The effect of exercise during sport training on levels of salivary diagnostic markers

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SUMMARY
Introduction/Objective The aim of this study was to determine the changes in concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in saliva samples collected before, immediately after, and 30 minutes after physical activity performed during basketball and mixed martial arts (MMA) training.

Methods Twenty-two athletes, 11 basketball players and 11 MMA fighters, 18 men and four women, aged 15–24 years, participated in the study. Saliva samples were collected using sterile saliva containers (Salivette®) from all participants before training (sample 1), immediately after (sample 2), and 30 minutes after training (sample 3). The levels of all investigated biomarkers were measured spectrophotometrically using a biochemical analyzer.

Results Statistically significant differences were present among samples 1, 2, and 3 in the concentrations of urea, AST, and CK in samples collected from MMA fighters (Friedman test). Among three samples taken from basketball players, the significant differences were not observed for the analyzed parameters. When concentrations of all diagnostic markers were compared between basketball and MMA independently for samples 1, 2, and 3, statistically significant differences (Mann–Whitney U-test) existed in concentrations of urea, uric acid, proteins, and AST.

Conclusion Based on the results of the present study, the influence of the exercise on the levels of salivary diagnostic markers, such as urea, AST, and CK, is more evident during MMA than basketball training. Saliva composition of MMA fighters and basketball players differ in terms of levels of urea, uric acid, proteins, and AST.

Keywords: exercise; sport training; saliva diagnostic markers; basketball; mixed martial arts

INTRODUCTION
Physical activity, unique physiological stress, triggers a systematic series of neuroendocrine and immune events directed at bringing the system back to a state of homeostasis. Various physiological changes occurring in the human body during physical exercise contribute to accommodating the increase in physiological demands. Two major neuroendocrine stress response arms are the hypothalamic–pituitary–adrenal and the sympathetic–adrenomedullary axis, with both axes modulating the function of immune system [1, 2]. Immune and stress responses work together to combat exercise stress.

While blood samples have historically been used to measure numerous parameters, indicators of physiological and pathological processes in the organism, many of them could be analyzed in a much easier, less complex, and completely non-invasive way in the saliva samples [3, 4]. Saliva has been used to examine hydration, electrolyte status, stress, and immune responses during and after physical activity [5, 6, 7].

The salivary glands are under the control of the autonomic nervous system, parasympathetic cholinergic nerves and sympathetic adrenergic nerves. The type of activated autonomic receptor, salivary flow rate, and intensity and duration of stimulation to the glands can influence saliva composition. During prolonged and intense exercise, due to the increased sympathetic stimulation, reduced salivary flow rate is expected. Qualitative and quantitative changes are described by the increased concentration of total protein, cortisol, and hormones in saliva during the stressful period, as well as by the alterations in ionic composition of the saliva [5–8]. Immediately after intense physical activity, saliva remains viscous for some time, although the control of saliva secretion is no longer under sympathetic nervous system. These changes are primarily explained by mouth breathing during physical activity, dehydration of the organism, and increased secretion of salivary mucin [6–9]. After physical
activity, the secretion of saliva is under control of the parasympathetic nervous system, which is active during the period of rest, and as these two systems have an antagonistic effect, increased secretion of the saliva, decreased protein concentration, and increased serosity are present.

Physical activity of any type may have implications for the immune system [10]. Changes in salivary secretion and composition and the alteration of the immune function that occur during intense physical activity may lead to the development of pathological changes. The occurrence of upper respiratory tract infections could be associated with systemic changes due to reduced immune response, as well as the lack of protective role of saliva in athletes due to reduced lubrication and IgA concentration [11, 12].

By measuring the levels of certain biomarkers in saliva samples, it is also possible to monitor changes in other organs whose functions may be affected by the intense physical activity. Among these biomarkers, the enzymes creatine kinase (CK), lactate dehydrogenase (LDH), or aspartate aminotransferase (AST) stand out as parameters that determine skeletal muscle injury and tissue damage in the muscles [13]. Previous studies reported changes in the levels of AST, CK, and LDH in saliva samples after intense exercise during different sports [14, 15].

