

THE EFFECT OF WRESTLING TOURNAMENT ON IMMUNE AND ENDOCRINE MARKERS IN BLOOD AND SALIVA OF MALE AND FEMALE ATHLETES

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Abstract. The aim of the present study was to determine the effect of one-day wrestling tournament on magnitude and direction of changes in selected immunological and endocrine status indices. The participants of the study were male (n = 12) and female (n = 13) wrestlers. The earlobe blood samples and unstimulated saliva were collected at three time points: in the morning before the competition (1), immediately after the last match (2) and in the next morning (3). Total protein, IgA and cortisol concentration and α -amylase activity in saliva, as well as concentration of cortisol and interleukin-6 in serum were measured. Significantly lower ratio of sIgA/protein and significantly higher levels of salivary and serum cortisol, interleukin-6, and α -amylase activity were observed at the second time point compared with the first and the third in both groups of athletes. Regardless of the examined time point, concentrations of serum and saliva markers were similar in female and male wrestlers. However, a tendency to lower sAA activity on the next morning after the tournament compared to basal activity of this enzyme was seen in both examined groups. In general, the changes of examined markers were short-lived, except for α -amylase activity, showing that tournament-wrestling matches had no sustained negative effects on endocrine and immunological body systems.

Key words: wrestlers, tournament, endocrine markers, immune markers, blood, saliva

Introduction

The mutual influence of the immune and nervous systems is well known (Webster et al. 2002). These systems communicate via two hormonal ways: the hypothalamic-pituitary-adrenal cortex axis and sympathetic-adrenal medulla. Depending on the duration, type and intensity of exercise, stress conditions may inhibit or stimulate the immune response, changing the direction and magnitude of the response and modulating the autoimmune phenomena. A mild short stress stimulates but a strong and prolonged one inhibits the immune response (Zimecki and Artym 2004; Hackney and Waltz 2013). Knowledge of the physiological response of the body to physical effort

is the basis for the rational procedures of training, so research on the effects of exercise on magnitude and direction of changes of many markers of endocrine and immune disturbances is very important.

The main immune system protein in the saliva is the immunoglobulin A (sIgA), responsible for bacterial agglutination, inhibition of bacterial adhesion to the mucosa, absorption of antigens, as well as neutralization of viruses, toxins and enzymes produced by microorganisms (Kazeeva and Shevelev 2009). It is generally accepted that sIgA, due to its dominance in the immune system of the mucous membranes, is the first line of defense against harmful external environmental factors (Bishop and Glesson 2009; Woof and Kerr 2006). Concentration of sIgA is influenced by various factors such as mental stress and physical activity (Daly et al. 2005). Results of several studies suggest that the immune system is affected by a single exercise or training session in athletes (Rahimi et al. 2010; Hübner-Woźniak et al. 1998). It is well known that intensive repetitive exercise causes a decrease in the concentration of secretory IgA, and an increase the susceptibility to upper respiratory tract infections (URTI) in athletes (Peters 1997). However, the moderate intensity physical loads reduce the risk of infection due to positive changes occurring in the immune system (Pacque et al. 2007) by potentiating the immune response to pathogens (Martin et al. 2009).

Measurements, observation and analysis of changes in physiological indicators can provide objective information on exercise-induced adaptation in athlete's organism and on many of immune or endocrine indicators, among others – cortisol in blood and saliva, reflecting metabolic processes in the body. Cortisol (C) is a steroid hormone produced by the adrenal cortex in the zona fasciculata (Acevedo et al. 2007). It is described as a catabolic hormone because it reduces protein synthesis and increases its degradation (Crewther et al. 2006). Cortisol secretion is regulated by the hypothalamic-pituitary-adrenal cortex (Eskandari and Sternberg 2002) in accordance with the circadian rhythm, with a maximum concentration in the morning and decreasing during the day (Kanaley et al. 2008). It is assumed that after exercise the level of cortisol in saliva changes in similar manner as free cortisol in the blood, and thus it may be an indicator of the body's response to physical effort (Rantonen et al. 2000).

