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Paper Accepted*

ISSN Online 2406-0895

Original Article / Оригинални рад

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

Утицај физичке активности током спортског тренинга
на ниво дијагностичких маркера пљувачке

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Received: September 13, 2021

Accepted: November 8, 2021

Online First: November 17, 2021

DOI: <https://doi.org/10.2298/SARH210913095B>

* **Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy-edited and/or formatted in the publication house style, and the text may be changed before the final publication.

Although accepted papers do not yet have all the accompanying bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: the author's last name and initial of the first name, article title, journal title, online first publication month and year, and the DOI; e.g.: Petrović P, Jovanović J. The title of the article. Srp Arh Celok Lek. Online First, February 2017.

When the final article is assigned to volumes/issues of the journal, the Article in Press version will be removed and the final version will appear in the associated published volumes/issues of the journal.

The date the article was made available online first will be carried over.

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

Утицај физичке активности током спортског тренинга на ниво дијагностичких маркера пљувачке

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 mixed martial arts (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

САЖЕТАК

Увод/Циљ Циљ 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).

Метод У истраживању је учествовало двадесет двоје спортиста, 11 кошаркаша и 11 ММА бораца, 18 мушкараца и 4 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 (Friedman test). 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 (Mann-Whitney U тест) у концентрацијама урее, мокраћне киселине, протеина и АСТ.

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

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

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-adreno-medullary 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 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 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 period of rest, and as these two systems have an antagonistic effect, increased secretion of saliva is present, decreased protein concentration and increased serosity.

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 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, 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. The null hypotheses were: (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 two sports, for all three samples separately.

METHODS

Participants

Twenty-two athletes, 11 basketball players and 11 mixed martial arts (MMA) fighters, 18 men and 4 women, aged 15–24 years, participated in the study. Basketball players worked out 5 times a week, while MMA fighters worked out 3 times a week. The duration of the training for basketball players was 2 hours, while for MMA fighters it was 1 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 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 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 (4,200 g, 10 min) 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, China). Appropriate reagents were used to obtain colored products (Human, Germany), 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 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). Data were statistically analyzed using IBM SPSS Statistical software, and significance level was set at $P < .05$ in all analyses.

RESULTS

Concentrations of urea, creatinine, uric acid, proteins, aspartate aminotransferase (AST), creatine kinase (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 ($p=.010$), proteins ($p=.023$) and AST ($p=.047$) between basketball and MMA samples. Immediately after training (sample 2) statistically significant differences were present in concentrations of urea ($p=.000$), proteins ($p=.019$) and AST ($p=.005$) between basketball and MMA samples. In samples taken 30 minutes after training (sample 3) statistically significant differences were present in concentrations of uric acid ($p=.013$), proteins ($p=.002$), and AST ($p=.002$). Concentrations of other examined parameters did not significantly differ between two sports.

DISCUSSION

Lack of statistically significant differences in the concentration of any of the parameters among saliva samples collected before, immediately after and 30 minutes after training, when all participants were analyzed, regardless of the sport, led to the acceptance of the first null hypothesis. The second null hypothesis was accepted for basketball, as no statistically significant difference was detected in the concentration of any of the parameters among three saliva samples, while the second null hypothesis was rejected for MMA, where statistically significant differences were present in the concentrations of urea, AST and CK among samples 1, 2 and 3. The third null hypothesis was also rejected, since significant differences existed in the concentrations of urea, uric acid, proteins, and AST between basketball and MMA samples.

In the present study, there were no statistically significant differences in the concentrations of salivary amylase and proteins in the total sample, nor when two sports were analyzed separately, which is in line with some previous findings [9]. Nevertheless, opposing results have also been reported [16]. Higher levels of these biomarkers were expected after

exercise, considering that their secretion is predominantly controlled by the sympathetic nervous system activated in stress [5, 6]. It could be assumed that sport trainings in this study had no significant effect on the protein and salivary amylase concentrations because the exercise intensity was below the anaerobic threshold, when salivary amylase and proteins were proved to increase [17]. It should be noted that the level of proteins in the MMA group was significantly higher than that in basketball group in all three samples, suggesting that MMA training is probably closer to the anaerobic threshold level.

Higher levels of serum enzymes CK, AST and LDH could be used as indirect markers of muscle damage, and in apparently healthy subjects they may be correlated with physical training status [13, 18, 19]. The results of this study showed that in the MMA group, AST and CK salivary levels increased immediately after training and their returned to their initial values in samples taken 30 minutes after, suggesting that this change is transitory.

Interestingly, the levels of salivary AST were significantly higher in all three MMA saliva samples than in basketball samples. Based on this finding it could be assumed that MMA fighters are more likely to have permanent increase of AST in saliva. Furthermore, analyses of key salivary electrolytes, stress and immune markers, and muscle damage markers in the saliva samples showed that male and female organisms have different response to exercise stress [15, 20]. A greater response to exercise stress was noticed in females, as there were significant increases in osmolality, salivary amylase activity and secretion rate and salivary IgA secretion rate, whereas such differences between rest and exercise were not present for any salivary analytes in males [20]. Also, the three enzymes indicating muscle damage (AST, LDH and CK) showed different responses in men and women playing rugby, with AST showing the most significant variations, which were more pronounced in men than in women [15]. In the present study, around 18% were females (4 out of 22) in the total sample, and all of them were in the MMA group, where they represented around 36% (4 out of 11), while in the basketball group all participants were males, which could explain the differences in the results among the studies. Further research should focus on the investigation of the influence of gender and type of sport on these salivary biomarkers at rest and during exercise.

