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The resting salivary antimicrobial proteins and cortisol concentration in wrestlers during 12-week training

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Summary

Study aim: the aim of the present study was to investigate the effect of 12-week training on saliva immunoendocrine response in collegiate male and female wrestlers.

Material and methods: the control group was composed of men and women of the same age, not engaged in any sports activity except for physical education classes at the university. The examined athletes participated in a 12-week training program, which consisted of two sub-phases (preparatory period and competitive period). Saliva samples were collected at three time points: at the beginning (the first point), after six weeks of the preparatory period (the second point, which was the start of the competitive period) and after six weeks of the competitive period (the third point). Immunoglobulin A and cortisol concentration, and α-amylase activity were measured in saliva by respective ELISA kits. Immunoglobulin A was expressed as relative to total protein concentration (sIgA/total protein).

Results: at the third time point, the sIgA/total protein ratio was significantly lower in female compared to male athletes. α-Amylase activity was lower in all examined athletes at all three time points compared to respective control groups.

Conclusions: hormonal and mucosal antimicrobial system response can provide helpful information of body adaptive processes to physical strain as well as indicators of magnitude of training-induced stress.

Key words: Wrestlers – Training – Saliva – Immunoglobulin A – α-amylase – Cortisol

Introduction

Saliva is a secretion of the salivary glands, acting in liquid mouth ecosystem environment. Thanks to its organic and inorganic components, it ensures the proper functioning of many processes determining the maintenance of homeostasis in the mouth. Saliva not only moistens the tissue of the mouth, enabling articulation, digestion and swallowing, but also covers the surface of the teeth and protects the mucous membranes against biological, mechanical and chemical factors. The composition of saliva varies depending on the physiological state of the organism and may be indicative of changes in the human body during physical activity [25]. The secretion of saliva is controlled by the autonomic nervous system, and during physical activity a severe stimulation of the sympathetic or parasympathetic nervous system might occur which may lead to a reduction or increase in salivation [29]. Collection of saliva is not associated with physical pain, mental stress and anxiety, as is the case for blood collection, which makes the measurement of the concentration of hormones, immunoglobulins and proteins in saliva a non-invasive alternative to blood tests [7].

In addition to changes in the rate of excretion, exercise may induce the changes in concentration of certain components of saliva, for example, immunoglobulins, hormones, lactic acid, proteins and electrolytes. The increase in the concentration of total protein in saliva during or after exercise has been demonstrated [4]. This is associated with the increased activation of β-adrenergic receptors in the salivary glands and an increase in the level of catecholamines in blood serum [25].

Immunoglobulins are a heterogeneous group of proteins of the immune system [34]. Immunoglobulin A (sIgA) is the main protein of the immune system associated with mucosa produced by salivary glands. It is widely recognized that the sIgA, due to its dominance in the immune system of the mucous membranes, is the first line of defense against harmful external environmental factors [3]. The secretory form of IgA is responsible for bacterial agglutination, inhibition of mucosal absorption of food antigens, as well as neutralization of viruses, toxins and enzymes produced by microorganisms [14]. Secretion of

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IgA into saliva is stimulated by a variety of factors, such as psychological stress or physical activity [9].

The degree and direction of change sIgA depends on the intensity, volume and duration of exercise [33]. Specific reduction of sIgA concentration is combined with incidents occurring during illnesses of the upper respiratory tract, so it could be an important and useful biological marker of predisposition to these diseases [26]. There is evidence to indicate that reduced levels of sIgA are related to the increased risk of upper respiratory tract infection (URTI) which could affect performance in sport [22]. Thus, it seems that the association of stress, sIgA, URTI, and sports performance should be addressed in the real competitive sport setting.

Cortisol is an important steroid hormone produced in the adrenal cortex layer band [1]. This hormone mediates training adaptations and is usually regarded as the primary catabolic hormone, because it decreases protein synthesis and increases protein breakdown [6]. Cortisol secretion is regulated by the hypothalamic-pituitary-adrenal axis [12] and depends on the circadian rhythm with maximal concentration early in the morning decreasing during the day [13, 24].

