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Effects of a Quasi-Formal Wrestling Match on the Humoral and Salivary Cortisol and Testosterone Levels of Elite and Ordinary Male Wrestlers

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ABSTRACT

Background & Objective: Researchers believe that long-term, high-intensity exercises may lead to the suppression of the immune system, whereas short-term, low-intensity exercises could strengthen the immune system. The present study aimed to compare the effects of wrestling competition on the changes in salivary immunoglobulin A (IgA), total protein, cortisol, and salivary testosterone in elite and ordinary male wrestlers.

Materials and Methods: This quasi-experimental study was conducted on 20 elite and ordinary male wrestlers selected via convenience targeted sampling. Subjects were divided into two groups of elite wrestlers (n=10) and ordinary wrestlers (n=10). Salivary samples were collected to determine the levels of salivary IgA, cortisol, and testosterone before the match, immediately after the first time, and immediately after the second time. Data analysis was performed using repeated measures to compare the intergroup and intergroup means at the significance level of $P<0.05$.

Results: A significant difference was observed in the stages before the competition, immediately after the first time, and immediately after the second time in terms of the salivary IgA and cortisol concentrations between the elite and ordinary wrestlers. Comparison of these changes indicated the significant reduction and increase of the baseline level, respectively. The peak of the changes was observed immediately after the second time.

Conclusion: According to the results, the immune system of the wrestlers could be affected by stress, as well as physical and psychological pressure, at the end of the match, thereby stimulating the immune system indicators in the bloodstream. This phenomenon may eventually expose athletes to the diseases of the upper respiratory tract.

Introduction

The human body is constantly exposed to the infectious microbial factors in the environment, such as viruses, bacteria, fungi, and parasites. These microorganisms have the potential for unrestrained birth, pathological damages, and the destruction of their host. In the past few decades, lymphocytes have been detected in the mucosa, under the mucous membrane of the gastrointestinal tract, and in the respiratory tract. A relatively new standpoint in this regard is the theory of the existence of a specific mucosal-specific immune system (1).

The mucosal immune system is the most important site for the production of immunoglobulin A (IgA), as well as an IgA secretion source (IgA-s). Salivary IgA is the only group of the antibodies that are actively secreted through the epithelial cells into the intestinal and respiratory tracts (2). IgA-s is an inherent component of the mucosal immune system. Furthermore, it is the leading line of defense and the first barrier against the entry, residence, and proliferation of pathogenic agents in the body, especially those that cause upper respiratory tract infections (3).

Cortisol is the primary catabolic hormone in the body. One of the functions of cortisol is the suppression of the immune system. Serum, plasma, and salivary cortisol levels increase during and after intense physical activity. Reduction of IgA-s levels has been attributed to the increased levels of cortisol since high cortisol affects B lymphocytes and reduces the production of immunoglobulins (4).

Cortisol has a catabolic function, while testosterone could be linearly enhanced in response to physical activity as an anabolic factor for stimulating skeletal muscle growth (5). Similar to cortisol, testosterone responds to the exercises involving a sudden, specific threshold. As a result, its concentration reaches plate linearly, which usually occurs at the end of the physical activity (6, 7). However, it should be noted that if low-intensity activities continue in the long run, they may lead to the significant increase in testosterone and cortisol (6, 7).

Several epidemiological studies have denoted that athletes, especially those involved with high-speed rates, are at the risk of infectious diseases, particularly upper respiratory tract infections (8-10). The humoral immune system is the foremost defensive line in the body, which is of paramount importance to the features of wrestling exercises, including the type of competition and physical and physiological characteristics of wrestlers (11, 12).

Wrestling is a fast sport involving endurance, strength, and remarkably intense physical activity, which may adversely affect the mucosal immune system of wrestlers, exposing them to a high risk of upper respiratory tract infections. In a study conducted on male wrestlers, the findings indicated that in the preceding season of the competition, IgG and IgA concentrations reduced by 40% and 30% during the league season, respectively (13).

In another research, Trochimiaket al. (2015) examined the salivary levels of antimicrobial and resting cortisol proteins in male and female wrestlers during a 12-week practice period. The saliva samples were collected at the beginning of preparation, after six weeks of preparation, and three weeks after the competitions. According to the findings, the third-stage levels of IgA and total protein were significantly lower in women compared to men. In addition, alpha amylase activity was reported to be lower in the third stage of sample collection compared to the control group (14). In this regard, Fisher et al. (2015) examined the reaction of salivary IgA in elite swimmers (10 males and eight females), claiming that IgA levels increased significantly in men after 61 hours of swimming. However, no significant change was reported in the IgA levels of between men and women after 90 minutes of endurance swimming (15).

