self-discipline or self-defence. Therefore, they have a significant impact on the physical and psychological state of people practicing this form of activity [1].

However, the authors point out that it should not be forgotten that beside many positive effects associated combat sports, negative consequences are also possible. Among them, the most frequently mentioned are an increased level of aggression and antisocial behavior, especially in adolescents [2].

Particular attention is paid to athletes representing a high level of sophistication. Sports competition induces a higher level of stress and negative emotions than training, which is often associated with a fear of failure, injury or with high expectations of the environment and the public [3, 4]. High performance athletes are often characterized by genetic predispositions, personality and environmental circumstances as well as mental toughness, which do not exclude the possibility of psychopathology [3]. The accumulation of tension, especially prior to the start in important events, can lead to impairment of optimal performance [5].

Anxiety disorders are surprisingly common, both in the context of the general population [6] and in relation to elite athletes [3]. They occur more often than affective disorders or substance abuse. Improperly treated, they can last for decades, or even a lifetime, which makes them the most chronic mental disorders [6]. Epidemiological studies have shown that the most common disorders in athletes of various disciplines (including martial arts) are generalized anxiety disorder (GAD), sleep problems, depression, eating disorders and self-harming behaviors [3].

Anxiety is defined as the persistent readiness to experience unreasonable negative emotional states and is the most common manifestation of stress. It is a multidimensional phenomenon consisting of cognitive and somatic subcomponents, which in various ways affect the course of the competition [7-9]. Fear appears in immediate danger and releases emergency defensive reactions, which allow for temporary, sometimes superhuman efforts. Anxiety, in turn, is connected with a focus on possible future threats, dangers or other potentially negative events. It can be characterized as a state of helplessness, due to the perceived inability to predict, control or achieve the desired results in the upcoming situations [6]. It is often characterized by enhanced skeletal muscle tone, unpleasant vegetative reactions as well as a feeling of constant tiredness, irritability, worry and loss of courage, compulsive thoughts and reactions. Anxiety depreciates experiencing pleasant feelings and contributes to the weakening of protective forces of the body, decreased self-esteem and, above all, to the incomplete use of potential possibilities. This condition interferes with the natural rhythm of the individual responsiveness and results in inadequate responses to specific situations [8, 9]. The lack of control over the process of worry is the defining feature of generalized anxiety disorders [6].

If combat sports athletes have appropriate psychological and tactical preparation and adequate levels of self-knowledge and self-control, anxiety may be reclassified as the so-called "sports anger" or anger at one's own fear. Such ability increases the probability of success with simultaneously maintaining high activation of the nervous system. It is inseparably connected with an increase in self-confidence, which is one of the essential conditions for effective action during a sport competition [9].

The aim of this paper is the usefulness of changes in physiological indicators during the diagnostic procedures of anxiety disorders and stress in combat sports athletes on the basis of the available literature reports. Particular attention was paid to the variability in hormonal balance, which undergoes dynamic fluctuations under conditions of increased mental stress as well as depending on the competition result (victory or defeat). Additional attention was paid to the limitations of the conducted analyzes as well as to the type of applied biological material. Both advantages and disadvantages of using different types of samples were taken into account.

Material and methods

This paper contains a thorough analysis of the literature concerning mental skills in combat sports practitioners. The primary reporting aspect is the phenomenon of anxiety or stress and its physiological components, whose correlation with the analyzed phenomena were scientifically evaluated. Particular attention was paid to hormonal balance and other diagnostic indicators, highly volatile due to fluctuations in mood and increasingly used to control the mental state of athletes in different sport disciplines.

The basic criterion for inclusion a study in a detailed analysis was a minimum of two measurements of a specific laboratory indicator (at rest and in relation to the competition or intensive training) in combat sports practitioners.

Articles qualified for the analysis were carefully selected from the available current Polish and English-language scientific original or review publications. They were retrieved primarily from online databases (PubMed, EBSCO) and from the available library resources.

Neuropsychological basis and diagnostics of anxiety

Anxiety is considered as a negative affective state, which is the most common symptom of stress. It is accompanied by strong physiological or somatic manifestations, which are reflected in the activation of distinct brain circuits, such as the corticotropin releasing factor system and Gray's behavioral inhibition system [6].

Chronic anxiety state is also characterized by persistent tension and arousal of the central nervous system, autonomic inflexibility and functional asymmetry of the brain, which seems to reflect the consequences of constant readiness to confront danger. Such systems can constitute a physiological basis of readiness to confront the stressful situation, to step up vigilance and to prevent helplessness [6].