It is believed that cortisol plays an important role in tissue remodeling in response to exercise, but not all the mechanisms are explained [16]. As a strong anti-inflammatory compound, cortisol enables combating stress factors [24], takes part in energetic metabolism control [36] and the function of motor cortex [31], and affects immune system functions [20]. Cortisol is one of the most important stress hormones, secreted in response to physical and psychological stress, and its level has been proved to increase after strenuous exercise. The mechanisms by which elevated cortisol concentration may improve athlete's performance are multi-factorial. It is assumed that the level of cortisol in saliva reflects the level of serum free cortisol in the blood, and thus may be an indicator of the body's adrenocortical response to exercise [30].

Salivary α-amylase (sAA) (α-1,4-α-D-glucan-4-glukohydrolase) is one of the key enzymes in saliva. It is a metalloenzyme containing calcium and it occurs in two isoforms, which differ in terms of the presence of carbohydrate. This enzyme hydrolyses the bond α-1,4-glycosidic linkage of starch to glucose and maltose, and inhibits bacterial adherence to epithelial surfaces [25]. Leicht et al. [17] showed increased sAA activity during physical exertion. However, the increase of this activity is noticeable even after written exams, and it is caused by changes in the concentration of adrenaline in response to stress [5].

It was demonstrated that the autonomic nervous system plays an important role in the secretion of salivary α-amylase and this marker can be regarded as an indirect indicator of autonomic-sympathetic nervous system activation in response to stress [11].

There are exist only incomplete data on the concentration immunoglobulin A, cortisol, and α-amylase activity in saliva at rest and as a result of long-lasting training in experienced athletes. Therefore, the aim of the present study was to examine the effect of 12-week training on saliva immunoendocrine response in collegiate male and female wrestlers.

Materials and methods

Participants of this study were male (group TM, n = 20) and female (group TW, n = 9) collegiate wrestlers. The control group was composed of men (group CM, n = 15) and women (group CW, n = 13) of the same age. They were physical education students whose only sports activity was participation in six hours per week physical education classes at the university. All participants were non smokers.

The inclusion criteria of the study were to give written free consent to participation and to be in good health. The athletes and students who fell sick or needed to take any medication during this study were excluded. The protocol of the study was approved by the local Ethics Committee according to the Declaration of Helsinki (SKE 21/2011).

Measurements of body height and weight with the accuracy of 0.1 cm and 0.1 kg, respectively, were made in the examined participants, and BMI (body mass index) was calculated. Percent of body fat was estimated by measuring of skinfold thickness at four sites – biceps, triceps, subscapular and supra-iliac, according to Durnin and Womersley [10]. Measurements were made with an accuracy of 0.5 mm using the Harpenden caliper, in standing position on the left side of the body. Measurements of body mass and height and percentage of body fat were very close (with the differences not exceeding 1%) at the three examined time points, so the general characteristics of participants shown in Table 1 are the average of the three measurements.

The experimental group of male and female wrestlers participated in a 12-week training program consisting of two sub-phases. The first sub-phase was the six weeks of preparatory period including aerobic training to develop general endurance and to improve technical and tactical skills. The second sub-phase, lasting also six weeks, was competitive and was characterized by decreasing training volume and increasing training intensity. The increase in training intensity was achieved by practicing specific exercises, i.e. wrestling contests and their elements. Immediately after the end of second sub-phase, male and female athletes participated in a competition. The study was performed in the winter/spring season. The participants trained

five times per week, and every training session lasted about two hours. Training loads in the phases of 12-week training were different for male and female athletes.

Saliva samples were collected at three time points: at the beginning (the first point), after six weeks of preparatory period (the second point, which was the start of the competitive period) and after six weeks of competitive period, just before participation in an actual competition (the third point). At each time point, three milliliters sample of unstimulated saliva was collected between 7 am and 8 am before breakfast. Participants were informed that chewing gum, eating candy and tooth brushing were prohibited for at least three hours prior to sampling. No samples were collected from the sick or after infection. To get rid of mucopolysaccharides, which could interfere with pipetting and reading, the saliva was immediately centrifuged for 15 min at 15 000 rpm at 4°C. Obtained supernatants were stored until the analysis at –70°C. All samples were defrosted only once.