Recently, the intensiveness of sports competitions and close performance of athletes to each other has led coaches to increase the frequency and timing of exercise sessions and their intensity. As a result, humeral immune responses have changed in intense workouts. Considering the nature of wrestling, which involves extremely physical contact, factors such as weight changes, muscle injuries, and maximum aerobic and anaerobic power distinguish wrestling from other sports. On the other hand, humoral safety plays a pivotal role in the fitness and durability of wrestlers before and during the competition season in terms of the features of wrestling exercises and physiological characteristics of wrestlers (13). Therefore, studying the effects of formal wrestling matches on the mucosal immune system of the athletes is of paramount importance.

Although some studies have been conducted in this regard, further investigation should be focused on ordinary subjects using serum sampling methods rather than salivary sampling, which is a noninvasive method, in elite athletes.

The present study aimed to review and compare the effects of a quasi-formal wrestling match on the humoral cortisol and salivary testosterone levels of elite and ordinary male wrestlers.

Materials and Methods

Study Subjects

This applied, quasi-experimental research was conducted based on the repeated measurements in two experimental groups, including the male wrestlers in the national league and ordinary wrestlers. Sample population consisted of 20 elite wrestlers (minimum of two years of regular league experience on the national level) and ordinary wrestlers (no experience in formal competitions), who were selected via convenience targeted sampling.

In the first stage, the nature, procedures, and objectives of the research were explained to the participants. Inclusion criteria for the elite wrestlers were a minimum of two years of participation in provincial and national leagues and regular attendance in trainings and competitions, and no experience in provincial and national competitions with a level of school skill for ordinary wrestlers. Other inclusion criteria of the study were favorable health status based on a health questionnaire, no drug use, and no smoking habits.

Participation in the research was voluntary, and written informed consent was obtained from all the subjects prior to the study. Participants were divided into two groups of elite wrestlers (n=10) and ordinary wrestlers (n=10).

Body Composition

A Seca stadiometer (Germany) was used to measure the height of the participants. The exact height was measured without shoes when the subject was standing and stretched on a device, so that the body weight would be evenly divided on both feet, and the eyes would be parallel to the horizon. At the extreme end of exhalation, the horizontal ruler of the device was placed on the head tangentially to create a vertical angle with the ruler. Height of the wrestlers was measured in centimeters.

A Beurer digital scale (model: PS06-PS07) was used to measure the weight of the eligible subjects. To do so, the subjects were without shoes with a hand wearing a lightweight sport outfit, standing on the scale to measure the body weight in kilograms. In order to measure the body mass index (BMI), the subjects were first weighed before the beginning of physical exercises. Afterwards, BMI was calculated by dividing the weight by square meter. In this formula, weight was measured in kilograms, height was measured in meters, and BMI was measured in kilograms per square meter.

Experimental Design

After dividing the subjects into the study groups, each wrestler combated all the members of the group. After a competition session with simulated conditions to official matches (the first three minutes, 30-second break, and the second three minutes), samples of IgA, cortisol, and salivary testosterone were obtained from the

subjects at the pre-match, immediately after the first three minutes, and immediately after the second three minutes.

At each stage of sampling, the subjects first washed their mouths with water and drank 200 milliliters of water to prevent dehydration. Salivary samples were placed into special tubes and preserved in a freezer compartment and frozen at the temperature of -20°C to be tested at appropriate times. The samples were frozen after each test and stored in the freezer.

After sampling, the salivary samples were transferred to the laboratory, and the required experiments and measurements were performed. Concentrations of the mentioned variables in the salivary samples were determined using the ELISA method and IBL commercial kit (Germany).

Statistical Analysis

Data analysis was performed in SPSS version 16. After verifying the normal distribution of the theoretical data, the Kolmogorov-Smirnov test and homogeneity of variances by the Levene's test were applied to compare the intra-group and inter-group means. In addition, inferential statistics (variance analysis with repeated measures) were used, and Bonferroni follow-up test was employed to compare the groups. In all the statistical analyses, P-value of less than 0.05 was considered significant.

Results

Characteristics of the elite and ordinary wrestlers are presented in Table 1. No significant differences were observed between the study groups in terms of age, height, weight, and BMI before the intervention (P>0.05).