In combat sports the phenomenon of anxiety is usually diagnosed through a variety of questionnaires, such as the Sport Competition Anxiety Test (SCAT) [7, 10], Competitive State Anxiety Inventory-2 (CSAI-2) [8, 11-13], State-Trait Anxiety Inventory (STAI) [11, 14-17] or the 8-color variant of Luscher's test [18] and other modified versions of available tools [19]. Some researchers estimate the players' emotions using the Profile of Mood States Questionnaire (POMS), which enables the analysis in six subscales: tension/anxiety, depression, anger, vigor, fatigue and confusion [10, 15, 20, 21].

Diagnosis of such disorders using only questionnaires can constitute a big problem, especially in elite athletes. Those contestants rather reluctantly provide information that might reveal an emotional disorder or mental fragility, which, in their opinion, could have a negative impact on their heavily developed image [3]. Therefore, it may appear that the commonly used for these purposes psychological tools may sometimes be inadequate. With regard to the current state of knowledge additional diagnostics methods, independent of the subjective athlete's opinion, are investigated.

Literature sources frequently refer to changes in physiological indicators related to anxiety in athletes, particularly during the competition [10, 16, 17, 22, 23], but also during intense training [22-25]. However, it is worth remembering that results obtained during simulations may differ from those gained in the course of competition [10, 22, 23]. The best diagnostic results are obtained by a combination of psychological tests with laboratory evaluation of physiological indicators [16, 17, 26]. In turn, some researchers are trying to conclude about stress and anxiety in competitors only on the basis of physiological markers [26, 27].

Summarized results from scientific research papers containing both psychological and physiological analysis of anxiety in martial artists are presented in Appendix.

Diagnostic markers for anxiety

Laboratory markers of stress and anxiety arouse a great interest not only in relation to sports competition. It is well known that two main systems are involved in stress response. The first one is hypothalamic-pituitary-adrenal axis (HPA), and the other one is the sympatho-adrenomedullary system (SAM). HPA activation causes an increase in cortisol in the adrenal cortex. In turn, the SAM is responsible, among others, for the release of noradrenaline from the sympathetic nervous system [28]. The researchers of various disciplines seek to explore the problem and undertake intensified activities in order to explain this phenomenon and find easy, reliable and non-invasive diagnostic methods.

It has been previously shown that a number of biological markers exhibit a characteristic variability, depending on the mood in healthy people and in people with the central nervous system diseases [29]. Cortisol, which is associated with stress, is considered to be the most acceptable and most widely used circuit marker, followed by adrenaline and noradrenaline [29, 30] and recently also testosterone [31]. However, it is worth remembering that the variability, depending on the psychological condition, can also be observed in the results obtained for the basic diagnostic indicators. Among others, it has been observed that higher levels of HDL cholesterol, free fatty acids (FFA), *dehydroepiandrosterone* sulfate (DHEA-S), or substance P may be associated with a deterioration in mood and increased tension-anxiety, whereas an increase in LDL-cholesterol and triglycerides may be connected with a decrease in this variable. Dependencies are also searched with regard to markers, such as glucose, C-reactive protein, fructosamine, ACTH, thyroid hormones or estrogens. Their fluctuations may also provide useful information about the functioning of the central nervous system, although generally they are related only to the functions of the brain or to metabolic disorders [29].

Among the most important hormonal markers of anxiety and stress which are commonly used in combat sports athletes, cortisol [10, 11, 13-16, 19-23] and testosterone [11, 13-16, 20, 31] should be mentioned. Additionally, researchers search for collateral links with other biomarkers, which among others include secretory IgA [10, 13, 23], α-amylase [21], or chromogranin-A [32]. Noteworthy are also the newly searched physiological compounds which are potentially related with the anxiety phenomenon, such as 2-heptanone [17]. Characteristics of selected, frequently used markers are presented below.