The DiaMetra (DKO078, Italy) test was used to determine the concentration of sIgA. This method allows for the determination of IgA from 0.5 to 400 µg/ml. The sensitivity of the ELISA kit is 0.5 µg/ml at the 95% confidence limit. The plate reading was performed on a micro-well plate reader at 450 nm within 20 minutes after addition of the stopping reagent. The concentration of sIgA was expressed as a ratio of sIgA to total protein in saliva, in order to avoid false high scores. Saliva protein was estimated by the Lowry's method using bovine serum albumin as a standard [19].

The DiaSource (KAPDB 290, Belgium) test was used to determine the concentration of salivary cortisol. The sensitivity of the ELISA kit is 1.0 ng/ml. The range for this test is from 1 to 100 ng/ml. Reading the plate was performed on a micro-well plate reader at 450 nm within 20 minutes after addition of the stopping reagent. A software product using 4-parameter curve was employed for the calculations.

α-Amylase activity was measured by the commercial test DiaMetra (MCJ 075, Italy). The saliva α-amylase assay is a kinetic colorimetric method for quantitative determination of this enzyme. The test ranges from 2.5 to 400 U/ml. Both the intra-assay and inter-assay variability were <1.5%. At the end of incubation, micro-plate reads the absorbance twice at 405 nm, after 1 minute and 5 minutes of incubation, each time subtracting the absorbance of blank.

Saliva samples were assayed for sIgA and cortisol using commercially available indirect enzyme immunoassays, following the manufacturer's recommended protocol. Each measurement was duplicated and reading of absorbance was performed on the ELISA absorbance reader Ledetect96 (Biogenet, Poland) correlated with the reader MicroWin2000.

Basic descriptive statistics were calculated; all values reported are mean and standard deviations. The normality and homogeneity of variances assumptions were verified by the Shapiro-Wilk test and the Levene test, respectively. Due to certain departures from normality, the data were log transformed prior to analysis. A three-way mixed design analysis of variance (ANOVA) with two between-group factors (sex and training) and one within-group factor (measurement) was used in data analysis; the post hoc Tukey HSD test was used to assess differences between individual means. The level of $p < 0.05$ was considered significant.

Results

Statistical analysis showed significant sex-related differences in body mass and height and in percentage of body fat (Table 1). The BMI value was higher in the group of male than in female athletes. In the present study, there were no significant differences in body mass and height and percentage of body fat between female groups (CW

Table 1. Physical characteristics of the examined groups (mean ± SD)

Parameter	Men		Women	
	CM (n = 15)	TM (n = 20)	CW (n = 13)	TW (n = 9)
Age (years)	20.0 ± 1.1	20.7 ± 2.9	20.1 ± 1.0	20.3 ± 2.2
Height (cm)	181.0 ± 3.7[A]	180.3 ± 4.7[A]	170.6 ± 5.6	163.5 ± 5.7
Body mass (kg)	72.9 ± 7.6[A]	85.7 ± 10.1[A]	60.3 ± 3.7	57.6 ± 7.5
BMI	22.2 ± 2.1	26.4 ± 2.7[B]	20.7 ± 1.6	21.3 ± 1.5
Fat content (%)	12.8 ± 3.4	12.9 ± 3.0	24.0 ± 2.9[C]	20.7 ± 3.1[C]
Training experience (years)	–	9.6 ± 5.2	–	7.4 ± 2.3

[A] Significantly higher compared to respective group of female ($p < 0.001$); [B] Significantly higher compared to female athletes ($p < 0.05$); [C] Significantly higher compared to respective group of male ($p < 0.05$)