α -Amylase (sAA) is one of the key enzymes present in saliva, it hydrolyzes α -1,4-glycosidic bonds of starch to glucose and maltose, and inhibits bacterial adhesion to the epithelial surface. It is therefore important for maintaining the mucosal immune defense (Nater and Rohleder 2009). Leicht et al. (2011) showed increased activity of the sAA during exercise and suggested that this marker can be regarded as an indirect indicator of autonomic sympathetic nervous system activation in response to physical stress (Ehlert et al. 2006).

Research conducted in the last decade has shown that during exercise the skeletal muscle fibers produce and release cytokines, exerting paracrine or endocrine effects in the body. These proteins, linked to the skeletal muscle metabolism, were named "myokines" (Pedersen et al. 2007; Febbraio and Pedersen 2005). One of the most important myokines is interleukin 6 (IL-6), which plays a crucial role in energy metabolism in contracting muscles, and its concentration is increased during and after exercise. When it was discovered that physical exercise induces alterations in the amounts of circulating cytokines, a correlation was found between physical activity and changes in the immune system (Pedersen and Febbraio 2008).

The saliva composition, as a body fluid obtained in a non-invasive manner, contributes to increasing knowledge of saliva and its homeostasis. The relationship between physical activity and the concentration of sIgA is fairly well known, however, exercise-induced changes in salivary cortisol concentration and α -amylase activity as well as serum cortisol and IL-6 concentrations are not fully explained. Therefore, the aim of the present study was to determine the effect of a one-day wrestling tournament on magnitude and direction of changes in selected

inflammatory and endocrine immune status indices and to establish whether wrestling matches leave permanent changes in the immune and endocrine systems.

Materials and methods

The participants of the present study were male ($n = 12$) and female ($n = 13$) free style wrestlers of different weight categories combating in international tournaments. General characteristic was shown in Table 1. All athletes were informed about the course of research and gave their written consent. The study protocol was approved by the local Ethics Committee (SKE21/2011).

Table 1. General characteristics of examined female and male wrestlers (mean \pm SD)

Parameter	Female wrestlers ($n = 13$)	Male wrestlers ($n = 12$)
Age (years)	20.3 \pm 2.6	20.7 \pm 2.9
Body mass (kg)	59.5 \pm 9.4	79.9 \pm 9.6
Body height (cm)	164.2 \pm 7.1	180.3 \pm 4.2
Training experience (years)	6.8 \pm 2.8	7.9 \pm 3.4

The earlobe blood samples and unstimulated saliva were collected at three time points: in the morning before the competition (1), immediately after the last match in tournament (2) and in the next morning (3). The wrestlers competed in two to four matches during the tournament. At first and third time points blood and saliva were taken in preprandial state.

The collected blood was transferred to Eppendorf's tubes and centrifuged at 4°C for 15 min at 3000 rpm (503 RCF) to obtain serum. Immediately after collection, the saliva was centrifuged for 15 min at 6000 rpm (1210 RCF) at 4°C to remove the mucopolysaccharides, which could interfere with pipetting and reading. Serum and saliva samples were stored at -70°C until assayed. All samples were thawed only once.

All assays were performed using enzyme immunoassays ELISA according to manufacturer's procedures. Salivary immunoglobulin A concentration was determined using the Diameter test (DKO078, Italy). This method allows for the determination of sIgA from 0.5 to 400 μ g/ml. The sensitivity of the ELISA kit is 0.5 μ g/ml at the 95% confidence limit. Reading was performed on a microplate reader at a wavelength of 450nm within 20 minutes after addition of stop reagent. The concentration of sIgA was expressed as a ratio of sIgA to total protein in saliva, in order to avoid false high scores. Salivary protein was determined by the Lowry's method using bovine serum albumin as a standard.

To assay the salivary cortisol concentrations the DiaSourceELISA test was used (KAPDB290, Belgium) with lower detection limit of 1.0 ng/ml. Reading the plate on a microplate reader was performed at a wavelength of 450 nm within 20 minutes after addition of stop reagent.