Cortisol

Cortisol is one of the most widely used physiological indicators in the assessment of anxiety and stress in combat sport athletes. This is a catabolic hormone secreted in response to stress, both physical and psychological [10]. Increased release of this hormone occurs when a stressful situation persists for at least a few minutes, days or even weeks and is conditioned by increased activity of the supra-adrenal cortex [26, 30]. The effect of cortisol is performed through specific receptors located in the cytoplasm of the target cells [33]. Cortisol makes us very vigilant, but a persistently increased level affects chronic anxiety and depression, hypersensitivity, emotional negativity, weight gain, heart disease, high blood pressure and a weakening of the immune system [30]. The studies which include an assessment of changes in the hypothalamic-pituitary adrenal axis activity combined with measurements of mental indicators are considered as useful tools in assessing stressful situations [26].

Increased levels of cortisol in combat sport athletes were observed both before the competition [11, 14, 26, 27] and through participation in competitions [10, 11, 14, 34]. The researchers suggest that the level of increase in cortisol concentration before the competition indicates psychological stimulation and depends on the training experience, age, physical fitness, skills, strategies to cope with emotions as well as on the rank of competition [26].

Scientists often analyze variations in cortisol levels depending on the results of competition. However, available data are very varied. Literature reports provide information that the dynamics of changes in cortisol concentration in athletes were not associated with winning or losing the competition. Such results were obtained in judo practitioners [14] as well as in athletes of various disciplines (including judo and taekwondo athletes) [26]. The probable explanation is the high homogeneity of the study groups (similar age, experience, training and skill levels of athletes) [26]. On the other hand, Serrano et al. [20], also in judo competitors, observed a higher level of cortisol in losers than in winners, but the difference did not reach statistical significance. A similar regularity was observed in karate athletes (kumite) [16] and judo practitioners [13], but the results were significantly higher in losers than in winners, both before and after the fight.

Diversified dynamics of changes in the cortisol level in blood, based on gender, also generate numerous controversies. In the group of judo and taekwondo athletes, it was observed that before competition cortisol concentration in women was significantly higher than in men. It should be noted that under neutral conditions cortisol concentrations in blood were similar in both sexes. This suggests that women are characterized by a stronger hormonal response to stress before the competition, probably because contests constitute a greater bioburden for them than for male athletes [26]. In turn, the inverse relationship was observed in taekwondo athletes. Since morning hours of the competition day male athletes were characterized by more elevated levels of cortisol than women,

but the conducted evaluation showed that the dynamics of changes were similar in both sexes. The highest concentrations were observed at 30 minutes of rest after the finished fight, and within 90 minutes normalization of this indicator took place [21].

Numerous speculations are also related to the assessment of correlations between cortisol and the anxiety level in relation to the competition. Evaluation of this indicator during a period of intense preparation for the contest and the concentration changes on the day of rivalry, but before the beginning, provides interesting, but differentiated information. Published reports showed that despite the significant increase in somatic anxiety in athletes, there was only a small increase in the cortisol level measured in the morning hours in the week before the competition [19]. No significant correlation between state anxiety and cortisol levels were found in studies on judo athletes either [14, 15]. On the other hand, in another group of judo competitors, positive a correlation between cortisol and somatic and cognitive anxiety before the competition was found [11].

Participation in competition and cortisol variability before and after the fight in jiu-jitsu athletes did not show significant correlations with the profile of mood or competition anxiety [10]. Similarly, studies of young taekwondo athletes found no relationship between hormonal and psychological variables [21], while in judokas the cortisol level was significantly related to negative mood [20]. Additionally, in two independent examinations of karate practitioners a significant correlation between high cortisol concentration and anxiety in failing athletes was demonstrated [13, 16]. Mechanisms responsible for the observed differences may be dependent on individual predispositions or developed techniques for coping with stress [16].

Testosterone

In men, testosterone is mainly produced in testicles by Leydig cells (95%). The remaining amount is formed in the adrenal glands, wherein, due to the peripheral conversion of adrenal androgens, mainly dehydroepiandosterone (DHEA) arises. In turn, in females, about 25% is derived from the adrenal glands, another 25% from the granular layer of the follicle, and the rest is converted from other androgens (mainly androstenedione) [35].

Testosterone is the main androgenic hormone that promotes development and maintenance of masculine characteristics. It also displays an anabolic effect, particularly increase in weight, strength and endurance of muscles or the skeletal system. Simultaneously, testosterone reduces body fat, increases muscles efficiency and facilitates their adaptation to exercise as well as improves their ability for regeneration [31, 35]. Among others, the level of circulating testosterone can affect neural activity, increase vigilance, visuospatial abilities, self-confidence as well as the willingness to take risks, aggressive behaviors and dominance [31].