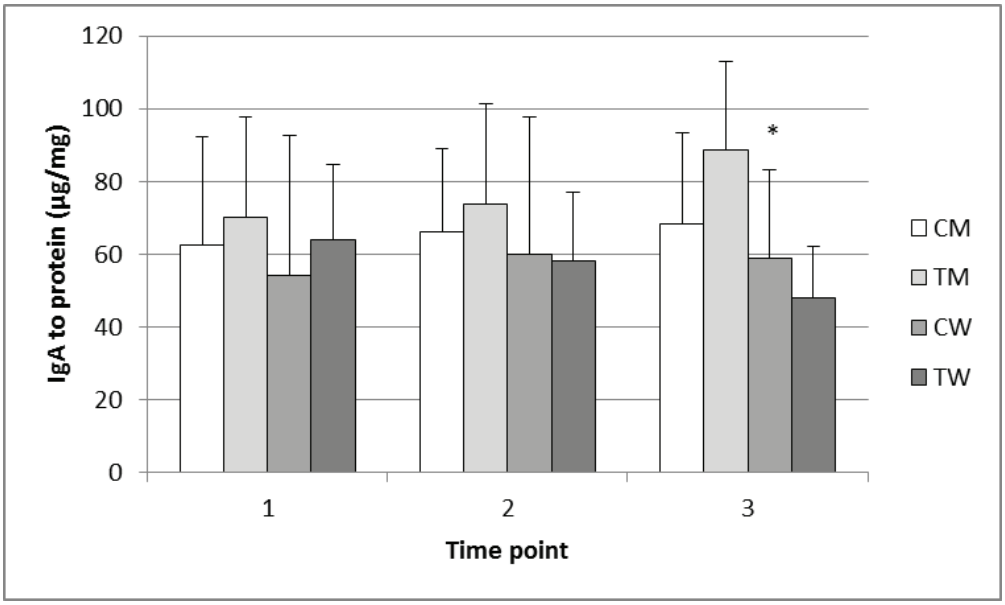


Fig. 1. IgA/total protein ratio in examined groups (mean ± SD)

* Significantly lower compared to male athletes at the respective time point in ($p < 0.001$)

vs. TW) and between male groups (CM vs. TM), independently of the level of physical activity. Training experience of male and female athletes was similar.

As shown in Fig. 1 the ratio sIgA/total protein in female wrestlers showed a tendency to decrease with time, and at the third time point (after 12 weeks of training) was significantly lower compared to male athletes. There were no differences in sIgA/total protein between control male and female groups.

Cortisol concentration in saliva at each time point was lower in control male group than in male athletes (Fig. 2), however no such differences occurred between examined groups of women with different physical activity levels.

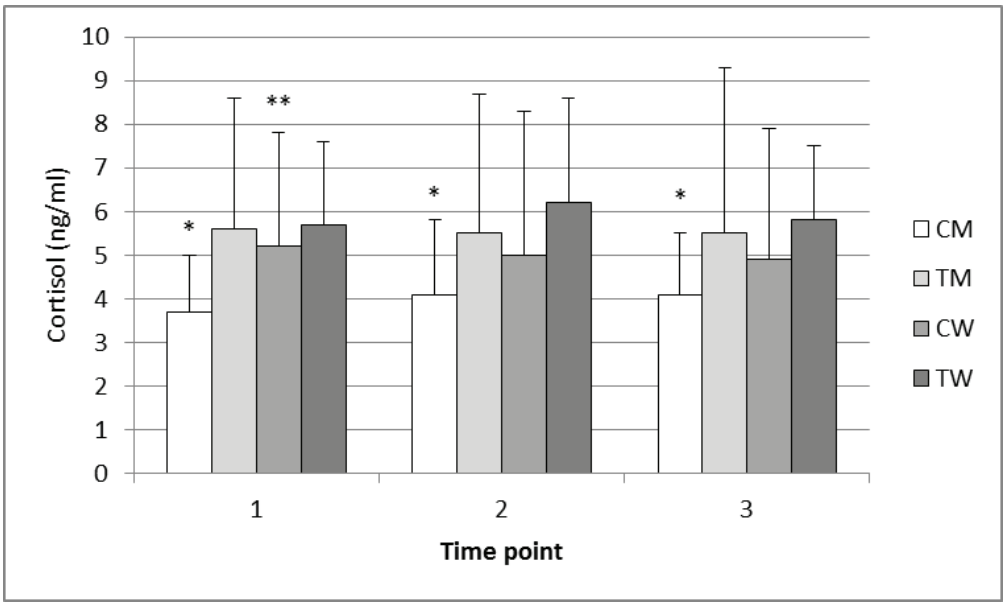


Fig. 2. Concentration of cortisol in examined groups (mean ± SD)