The aim of this study was to determine the changes in concentrations of urea, creatinine, uric acid, proteins, AST, CK, and salivary amylase in saliva samples collected before, immediately after, and 30 minutes after physical activity performed during basketball and mixed martial arts (MMA) training. The null hypotheses were the following: (1) there are no statistically significant differences in the concentration of the mentioned biomarkers in saliva samples collected before, immediately after, and 30 minutes after physical activity performed during basketball and MMA training. The null hypotheses were the following: (1) there are no statistically significant differences in the concentration of the mentioned biomarkers in saliva samples collected before, immediately after, and 30 minutes after training, regardless of the sport; (2) there are no statistically significant differences in the concentration of the biomarkers in saliva samples collected before, immediately after, and 30 minutes after intense physical activity, within each sport separately; and (3) there are no statistically significant differences in the concentration of the biomarkers in saliva samples collected before, immediately after, and 30 minutes after training, between the two sports, for all three samples separately.

METHODS

Participants

Twenty-two athletes, 11 basketball players and 11 MMA fighters, 18 men and four women, aged 15–24 years, participated in the study. Basketball players worked out five times a week, while MMA fighters worked out three times a week. The duration of the training for basketball players was two hours, while for MMA fighters it was one hour and 30 minutes. Distribution of the participants and their characteristics among the two groups is shown in Table 1.

All participants were given verbal and written explanation of the purpose and the protocol of the research previously approved by the institutional ethics committee, and their written informed consent for participation in the study was obtained. Main anamnestic data collected from the athletes indicated that all the participants were healthy, they did not suffer from any chronic disease, they had adequate oral hygiene habits, at least five balanced meals per day, and they all declared themselves as non-smokers. Also, basic dental examinations with a mirror and a probe were performed, and it was established that the participants did not have active pathological processes in the mouth.

Prior to sample collection, all the participants were familiarized with the experimental protocol, they were explained the rules of behavior, and shown the correct technique for saliva specimen collection. According to the instructions, they had to brush their teeth at least 30 minutes before their scheduled training and then refrain from taking food, caffeine, alcohol, tobacco, chewing gums, juice, and energy drinks. Consuming water before and in-between sample collection was allowed.

Saliva samples

Saliva samples from all the participants were collected before training (sample 1), immediately after (sample 2), and 30 minutes after training (sample 3). For each sample, the athletes were asked to wash their hands and they were each given a sterile saliva container (Salivette®, Sarstedt, Germany) containing a sterile plain cotton swab. They were asked to open the lid of the container, take the cotton swab, and put it under the tongue for 3 minutes, while performing minimal orofacial movement. The cotton swabs were then placed back into Salivette® containers, the lid was closed, and each container was properly labeled. Saliva samples were immediately transferred to the laboratory for centrifugation (4200 × g, 10 minutes) and stored at the appropriate temperature (-20°C) before analyses were performed.

Saliva analyses

The levels of all investigated parameters (urea, creatinine, uric acid, proteins, AST, CK, and salivary amylase) were measured spectrophotometrically using a biochemical analyzer Rayto 1904-C (Rayto Life and Analytical Sciences Co., Ltd, Shenzhen, China). Appropriate reagents were used to obtain colored products (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany).

Table 1. Distribution of the participants and their characteristics among the groups

| Sport     | n   | Sex     | Age (years) (Mean ± SD) | BMI (Mean ± SD) | Number of trainings per week | Training duration |
|-----------|-----|---------|-------------------------|----------------|-----------------------------|------------------|
| Basketball| 11  | 11 males| 16.54 ± 1.03            | 21.7 ± 1.49    | 5                           | 120 minutes      |
| MMA       | 11  | 7 males 4 females | 21.82 ± 1.83            | 23.75 ± 2.73   | 3                           | 90 minutes       |
| Total     | 22  | 18 males 4 females | 19.18 ± 3.06            | 22.73 ± 2.38   | -                           | -                |

MMA – mixed martial arts
using the same methodology as for the serum measurements. The absorbances of the obtained colored compounds at certain wavelengths were measured, and then converted into quantitative concentration, mass concentration, or enzyme activity, depending on the biomarker.