It is noteworthy that, in relation to sports competition, testosterone appears to play a significant role in reducing anxiety [31] and in the preservation of appropriate motivation level [35]. If tension appears too early before the competition (2-3 days), it decreases blood testosterone levels and a number of other adverse physiological changes. It is particularly important in male competitors, because it contributes to a decline in vigor, deterioration in mood and sleep disturbances at night before the competition [5]. Evaluation of the testosterone level in relation to competition is of great interest, mainly due to the disturbing fact that some athletes abuse anabolic androgenic steroids [36].

Considerable attention is paid to testosterone levels in relation with the results of rivalry. Observations of judo [20] and karate competitors [16] during an event showed higher concentrations of testosterone in winning than in losing athletes, especially after the contest, but the results did not reach statistical significance. Another research team observed that among judo athletes there was a subgroup characterized by elevated concentrations of testosterone and cortisol as well as increased motivation to win the fight. Ultimately, they obtained better results than others, which might suggest that a correlation between hormonal and psychological indicators can affect the competition outcome [15].

A similar conclusion was found during a study of another judoists group, where on the day of the competition an approximately 20% increase in testosterone concentration was observed. Contestants, particularly winners, were characterized by an offensive behavior and determination during the competition. However, the results of one of the contestants aroused particular interest. Observations showed that testosterone concentration in him was 5 times lower compared to the rest of the group and about 3.5 times lower than his own level measured four days before

the competition. He declared poor quality of sleep before the competition and depressed mood for a few days before the first fight. During the competition he behaved passively and suffered defeat. This observation supports the fact that an appropriately high level of testosterone is a necessary condition for successful rivalry [27].

However, some researchers found different relationships. Papacosta et al. [13] studying a judoists group, found that concentrations of salivary testosterone presented no differences between winners and losers. In turn, Filaire et al. [14], also in the group of judo athletes, observed significantly higher levels of testosterone in losers after the last fight than those obtained in winners . There are also some reports in which there was no clear variability of this indicator in relation to the competition, independently of the outcome [11, 15].

In females, testosterone levels are significantly lower than in males. Concentrations may be modified by the female sex hormones (e.g. estradiol), which definitely hinders a realistic assessment of its volatility. Therefore, in female athletes it is difficult to assess the impact of testosterone on the course of competition [31].

In practice, it is extremely hard to assess the correlation between athletes' testosterone level and their psychological state. However, there are some reports confirming a positive correlation between concentration of this hormone and the cognitive appraisal of performance which was observed in judo athletes [20]. A positive association of this hormone concentration with offensive behavior was also observed in judokas. The higher the level of testosterone in a competitor, the greater the number of threats, fights, and attacks was observed. This supports the hypothesis that testosterone may affect the expression of aggressive beh indicator aviors in competitors [36].

In summary, the available data suggest that the physiological role of testosterone in athletes' performance should be better examined [13].

Testosterone/cortisol ratio (T/C ratio)

Recently, repeatedly more often the T/C ratio is used in research, which should enable prediction of aggressive and dominant behavior. However, results of some studies do not fully confirm this theory. In particular, it was shown that the interaction between testosterone and cortisol significantly provides dominant behavior, but the ratio of testosterone/cortisol does not. The authors proposed a hypothesis concerning the interaction of these hormones, which says that the testosterone levels can affect aggression and dominance only at low concentrations of cortisol and, vice versa, when the level of cortisol increases, the effects of testosterone during the competition may be blocked [37].

Observations of judoists during the competition showed that winners have a lower mid-competition salivary T/C ratio than losers. A probable reason for such a result lies in the low physiological value of salivary cortisol [13], which confirms the above hypothesis.

Secretory IgA

Participation of the immune system component in response to psychological stress is very complex and closely correlated with the functioning of the HPA endocrine system. Factors that cause stress also increase activity of the adrenal cortex. This leads to an enhanced level of corticosteroids, which in turn have an inhibitory effect on the function of lymphocytes, macrophages, and monocytes, and increase the probability of infection [38].

IgA is part of the initial protective barrier of the immune system. Salivary secretory IgA is the most frequently determined. Changes in the concentration of this parameter in saliva may indirectly indicate the activity of the immune system [10]. Secretory IgA in saliva is the predominant antibody and exists as a dimer of IgA molecules linked to the polypeptide (J-chain), which makes it resistant to proteolysis in an enzyme-rich environment [38].