* Significantly lower compared to male athletes at the respective time point ($p < 0.001$); ** Significantly higher compared to male control at the respective time point ($p < 0.05$)

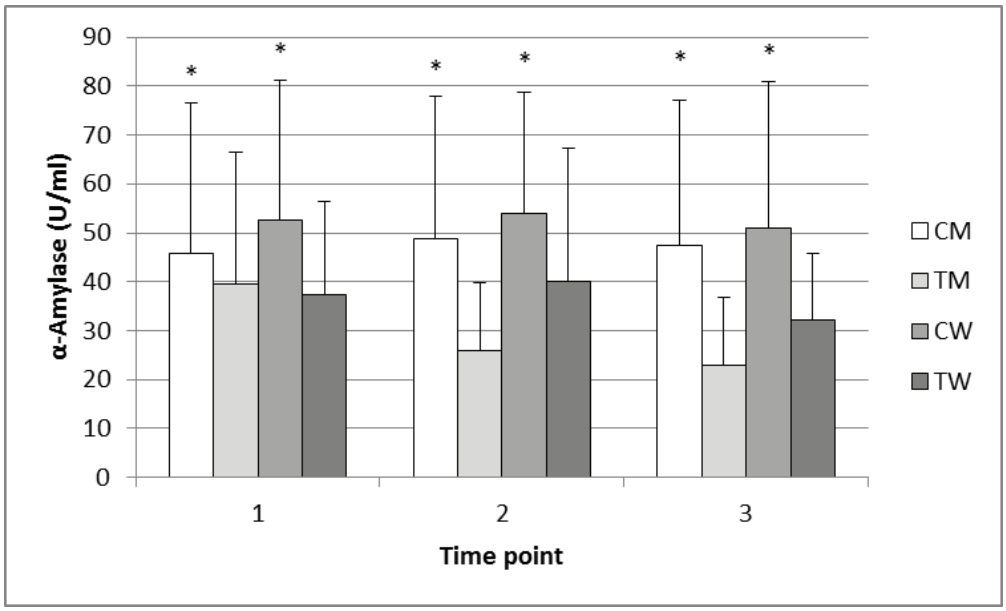


Figure 3. α-Amylase activity in examined groups (mean ± SD)

*Significantly higher compared to athletes group at the respective time point ($p < 0.001$)

It was demonstrated that saliva α-amylase activity at every time point was significantly lower in male and female athletes compared to respective control groups (Fig. 3). At all time points, activity of α-amylase was similar for all athletes (both female and male groups). It was also similar for both female and male control groups.

Discussion

The BMI value was higher in male compared to female athletes, possibly as a consequence of their greater musculature.

It was noted that exposure of athletes to biological, physical and psychological stressors triggered a neurological and endocrine response affecting immune parameters, which contribute to the emergence of symptoms of respiratory diseases [28]. Currently, however, there is not enough direct evidence concerning the mechanisms associated with susceptibility to infections.

Decreased levels of salivary immunoglobulin A are associated with an increase in the incidence of upper respiratory tract illness (URTI), so is a useful biological marker for predisposition of respiratory diseases. The incidence of upper respiratory tract disease is often associated with the sport activity, but it does not fully explain the nature of upper respiratory tract infections, particularly among competitive athletes [27].

It was shown that psychological stressors imposed by training within a short timeframe might be contributing factors to increase in URTI [23] and prolonged strenuous exercise suppressed sIgA secretion in elite athletes [18]. Moreira at al. [21], examining the top futsal players after two highly competitive games separated by seven days, showed that a competitive training match induced a decrease in sIgA/total protein ratio, which suggests an increment of the vulnerability to infections mediated by the training stimulus. In addition, Li et al. [18] examining young volleyball players, showed that sIgA concentrations and the ratio of sIgA/total protein in athletes were significantly lower compared with sedentary controls. On the other hand, Tsai et al. [35] described an increase in the sIgA/total protein ratio in elite male athletes practicing weightlifting during training with increased intensity.