Statistical analysis

The concentrations of the investigated biomarkers in the samples collected at three different time points relative to the physical activity (sample 1, sample 2, and sample 3) were statistically analyzed with Friedman test, regardless of the sport (all basketball and MMA samples together) and within each sport separately (basketball and MMA samples separately). Wilcoxon signed ranks test was used for post-hoc between-group comparisons when significant differences among three samples were detected by the Friedman test. Mann–Whitney U-test was used to assess the differences in the concentrations of the investigated parameters in samples 1, 2, and 3 independently, between two sports (basketball vs. MMA). The data were statistically analyzed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA), and significance level was set at p < 0.05 in all analyses.

RESULTS

Concentrations of urea, creatinine, uric acid, proteins, AST, CK, and salivary amylase in samples 1, 2, and 3 did not statistically differ among each other when all collected saliva samples were compared regardless of the sport (Friedman test, Table 2).

When concentrations of examined parameters were statistically analyzed among samples 1, 2, and 3 separately for basketball and MMA, there were no significant differences for any of the parameters in the samples collected from basketball players (Friedman test, Table 3), whereas statistically significant differences were present in the concentrations of urea, AST, and CK in the samples collected from MMA fighters (Friedman test, Table 4). Between-group comparisons revealed that the concentration of urea was significantly higher in sample 3 than in samples 1 and 2, while concentrations of AST and CK were significantly different only between samples 2 and 3 (Wilcoxon signed ranks test, Table 4).

Additionally, concentrations of all examined parameters were compared between basketball and MMA (Mann–Whitney U-test) independently for samples 1, 2, and 3. In samples taken before training (sample 1) statistically significant differences existed in concentrations of urea (p = 0.010), proteins (p = 0.023) and AST (p = 0.047) between basketball and MMA samples. Immediately after training (sample 2), statistically significant differences were present in concentrations of urea (p = 0.000), proteins (p = 0.019), and AST (p = 0.005) between basketball and MMA samples. In samples taken 30 minutes after training (sample 3), statistically significant differences were present in concentrations of uric acid (p = 0.013), proteins

Table 2. Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in samples 1, 2, and 3, taken from MMA fighters and basketball players, statistically analyzed with Friedman test, Table 2.

|          | MMA fighters (n = 11) | Basketball players (n = 11) | p-value (Friedman test) |
|----------|-----------------------|-----------------------------|-------------------------|
| Urea (mmol/l) | 4.99 ± 2.273          | 4.99 ± 2.273                | 0.103                   |
| Creatinine (μmol/l) | 23.24 ± 6.331          | 23.24 ± 6.331               | 0.643                   |
| Uric acid (μmol/l) | 250.82 ± 82.014        | 268.54 ± 109.297            | 0.441                   |
| Proteins (g/l) | 1.71 ± 1.181           | 1.16 ± 0.612                | 0.294                   |
| AST (U/l) | 19.73 ± 19.942         | 16 ± 13.550                 | 0.461                   |
| CK (U/l) | 4.59 ± 6.741           | 3.12 ± 2.982                | 0.695                   |
| Salivary amylase (U/ml) | 143.91 ± 94.159        | 147.82 ± 89.334             | 0.234                   |

Table 3. Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in samples 1, 2 and 3, taken from basketball players before, immediately after, and 30 minutes after training, respectively.

Table 4. Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (CK), and salivary amylase in samples 1, 2 and 3, taken from MMA fighters before, immediately after, and 30 minutes after training, respectively.

The results are presented as mean values ± standard deviation; values in bold indicate statistically significant differences relative to the corresponding values in the samples collected from the same sport, sample, and time point.

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and salivary IgA secretion rate, whereas such differences in osmolality, salivary amylase activity, and secretion rate was noticed in females, as there were significant increases in response to exercise stress [15, 20]. A greater response to exercise stress is probably closer to the anaerobic threshold level. It is well-known that saliva also contains urea, creatinine, and other markers of muscle damage. Studies have shown that the salivary concentrations of these markers are useful for the assessment of renal function, and one study investigated them as possible markers for periodontal disease [23, 24]. To the best of the authors’ knowledge, the assessment of salivary concentration of these parameters has not been used in relation to the physical activity, sport, and exercise. While the levels of creatinine did not show significant changes among the collected samples and groups, concentration of urea in MMA samples 1 and 2 was significantly lower than in sample 3, and it was also lower in MMA samples 1 and 2 compared to that of basketball samples 1 and 2. These differences could probably be associated with urea excretion through sweat and individual characteristics that affect sweating.