The DiaSourceELISA (KAPDB270, Belgium) test was used to determine the concentration of cortisol in serum. The sensitivity of this kit is 0.4 μ g/dL. The intensity of the resulting color is inversely proportional to the concentration of cortisol in the sample. Absorbance was measured at 450 nm within 20 minutes after addition of stop reagent.

To measure the concentration of IL-6, enzyme-linked immunosorbent assay was used with monoclonal antibodies directed against different epitopes of IL-6 (DiaSource, KAP1261, Belgium) with sensitivity of 2 pg/ml.

The IL-6 concentration was read within 20 minutes after addition of stop reagent, and the absorbance was measured at a wavelength of 450 nm.

Automatic result processing was used a 4-parameter logistic function curve fitting for calculation of the serum and salivary cortisol and IL-6 concentration.

α -Amylase activity was measured using the commercial test DiaMetra (DKO075, Italy). The absorbance at 405 nm was measured after 1 and 5 minutes of incubation. The sensitivity of this method is 2.5 U/ml.

The variation coefficients of the intra-assay were 2.9, 4.3, 8.0 and <1.5% for serum cortisol, IL-6, salivary cortisol and α -amylase, respectively.

All measurements were performed twice, and the absorbance readings were performed on the ELISA reader Ledetect96 (Biogenet, Poland) correlated with 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 two-way mixed design analysis of variance (ANOVA) with two between-group factors 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

Figures 1, 2, 3, 4 and 5 presents the results of measurements of sAA activity, sC, C and IL-6 concentrations and the sIgA/protein ratio at the examined time points: before the start to the tournament (1), immediately after the last tournament match (2) and in the next morning (3).

Significantly lower ($p < 0.01$) ratio of sIgA/protein was observed at the second time point compared to the first and the third, in both groups of wrestlers, male and female (Figure 1).

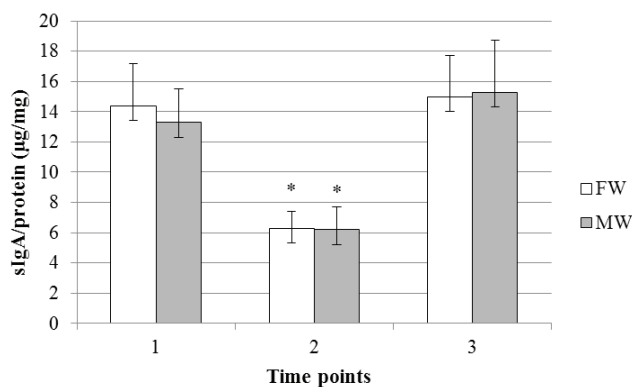
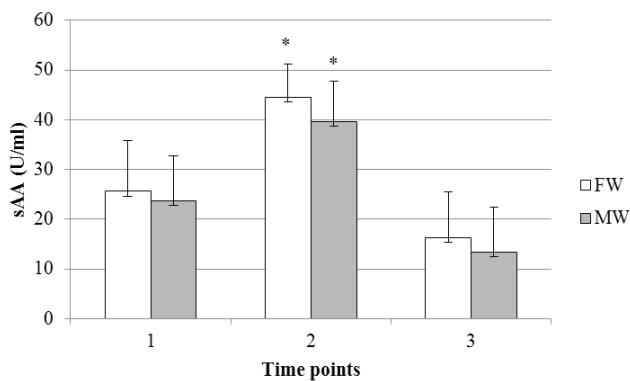