In the literature, various reports about variability of this indicator in response to participation in the competition can be found. In kickboxing competitors, no significant changes in this parameter were observed during a match [34]. Significantly elevated sIgA secretion rates were observed after the fight in jiu-jitsu [10] and in judo competitors [13]. Increased sIgA concentration occurred with increased concentrations of cortisol, despite the prevailing opinion that growth glucocorticoid levels can suppress the immune system. Therefore, the observed changes are explained by the increased level of tension and psychological stress [10].

In turn, a reduction in sIgA was observed in female taekwondo athletes who intensively reduced their weight before the competition. The decrease in the value of sIgA was characterized by a gradual decline after the beginning of intensive training, with the most significant results in the face of competition. This effect subsided quickly after the cessation of stress and returned to normal values after two consecutive days. It indicates that mucosal immunity in athletes can be significantly modified by a combination of intense training with rapid weight reduction [23].

α- amylase

α- amylase is one of the major salivary enzymes in humans, secreted from salivary glands in response to stimulation of the adrenergic system [28, 38]. It is responsible for the degradation of starch to oligosaccharides and, subsequently, to maltose and glucose by the hydrolysis of the alpha-1,4-glucan bonds. This enzyme also occurs in the pancreas, fallopian tubes, lungs, prostate and ovarian tissues [38].

Literature reports suggest that α-amylase can serve as a marker for mild psychological and physical stress [28, 32], because there is a positive relationship between the concentration of α-amylase and the level of catecholamines in blood. It is also believed that the measurement of this enzyme in saliva is a useful tool for assessing the function of the sympatho-adrenomedullary system. In experimental conditions, a significant increase in the activity of this enzyme was observed immediately after the implementation of a specific stressor stimulus. The activity of this enzyme probably changes faster and to a greater extent than cortisol and highly correlates with symptoms of anxiety [28].

The research project with the participation of taekwondo athletes demonstrated a significant increase in the α-amylase activity at the end of competition, whereas after 30 minutes of rest it returned to the primary values. This confirms the assumption that events are highly stressful for young athletes. Additionally, no differences based on sex were found, which means that intense competition causes a similar reaction in both young male and female athletes [21].

2-Heptanone

Urine of some primate species includes chemicals that occur during confrontations between males competing for dominance or between females that compete for food resources. The preputial gland of dominant male rodents releases, among others, 2-heptanone, which is capable of producing anxiety by acting on an appropriate receptor. It seems that the production of this ketone is closely related to the metabolism of fatty acids that involves participation of so-called stress hormones. This may lead to an increase in lipolysis of fatty acids and production of their metabolites, such as 2-heptanone. An attempt to assess this phenomenon was made in athletes during a fight, because such conditions can serve as a natural model of competition. In martial artists it was shown that the peak of urinary 2-heptanone appeared only in non-winners immediately after the competition. However, changes in concentration of that indicator showed no correlation with the levels of state and trait anxiety. It was suggested that it might be a type of an alarming signal which indicates immediate danger [17].

Other available methods

Among other, reliable methods for detecting stress and anxiety associated with waiting for the fight measurements of the heart rate and blood pressure can be mentioned [5]. In judo athletes, it was demonstrated that the heart rate variability is sensitive to changes in precompetitive anxiety and truly reflects its level [12]. The usefulness of this indicator was also positively rated in taekwondo athletes [21]. Additionally, some simple methods to detect start-up preload are recommended. Among others, a discreet observation of competitor's behaviors by the coach is recommended. This is due to the fact that strong emotions, such as anxiety, significantly alter facial expressions [5].

Limitations in the evaluation of laboratory markers

During the analysis of biological material, of both professional and amateur athletes, it should be remembered that not only the psychological state but also training procedures and physical exercise might affect changes in the concentration of numerous biomarkers [22-24]. In the research project with the participation of judokas, variations in concentrations of cortisol, testosterone, and some elements of the immune system were observed under the influence of physical activity during the training period [25]. It is worth taking into account such a possibility, especially in an assessment of hormonal balance in situations where an increase in concentrations of the measured indicators is observed in short intervals, directly after competitive bouts [20].

The researchers also showed that it is reasonable to draw attention to the type and the intensity of effort, especially when the assessed indicators include cortisol, testosterone and dehydroepiandrosterone-sulfate. Intense exercises performed during training in taekwondo female athletes brought about changes that are more pronounced in hormonal indicators than moderate effort [39].