It was expected that exercise would cause an increase in the concentration of uric acid, a salivary antioxidant, as antioxidant responses are promoted by physical activity and the antioxidant profile of saliva samples showed to be very similar to that of plasma [25]. The results of this study showed that concentration of uric acid was higher in samples taken after training than in samples taken before training, but the difference was not statistically significant in any of the analyses. However, a significantly higher concentration of uric acid 30 minutes after training observed in the saliva of basketball players than in that of MMA fighters suggests that activities similar to basketball may lead to more pronounced antioxidant responses and consequently to the physiological processes related to redox. It may also be because basketball players trained more often, they were probably in better physical shape, and they were younger than MMA fighters that participated in this study.
Lack of significant difference in the concentration of some markers before and after training could be explained by an early activation of stress response that is not directly related to the physical activity during training, but to the research itself, i.e., excitement or fear of the unknown which participants may have had upon entering the study. On the other hand, sample 1 was taken prior to training, but after participants' arrival at the training site, hence one should have in mind that they had certain activity (e.g., walking, fast walking, public transportation) during arrival, so sample 1 could not be entirely considered a sample at complete rest.

CONCLUSION

In conclusion, the influence of the exercise on the levels of salivary diagnostic markers, such as urea, AST and CK, is more evident during MMA than basketball training. Saliva composition of MMA fighters and basketball players differ in terms of levels of urea, uric acid, proteins, and AST composition. Saliva may be an alternative and noninvasive tool in sports medicine for the study of salivary proteins, stress and immune markers, antioxidants, and muscle damage enzymes in different physical exercise protocols.

Conflict of interest: None declared.

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Утицај физичке активности током спортског тренинга на ниво дијагностичких маркера пљувачке

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САЖЕТАК

Увод/ЦиљЦиљ ове студије био је да се утврде промене у концентрацији урее, креатинина, мокраћне киселине, протеина, аспартат-аминотрансферазе (АСТ), креатин-киназе (КК) и саливарне амилазе у узорцима пљувачке прикупљеним пре, непосредно након и 30 минута након физичке активности која се изводила током тренинга кошарке и мешовитих борилачких вештина (ММА).

Методе У истраживању је учествовало двадесет двоје спортиста, 11 кошаркаша и 11 ММА бораца, 18 мушкараца и четири жене, старости 15–24 године. Узорци пљувачке сакупљани су у стерилне епрувете за пљувачку (Salivette®) од свих учесника пре тренинга (узорак 1), непосредно након (узорак 2) и 30 минута након тренинга (узорак 3). Нивои свих испитиваних биомаркера измерени су спектрофотометријском методом у биохемијском анализатору.

Резултати Статистички значајне разлике биле су присутне међу узорцима 1, 2 и 3 у концентрацијама урее, АСТ и КК у узорцима прикупљеним од ММА бораца (Фридманов тест). Међу три узорка узета од кошаркаша нису уочене статистички значајне разлике у анализираним параметрима. Када су упоређиване концентрације свих дијагностичких маркера између кошарке и ММА, независно за узорке 1, 2 и 3, постојале су статистички значајне разлике (Ман–Витни U тест) у концентрацијама урее, мокраћне киселине, протеина и АСТ.

Закључак На основу резултата ове студије, утицај физичке активности на ниво дијагностичких маркера пљувачке, као што су уреа, АСТ и КК, израженији је код ММА у односу на кошаркашке тренинг. Састав пљувачке ММА бораца и кошаркашке маркерна разликује се у погледу нивоа урее, мокраћне киселине, протеина и АСТ.

Кључне речи: физичка активност; спортски тренинг; дијагностички маркери пљувачке; кошарка; мешовите борилачке вештине