The present study demonstrated the gradual decrease of sIgA/total protein ratio in a group of female athletes during the 12-week training period, and at the third time point, this ratio was significantly lower compared to male athletes. This decrease suggests that the female wrestlers were at an increased risk of developing an upper respiratory tract infection, and therefore, it could be necessary to reduce their training load and take protective actions to minimize contact with cold viruses [21]. Physiological stress in female athletes demonstrates itself in the form of discernible decrease in the sIgA/total protein ratio at the end of competition period and may be due to their worse adaptation to exercise loads. Smith [32] suggested that decrease of blood glutamine, crucial to functioning of immune cells, is one of the underlying mechanisms of overtraining syndrome. Cell mediated immunity is then

suppressed in athletes resulting in the increased susceptibility to illness and colds.

Allgrove at al. [2] showed that the concentration of salivary cortisol immediately after exercise did not change compared to baseline levels. This is not surprising because the appearance of cortisol in the circulation normally occurs after a time lag, which in some cases could be more than 1 h [36]. On the other hand, Crewther et al. [8], studying the rugby players during six-week training program focused on endurance loads with parallel training workouts integrating agility, speed, and improving cardiovascular fitness, reported no significant differences in any of cortisol concentrations in saliva. In the present study, saliva cortisol concentration was higher in male athletes compared to control male group at each of the examined time points, while there was no such differences between female groups.

Tsai et al. [35], examined eleven Taiwanese elite male weightlifters, who trained through three training stages before a national weightlifting competition, followed by a two-week recovery stage. The athletes showed a significant increase of serum cortisol concentration in the training stages compared to the recovery stage. These authors suggested that prolonged, strenuous exercise causes increase in the immunoendocrine responses in athletes. Li et al. [18] demonstrated that the salivary cortisol concentrations and the ratio of cortisol/total protein in volleyball players were markedly higher compared with control groups, and revealed that the prolonged strenuous training may elicit a sustained stress and induce a suppressive effect on mucosal immunity in regularly and intensively trained adolescent athletes.

It was demonstrated that activity of salivary α-amylase is linked to the autonomic nervous system component of the stress response, and can be used as a biological marker of autonomic nervous system activation [25]. The studies showed that activity of salivary α-amylase increased under both physical and psychological stressful conditions [25], and that salivary α-amylase activities were associated with norepinephrine change in response to stress [5].

In the present study, saliva α-amylase activity in male and female athletes at all examined time points was lower compared to respective inactive groups suggesting higher susceptibility to bacterial or viral infections. Leicht et al. [17] studied a group of wheelchair athletes and showed an increase in this enzyme activity directly after exercise, but, after 30 min of recovery, activity of α-amylase was lower than the initial value. Also Kivlighan and Granger [15], in a study of athletes, showed an increase of salivary α-amylase activity in response to rowing ergometer exercise and decrease in this activity to pre-exercise level after 40 min recovery and stated that changes in this enzyme's activity were associated with previous training experience and player's training status. In addition, Allgrove et al. [2] demonstrated that increased α-amylase activity after exercise returned to baseline values at 1 h post exercise, independently of exercise intensity.

It is generally accepted that the sympathetic nervous system may be at least partly responsible for the changes in salivary α-amylase activity as well as sIgA concentrations [2, 3]. Data obtained in the present study, concerning lower resting α-amylase activity in male and female athletes compared to inactive participants, were unexpected and this aspect of antimicrobial defense requires additional research.

In summary, the training loads in the competition period for women caused a clear tendency to decreased sIgA/protein ratio, which could indicate an increased susceptibility to infections of the upper respiratory tract. Intensive training loads resulted in a decrease of α-amylase in saliva in all examined athletes. The obtained data suggest that intensive training may have cumulative effect on sIgA and cortisol levels in elite wrestlers. This finding may provide useful information for coaches and athletes in the development of training programs, which are effective for improving performance while reducing adverse physiological effects. Evaluation of hormonal and mucosal antimicrobial system response to training can be helpful in recognizing body adaptive processes to physical strain and can serve as indicators of magnitude of training-induced stress.

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