Very important is also the fact that researchers frequently attempt to measure hormonal changes in athletes only on the day of the competition, which makes it impossible to compare the results with a non-stressful situation [13]. Additionally, measurements are often made at different times of the day, depending on the contest plan, which makes proper consideration of the circadian rhythm of markers secretion (e.g. cortisol, testosterone, alpha-amylase, sIgA) difficult in the interpretation of results [38].

Information on the diet and a significant reduction in body weight before the competition is also very important, as this can affect both the psychological state and some physiological indicators [40]. It should be remembered that the demand for various nutrients varies and depends on the sport discipline and the intensity or the type of training, and athletes often apply very restrictive diets focused on rapid weight reduction [25, 35, 40]. Weight loss through dehydration is also very common, especially among combat athletes, and exerts a number of adverse effects on the body [41].

Among the psychological aspects of intensive weight loss and dehydration in combat sports competitors, the most frequently mentioned are: increased anxiety, fatigue and anger, depressed mood, a decrease in motivation [41], short-term memory, concentration and self-esteem as well as depression and isolation, which can reduce the competitive capacity [40]. On the other hand, studies concerning their effect on competitors' hormonal balance and the psychological and health state are limited [41]. Tsai et al. [23] have observed a number of changes in taekwondo athletes intensively reducing weight both in their hormonal balance and in immunological markers. In female athletes during the training, competition and recovery period, the observed changes mainly involved sIgA and cortisol, while in male competitors, sIgA varied significantly, and cortisol was relatively stable [22]. This raises another suggestion that during the assessment of hormonal indicators the diversity resulting from sex should not be forgotten, especially due to the fact that in women the phase of the menstrual cycle or oral contraceptives may also play an important modulatory role in this field [26, 35].

Additionally, athletes under huge pressure to achieve better results often use substances intended to support the improvement of condition, which may lead to hormonal disorders [35].

All these elements usually coexist, and it is difficult to consider them separately. Therefore, before the analysis of results it seems reasonable to collect detailed information on the types and the frequency of undertaken physical activity and on the diet and significant fluctuations in body mass.

Diagnostic materials in stress analysis

Diagnostic procedures of different diseases and their monitoring often requires painful and invasive procedures such as repeated blood sampling or biopsies, which causes unnecessary stress and unpleasant experience for a competitor [32, 42]. Routinely used diagnostic tests are usually based on blood or urine components analysis, which are well-known and standardized diagnostic materials [43, 44]. It is well known that they contain a wide range of molecular components, including enzymes, hormones, antibodies, growth factors, and many others. However, it often happens that the procedures required for the collection and analysis of blood samples can be expensive, problematic and physically intrusive [42].

Along the advancement of science, the circle of usable biological materials widens, with new, easier in acquisition, more durable and giving the possibility of multiple extraction in a short time period [43, 44]. In recent years, it has been found that the majority of substances present in the blood serum are also present in the saliva, which becomes a more popular diagnostic material among researchers. In addition, a correlation between changes in the concentration of substances present in the saliva and their variability in the plasma has been shown [44]. Explanation of this mechanism is based on the theory which says that each of the salivary glands is highly permeable and surrounded by capillaries. This allows for free exchange of blood molecules to adjacent cells producing acinus saliva. Researchers postulate that molecules from the blood penetrate to the saliva by transcellular active and passive transport or through extracellular ultrafiltration, which may potentially

affect the molecular composition of the mouth fluid. Thanks to that, the oral cavity discharge can contain the information on the current state of health [42].

The diagnostic usefulness of compounds contained in the saliva frequently depends on the location of their production. The compounds produced in salivary glands (intra-glandular) are considered to have a lower diagnostic potential (except for a secretory immunoglobulin A). In turn, the substances synthesized extra-glandular and then transported from plasma to saliva are considered as factors with a greater diagnostic potential, because they reflect the physiological state of the body [44]. However, proving their effectiveness in saliva compared to blood requires further analysis [42].

Particularly emphasized are the advantages of saliva as a diagnostic material, such as non-invasive, painless and easy collection, and because of the material stability, work safety (saliva contains agents that inhibit HIV infectivity) and low costs of the collection and storage of samples [28, 42-44]. However, should not be forgotten that the concentration of most analytes in saliva is considerably lower than in the blood and requires the appointment of new reference values as well as assays with higher diagnostic sensitivity [42].