According to the information in Table 2, comparison of the means with various stages of official wrestling competitions (before the match, immediately after the first time, and immediately after the second time), intra-group changes in salivary IgA (F=10.47; P=0.01), salivary cortisol (F=13.14; P=0.01), and salivary testosterone (F=1.98; P=0.18) were statistically significant in the elite male wrestlers. Moreover, the intra-group changes in salivary IgA (F=1.30; P=0.29), salivary cortisol (F=0.37; P=0.61), and salivary testosterone (F=1.51; P=0.25) were not statistically significant in the ordinary wrestlers.

In the inter-group comparison, a significant difference was observed in the mean changes in salivary cortisol (F=4.55; P=0.04). However, no significant differences were denoted in the salivary immunoglobulin variables (F=2.04; P=0.17) and salivary testosterone levels (F=0.05; P=0.81).

Discussion

According to the findings of the current research, the formal wrestling competition had a significant effect on the concentration of salivary IgA at the pre-match, immediately after the first time, and immediately after the second time in the elite wrestlers. In the case of the significant reduction of these changes compared to the baseline levels, the peak of the decline is observed immediately after the second time of the match. These findings are consistent with the results obtained by Moreira et al. (2013) (16), while inconsistent with the findings of Fisher et al. (2015) and Hejazi et al. (2012) (15, 17).

In their study, Moreira et al. (2013) reported that salivary cortisol levels were higher before and after the final volleyball match, whereas the IgA values were significantly lower in the final match, compared to the other stages (16). In addition, Fisher et al. (2015) evaluated the reaction of salivary IgA in elite swimmers (10 males and eight females), stating that IgA levels increased significantly in men after 61 hours of swimming. However, no significant changes were reported in IgA levels in male and female athletes after 90 minutes of endurance swimming (15).

On the other hand, Hejazi et al. (2012) evaluated the effects of preparation and the pre-competition phase on the levels of A, M, and G immunoglobulins in 13 elite mid-distance runners, concluding that 14 weeks of training and 12 training sessions per week led to a significant decrease in the IgM and IgG levels at the end of

the course, while no significant changes were denoted in IgA levels (17).

Research on the changes in the immune system factors has yielded controversial findings, which are often influenced by several factors, such as the age, gender, health status, and physical fitness of the subjects, as well as the duration, intensity, and type of the exercises; therefore, the contradiction in this regard could be justified. Considering the limited observations in wrestling, further research is required to thoroughly identify the mucosal immunity of wrestlers.

The response of the mucosal immune system to acute psycho-physiological stress has been shown to increase salivary immunoglobulin levels (18), while these levels may reduce due to chronic stress (19). The proposed hypotheses in this regard denote that the responsible mechanisms for the changes in salivary IgA include the changes in the level of oral mucosa due to intense breathing during exercise, inhibition of IgA secretion or producing the secretion unit responsible for the transmission of antibodies to the mucosa, and changes in the secretion of the cells in the IgA implantation in the mucosal areas in elite athletes.

Reduction of total IgA levels in oral mucosal surfaces after intensive exercise is due to the decreased concentration of IgA and the saliva flow. The adjustment of the saliva flow is complicated and involves the stimulation of the parasympathetic and sympathetic nervous system. Decreased sympathetic range reduces the volume or flow of the saliva by the restriction of the saliva or contraction of the blood vessels in the salivary glands. The intake of nervous stimulation from the higher centers of the nervous system seems to slow the saliva flow (e.g., in the presence of the psychological stress caused by official tournaments). However, tension hormones (e.g., catecholamines) do not seem to regulate the saliva flow, while they may sympathetically control the B cell migration that secretes IgA into the oral mucosa through blood vessels (20-22).

According to the present study, the formal wrestling competition had a significant effect on the salivary cortisol concentrations at the pre-match, immediately after the first time, and immediately after the second time in the elite wrestlers. If these changes significantly increase compared to the baseline levels, the peak of the increase is observed immediately after the second time.

The results of the present study are in congruence with the findings of Doan et al. (2007) and Edwards et al. (2006) (23, 24), while inconsistent with the studies by Ormsbee et al. (2013) and Moreira et al. (2009) (25, 26). It activates the physical and psychological pressures of the pituitary and adrenal axis, thereby increasing the levels of adrenocorticotropic hormone (ACTH) and cortisol. One of the main properties of cortisol is the suppression of the immune system and inhibition of the normal response of the immune system, which result in the gradual degradation of the lymphoid tissue, followed by the reduced production of antibodies and activities of eosinophilic, lymphocytic, and basophilic cells (17, 27).