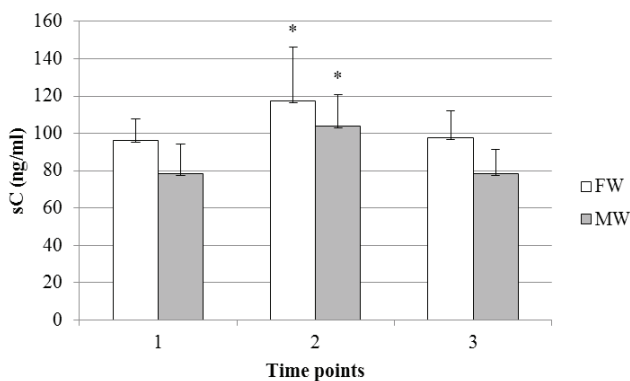
Figure 1. The sIgA/protein ratio at three time points in examined female (FM) and male wrestlers (MW) (mean \pm SD)

Significantly higher ($p < 0.05$) of α -amylase (Figure 2) activity, levels of salivary cortisol (Figure 3), serum cortisol (Figure 4), interleukin-6 (Figure 5) were also observed for both groups at the second time point compared with the first and the third. There were no significant differences in any examined marker between the first and the third time points in male as well as in female athletes. Regardless of the examined time point, concentrations of markers in serum and saliva and α -amylase activity were similar in female and male wrestlers. However, a tendency to lower sAA activity on the next morning after the tournament compared to basal activity of this enzyme (the first time point) was seen in both examined groups.



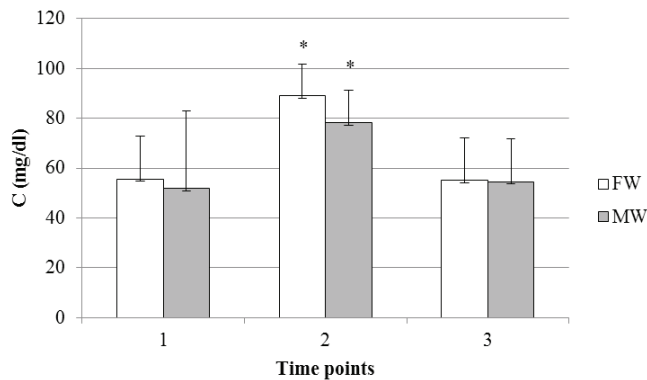
*Significantly higher compared to 1 and 3 time points

Figure 2. The sAA activity at three time points in examined female (FM) and male wrestlers (MW) (mean \pm SD)



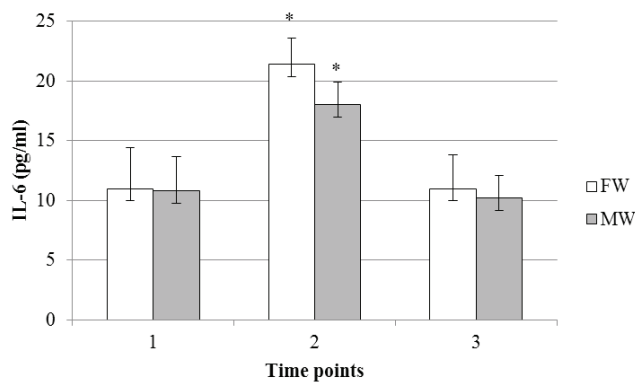
*Significantly higher compared to 1 and 3 time points

Figure 3. The salivary cortisol (sC) concentrations at three time points in examined female (FW) and male wrestlers (MW) (mean \pm SD)



* Significantly higher compared to 1 and 3 time points

Figure 4. The serum cortisol (C) concentrations at three time points in examined female (FW) and male wrestlers (MW) (mean \pm SD)



* Significantly higher compared to 1 and 3 time points

Figure 5. The interleukin-6 (IL-6) concentrations at three time points in examined female (FM) and male wrestlers (MW) (mean \pm SD)

Discussion

Over the last decade numerous studies have shown that short-term, intense physical activity can contribute to the occurrence of respiratory diseases, or to reduced resistance. The present study demonstrated a decrease in the ratio of sIgA/protein in both men and women, with the increase in the activity of α -amylase immediately after the last wrestling match. This is consistent with the data reported by Gill et al. (2013) who examined athletes during 230 km ultra-marathon conducted in high ambient temperatures. They found the 32% decline in salivary IgA concentrations, but also an 187% increase in α -amylase activity after the end of exercise. The same authors noted that the decrease in sIgA concentration is accompanied by the increase in compensatory α -amylase activity, which plays an important role as an antimicrobial protein. However, there are other studies, which demonstrated no

changes in the concentration of sIgA after exercise. Moreira et al. (2013) examined twenty basketball players before and after the official match and found that the effort expended by the players during the competition was too small to lead to changes in the sIgA concentration.