Regarding the search for optimal methods of determining markers associated with stress and anxiety, particular attention is paid to cortisol, because it is one of the most problematic indicators.

Analysis of cortisol concentration is conducted in various types of biological material (blood, urine, saliva) [26, 45]. Most frequently, blood (serum, plasma) is used for this purpose, but it has a few certain defects. The main one is the fact that this indicator undergoes quite rapid concentration changes and the process of blood sampling from the vein is often associated with additional stress, which can cause false positive results [32, 33]. Another disadvantage is fact that overall cortisol, not biologically active free fraction, is measured in serum or plasma [33]. Determination of cortisol in saliva appears to be reasonable, because its transfer from blood to saliva takes place quite quickly, within less than 2-3 minutes. However, it requires further accurate standardization [33]. Comparative studies, based on concentrations in overnight urine, serum and saliva samples, showed a high correlation coefficient between analyzed materials, which indicates that all of them can be used to monitor cortisol levels. Yet, non-invasive collection procedures and the possibility of multiple repetitions convince to using saliva as a chosen diagnostic material [45].

Similar problems are encountered during the determination of other stress markers, particularly α-amylase activity [32]. Therefore, it is worth considering them before conducting a research project and before the collection of biological material.

Conclusions

Emotional control before competition does not have to completely eliminate anxiety and stress, but keeps them at a level that will allow competitors to maintain the best possible performance and to fully exploit their potential [4, 5]. The basic premise of such an approach is the fact that emotions determine the rules of responsiveness in certain situations, through which they have great functional significance. Their primary function is adaptive values, because they determine not only preparation for action, but also interpersonal communication [6].

An appropriate assessment of anxiety and other emotional disorders is very important for the course of sports competition, especially in combat sports. The impact of this emotional state is differentiated in athletes depending on successes and failures [13, 14, 16, 17, 20] or the gender [21]. It was also shown that it could affect the competitor's fighting style [18, 36]. In the group of boxers, it was observed that the attacking style of boxing is associated, among others, with low anxiety, tiredness, high workability and depends on the vegetative functions [18].

Therefore, it seems legitimate to assess changes in laboratory indicators, concerning hormonal balance [15, 16, 20, 21] and the immune system function [10, 13] within the context of competition in athletes practicing combat sports. Reported differences in available data concerning the correlation of laboratory indicators with an assessment of anxiety and stress may suggest that physical exercise and participation in important events produce very strong psychological burden and activate the differentiated stress-related mechanisms [21]. Authors suggest the existence of multi-level dependencies between the competition results, the anxiety level and physiological markers. Variability, especially in the hormonal balance, *inter alia*, can be caused by expectations of a high result and by uncertainty, which also generates an anxiety state [16]. However, the results are varied and need further standardization [16, 21].

Despite often achieved, quite controversial results, the researchers support the theory that the outcome of the competition depends on the level of psycho-physiological arousal [13].

The exact determination of the nature and the severity of anxiety disorders is extremely important because it determines the selection of an appropriate therapy form and the adaptation of the training process to competitors' individual needs [46]. Numerous projects conducted to evaluate competitors' psychological preparation [46, 47] and those performed to find effective forms of discharging negative emotions [48-51] indicate a frequent occurrence of this phenomenon in combat sports competitors and in the community.

It is essential to remember that success in combat sports depends not only on the tactical, physical and technical preparation to competition, but psychological preparation is also important, which is why it should be integral part of the training process [4, 5, 46]. The realization of these principles requires close involvement of the athlete and the coach as well as the psychologist and the physiologist, because a full understanding of anxiety and stress reactions requires knowledge not only about his psychology but also about physiology [5]. Therefore, knowledge of the fundamental laboratory markers of anxiety and stress should be extended.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix. Number of observations for each group of individuals for all measured biochemical indicators