Cortisol is a primary stress hormone, which is secreted in response to the physical and psychological pressures imposed on the organism through the cortical part of the adrenal glands and enhances the effects of catecholamines. Intense physical activity is considered to be the most important cortisol stimulant depending on the type, intensity, and duration of the activity (17, 28). Reduced blood glucose concentrations increase the circulation of cortisol, thereby triggering the hypothalamus-pituitary-adrenal axis and leading to ACTH release, which stimulates adrenal production and cortisol secretion. It is notable that the intensity of physical activity and the subsequent pressure mainly depend on the anaerobic threshold of the individual. Factors such as the increased threshold of stimulation and production of the

derivatives of biliary metabolism (e.g., lactate accumulation), pH reduction, and hypoxia have been reported to stimulate the hypothalamic-pituitary-adrenal axis to increase cortisol (17, 29, 30).

According to the findings of the current research, the formal wrestling competition had no significant effect on the testosterone concentrations in the elite and ordinary wrestler at the pre-match, immediately after the first time, and immediately after the second time. If these changes are lower than the baseline levels, they are not considered statistically significant.

Testosterone is an anabolic hormone that increases after intense, short-term exercises; however, it often decreases during long-term exercises, especially endurance training, as it is required during long-term endurance in order to maintain muscle performance in athletes (31). The mechanism of testosterone response to exercise remains unclear; some studies have attributed the increased concentration of testosterone to the aggressive nature of sports competitions (32).

Total salivary protein content represents the total amount of protein in the saliva. Increased total protein content following physical activity has been attributed to the reduction of saliva due to the increased ventilation of the lungs and evaporation of the water in the saliva. Increased secretion of protein into the salivary duct due to sympathetic stimulation is another cause of increased salivary protein, which may have affected the results of the present study (33). Increased testosterone concentrations in the current research and previous studies in this regard might be associated with the aggressive nature of the wrestling competition and its impact on the athletes.

Conclusion

According to the results, physical activity is a significant influential factor in the defense mechanisms of the body depending on the exercise intensity, duration, and planning, as well as the fitness status of the individual. Our findings also indicated that official wrestling competitions could affect the investigated variables; for instance, they could significantly decrease salivary IgA, increase cortisol, and reduce salivary testosterone in male wrestlers.

Despite the discrepancies in the results of the studies in this regard, researchers believe that long-term, high-intensity exercises suppress the immune system, while short-term, low-intensity exercises could strengthen the immune system. In general, it could be concluded that official competitions between elite male wrestlers may weaken their defense mechanisms. Therefore, it is recommended that trainers, coaches, and sports practitioners take proper measures to improve the immune system of athletes during long-term, high-intensity exercises.

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Table 1. Baseline Characteristics of Participants

Groups	Variations (M±SD)			
	Age (year)	Height (m)	Weight (kg)	Body Mass Index (kg/m^2)
Elite	20.70±1.94	1.73±2.11	63.30±1.63	20.98±0.69
Ordinary	21.40±2.06	1.75±3.30	62.80±2.25	20.45±1.11

Table 2. Intra-group and Inter-group Comparison of Salivary Immunoglobulin A, Cortisol, and Testosterone Levels in Elite and Ordinary Wrestlers

Variables	Groups	Before Competition M±SD*	Immediately after First Time M±SD*	Immediately after Second Time M±SD*	Variations	
					Intra-group Comparison	Inter-group Comparison
					P-value	P-value
Immunoglobulin A (ng/ml)	Elite	37.16±6.69	28..23±4.00	27.62±8.73	0.01‡	0.17
	Ordinary	38.57±1.03	35.24±8.67	34.93±1.42	0.29	
Cortisol (ng/ml)	Elite	4.80±1.75	8.12±3.72	9.31±3.09	0.01‡	0.04‡
	Ordinary	5.55±1.83	5.64±1.94	5.06±2.42	0.61	
Testosterone (ng/ml)	Elite	309.97±15.93	318.23±16.94	267.97±13.29	0.18	0.81
	Ordinary	245.56±14.06	307.56±21.20	292.39±19.80	0.25	

**Data presented as mean±standard deviation; ‡: significant mean difference at 0.05*