Increased activity of the sAA caused by exercise is short-lived, and this was confirmed in this study. Leicht et al. (2011), who studied a group of disabled athletes, also found increased activity of this enzyme immediately after exercise, but this activity decreased below the initial value after 30 minutes of recovery. Zauber et al. (2012) showed a significant increase in sAA activity in men and women after 1060-kilometer cycling compared to the regeneration phase, but the decrease in this enzyme activity was observed at rest (two days after the event). The present study indicated a tendency of decrease in the activity of sAA persisting to the next morning after competition in both male and female wrestlers.

The present study found a significant increase in the concentration of cortisol in blood and saliva immediately after the last tournament match, which was caused by both mental and physical stress. Importantly, the changes in concentration of cortisol in saliva are parallel to its concentration in serum, but the levels of this hormone in saliva are significantly higher than in serum. The study of Moreira et al. (2013) showed a significant increase of salivary cortisol concentration immediately after a basketball game, which was stressful enough to increase the levels of salivary cortisol at a constant level of sIgA. Kivlighan and Granger (2006) found a relationship between the concentration of α -amylase and salivary cortisol and observed the cortisol levels increase of 87% and α -amylase increase of 156% after a single short-ergometer exercise. However, the changes in cortisol levels are short-term as evidenced by Chatzinikolaou et al. (2014), who showed that serum cortisol levels dropped to baseline within 24 hours, which is also confirmed in the present study. In addition, Barbas et al. (2011), who measured serum cortisol concentration following each match during one-day wrestling tournament, demonstrated that just after one hour recovery (between matches) their levels returned to pre-match (basal) values.

It was found repeatedly that the concentration of IL-6 in serum increases during exercise (Febbraio and Pedersen 2002; Febbraio and Pedersen 2005; Fischer et al 2007; Nielsen et al. 2007; Pedersen et al. 2004) but it has been shown that changes in the levels of IL-6 occurring under the influence of physical activity are reversible and of short duration. Meckel et al. (2011) evaluated the impact of different types of sprint interval sessions on the balance of circulating inflammatory cytokines. For this purpose, twelve healthy outstanding young handball players aged 17–25 years were studied. Blood samples were collected before and one hour after the workout. These authors noted a significant increase in IL-6 after two training sessions, lasting only one hour after exercise. Similar results were obtained by Nivaldo et al. (2012), who tested a group of sixteen players before and immediately after the futsal match, and showed a significant increase of IL-6 in serum. In addition, Scherr et al. (2011) demonstrated that, after a marathon run, concentrations of IL-6 were increased and returned to the initial level within 72 hours. Barbas et al. (2011) showed that serum IL-6 concentration increased following all (five) wrestling matches during tournament, independently of their level before the match, but declined after 5–6 hours of recovery between the fourth and the fifth match.

Conclusions

Saliva is a convenient biological fluid to examine exercise-induced endocrine and immune systems response. One-day wrestling tournament induced a pro-inflammatory environment by altering cortisol and immune response. Therefore, female and male athletes had a higher, although short-lasting, risk of upper respiratory tract infection.

The tendency to lower α -amylase activities in the next morning compared to baseline values suggests that effort of wrestling matches may decrease antimicrobial potential of saliva. Post-tournament elevated levels of sC, C and IL-6 as well as the sIgA/protein ratio returned to baseline values by the next morning. In general, the changes of examined markers were short-lived, except for α -amylase activity, showing that wrestling matches during tournament had no sustained negative effects on endocrine and immune body systems.

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