References	Study group		Anxiety evaluation				Observations
	sport	gender	psychological	physiological			
	(number of subjects)			measured indicator	sample	time of collection	
Gutiérrez-García, 2015 [17]	Karate (6) Judo (14)	Male	STAI STAI-S, STAI-T	2-heptonone	urine	- before the first match, - 20 min and 1 week after the competition	- highest values observed only in non-winners shortly after the competition, - no relation to STAI scores
Salvador, 2003 [15]	Judo (17)	Male	STAI POMS	testosterone cortisol	saliva	- 1 h and 30 min before the competition	- hormonal levels were stable in resting conditions - higher levels of cortisol and anxiety scores observed before the competition - non-significant correlation between state anxiety and the cortisol level - testosterone increases observed in strongly motivated athletes
Serrano, 2000 [20]	Judo (12)	Male	POMS	testosterone cortisol	saliva	- before weigh-in and 30 min afterwards but before the first competition - 10-, 30-, 45 min after the last competition	- non-significant differences in hormones and mood - cortisol consistently related to negative mood (losers showed higher levels than winners - non-significant relationship) - testosterone associated with cognitive appraisal of performance (winners showed increased levels until 30 min after the competition, whereas losers showed decreases – non-significant effect)
Chiodo, 2009 [21]	Taekwondo (16)	Male (10) Female (6)	POMS	α-amylase cortisol	saliva	- in the morning, - 15 min before and directly after the competition, - during the recovery phase (30 and 90 min),.	- no difference in POMS and α- amylase according to gender - higher levels of cortisol observed in male athletes - the highest cortisol values observed at 30 min of recovery, normalization after 90 min (in both sexes) - a significant increase in α- amylase observed at the end of the match, normalisation at 30 min of recovery - tension/anxiety lower after the competition than before (non-significant relationship) - no relationship between hormonal and psychological variables

References	Study group		Anxiety evaluation				Observations
	sport	gender	psychological	physiological			
	(number of subjects)			measured indicator	sample	time of collection	
Parmigiani, 2009 [16]	Karate (24)	Male	STAI	testosterone (T) and cortisol	venous blood (serum)	- 10 min before and 10 min after kumite (real fight) and kata (ritualized form) sessions	-testosterone and cortisol increased only during kumite contest - losers showed higher cortisol and anxiety levels than winners (pre-kumite session levels positively correlated)
Papacosta, 2015 [13]	Judo (23)	Male	CSAI -2	sIgA, cortisol testosterone T/C	saliva	- in the morning - mid- and post- competition	- winners showed a higher cortisol level in the morning of the competition, higher rates of sIgA secretion mid-competition and higher levels of cognitive anxiety than losers. - no differences were found in levels of somatic anxiety and self-confidence according to the outcome
Filaire, 2001 [14]	Judo (18)	Male	STAI-Y-2	cortisol testosterone	saliva	- resting day (3samples) - competition day: after awakening, 5 min before and 5 min after the competition	- cortisol levels were significantly higher on the day of the competition than the resting values; no differences were observed according to the outcome - competition did not induce statistically significant increases in testosterone, while post-competition values was significantly higher in losers than in winners - no correlation was observed between hormonal and psychological variables
Filaire, 2001 [11]	Judo (12)	Male	STAI-Y-2 CSAI-2	cortisol testosterone	saliva	- resting day - competition day: 5 min before and 5 min after each competition in interregional and regional championships	- no significant changes in the testosterone level were observed - cortisol levels were increased in both interregional and regional championships - anxiety components were higher in interregional championships than in the regional ones and were positively correlated with cortisol levels
Andreato, 2014 [10]	Jiu-jitsu (12)	Not defined	SCAT POMS	IgA cortisol	saliva	- before and immediately after competitions	- a significant correlation was observed between IgA and cortisol; both were significantly increased after the competition - no other significant correlations were found between IgA, cortisol, anxiety and profile of the mood state (except the confusion score).
Starhler, 2010 [19]	Jiu-jitsu (10) Karate (4) Kickboxing (3)	Male	WAI	Cortisol (CAR)	saliva	- three days prior to the competition, including the morning of the competition	- there was non-significant increase in CAR across the week - a significant rise was observed in somatic anxiety - no significant association between neuroendocrine response and state anxiety

Explanations: **STAI** State-Trait Anxiety Inventory (evaluation of state anxiety and trait anxiety); **CSAI-2** competitive state anxiety inventory (somatic anxiety, cognitive anxiety and self-confidence); **SCAT** Sport Competition Anxiety Test; **WAI** Wettkampfangstinventar Trait, a German questionnaire similar to Sport Anxiety Scale (SAS) (evaluation of competitive trait anxiety); **sIgA** salivary secretory immunoglobulin A; **RWL** rapid weight loss; **POMS** Profile of Mood States (six subscales: Tension/Anxiety, Depression, Anger, Vigour, Fatigue, and Confusion); **T/C** testosterone/cortisol ratio; **CAR** cortisol awakening response.

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