Differences in the levels of biomarkers between basketball and MMA samples observed in the present study may be due to several other reasons, such as younger age, lower BMI, more frequent and longer trainings of basketball players than those of MMA fighters, as well as due

to basic differences in these two sports. Basketball is a team play with a ball characterized mainly by running and shooting, while MMA is a combat sport with two distinct categories, grappling and striking. Therefore, these two sports develop different physical and physiological profiles, in terms of aerobic and anaerobic capacities, strength, kinematic and neuromuscular variables [21, 22].

It is well-known that saliva also contains urea, creatinine, and other markers of renal function. Studies have shown that the salivary concentrations of these markers are useful for the assessment of kidney 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 sample 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 basketball kind of activities 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 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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Paper accepted

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

Sport	n	Sex	Age (years) (Mean \pm SD)	BMI (Mean \pm SD)	Number of trainings per week	Training duration
Basketball	11	11 males	16.54 \pm 1.03	21.70 \pm 1.49	5	120 minutes
MMA	11	7 males 4 females	21.82 \pm 1.83	23.75 \pm 2.73	3	90 minutes
Total	22	18 males 4 females	19.18 \pm 3.06	22.73 \pm 2.38	-	-

MMA – mixed martial arts

Paper accepted

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 all participants before, immediately after and 30 minutes after training, respectively

All participants (N=22)	Sample 1 Before training	Sample 2 Immediately after training	Sample 3 30 minutes after training	p-value (Friedman test)
Urea (mmol/l)	4.98 ± 2.105	3.85 ± 2.398	4.89 ± 1.839	0.202
Creatinine (µmol/l)	23.03 ± 5.030	22.56 ± 4.465	23.49 ± 4.601	0.989
Uric acid (µmol/l)	212.23 ± 76.998	233.82 ± 92.464	246.04 ± 129.616	0.170
Proteins (g/l)	2.74 ± 2.008	6.19 ± 8.379	6.24 ± 8.268	0.795
AST (U/l)	23.27 ± 15.520	25.59 ± 18.477	20.64 ± 16.580	0.063
CK (U/l)	4.48 ± 5.577	4.64 ± 6.721	2.83 ± 3.339	0.086
Salivary amylase (U/ml)	148.68 ± 102.448	159.54 ± 118.983	169.18 ± 117.247	0.351

The results are presented as mean values ± standard deviation

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

Basketball players (n = 11)	Sample 1 Before training	Sample 2 Immediately after	Sample 3 30 minutes after training	p-value (Friedman test)
Urea (mmol/l)	4.99 ± 2.273	5.74 ± 1.374	5.01 ± 1.126	0.103
Creatinine (µmol/l)	23.24 ± 6.331	22.37 ± 3.430	21.66 ± 3.663	0.643
Uric acid (µmol/l)	250.82 ± 82.014	268.54 ± 109.297	290.36 ± 105.489	0.441
Proteins (g/l)	1.71 ± 1.181	1.16 ± 0.612	1.13 ± 0.683	0.294
AST (U/l)	19.73 ± 19.042	16.00 ± 13.550	12.64 ± 10.585	0.461
CK (U/l)	4.59 ± 6.741	3.12 ± 2.982	3.76 ± 3.111	0.695
Salivary amylase (U/ml)	143.91 ± 94.159	147.82 ± 89.334	170.54 ± 128.543	0.234

The results are presented as mean values ± standard deviation; the grey highlighted values indicate the statistically significant difference with the corresponding values in the MMA samples shown in Table 4

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

MMA fighters (n = 11)	Sample 1 Before training	Sample 2 Immediately after training	Sample 3 30 minutes after training	p-value (Friedman test)
Urea (mmol/l)*	2.97 ± 1.377 ^B	1.96 ± 1.536 ^B	4.78 ± 2.410 ^A	0.020
Creatinine (µmol/l)	22.82 ± 3.600	22.75 ± 5.480	25.32 ± 4.868	0.529
Uric acid (µmol/l)	173.64 ± 49.472	199.09 ± 57.923	201.73 ± 140.826	0.148
Proteins (g/l)	3.78 ± 2.171	11.22 ± 9.564	11.36 ± 9.245	0.175
AST (U/l)*	26.82 ± 10.750 ^{AB}	35.18 ± 18.192 ^A	28.64 ± 18.013 ^B	0.026
CK (U/l)*	4.37 ± 44.454 ^{AB}	6.15 ± 8.994 ^A	1.90 ± 3.439 ^B	0.021
Salivary amylase (U/ml)	153.45 ± 114.563	171.27 ± 146.447	167.82 ± 111.091	0.534

The results are presented as mean values ± standard deviation; asterisk (*) indicates the presence of statistically significant difference; different superscript letters indicate statistically significant difference among the values presented in the same row; the grey highlighted values indicate the statistically significant difference with the corresponding values in the basketball samples shown in Table 3