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Original Article

Exercise affects salivary biomarkers of creatine metabolism in healthy adults

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ABSTRACT

Background: We monitored changes in salivary creatine pre- and post-high-intensity exercise in young adults while also investigating the potential correlation between salivary and serum creatine levels.
Method: Saliva and serum samples were collected before and immediately after an incremental running-to-exhaustion treadmill test in fifteen young adults (mean age [23.9 ± 2.9] years, eight females), with samples analyzed for guanidinoacetic acid, creatine, and creatinine using a liquid chromatography–tandem mass spectrometry method.
Results: Following exercise, there was a substantial elevation in salivary creatine levels from (17.5 ± 14.2) μmol·L^{-1} to (43.6 ± 30.4) μmol·L^{-1} ($p < 0.001$), coupled with a significant increase in salivary creatinine from (11.3 ± 5.8) μmol·L^{-1} to (17.0 ± 9.3) μmol·L^{-1} ($p = 0.04$). In contrast, serum creatine levels were unaffected by exercise ($p = 0.80$), while creatinine levels exhibited a strong tendency to decrease post-exercise (from [81.8 ± 17.5] μmol·L^{-1} to [73.1 ± 11.6] μmol·L^{-1}; $p = 0.06$). A comparison of the slopes of the two regression lines (saliva *vs.* serum) revealed significant differences for both creatine ($p = 0.01$) and creatinine ($p = 0.03$).
Conclusions: The above findings suggest a potential difference in the dynamics of creatine metabolites in these two bodily fluids, both pre and post-exercise.

1. Introduction

During physical exertion, particularly during high-intensity exercise, the body's energy requirements escalate. Creatine, predominantly stored in skeletal muscles, plays a crucial role in supporting cellular energy production during heavy exercise by maintaining adenosine triphosphate (ATP) levels. This process can lead to the well-documented temporary depletion of muscle creatine stores[1]; however, there is limited information available regarding exercise-induced changes in creatine metabolism in other tissues. High-intensity exercise could result in fluctuations in creatine levels in bodily fluids such as serum and saliva. For example, an acute session of exhaustive exercise induces transient changes in circulating biomarkers of creatine metabolism,[2–4] suggesting a significant exercise-induced disruption in bioenergetics. Using blood as a sample for monitoring creatine biodynamics is not always feasible due to concerns regarding invasiveness, hygiene, and the potential infection risks associated with its collection and handling.[5] In contrast, saliva appears relatively clean, and the samples can be quickly and noninvasively collected and easily stored.[6] Still, no study so far has evaluated whether exercise affects salivary biomarkers of creatine metabolism and do creatine levels in saliva mirror those found in serum before and after exercise. The primary objective of this study was to observe the variations in salivary creatine levels before and after a single session of high-intensity exercise among young adults. Additionally, the study aimed to assess the potential correlation between salivary creatine levels and those present in the serum of the study participants. We hypothesized that high-intensity exercise induces transient changes in salivary creatine levels, with fluctuations that correspond to alterations observed in serum creatine levels before and after exercise. These variations in salivary creatine could potentially serve as a non-invasive biomarker for monitoring exercise-induced shifts in creatine metabolism within the human body.

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Abbreviations

ATP	adenosine triphosphate
BMI	body mass index
GAA	guanidinoacetic acid

2. Methods

2.1. Ethical approval

Ethical clearance for conducting the study was obtained from the local Institutional Review Board at the University of Novi Sad (Approval # 49-03-14/2023-1), with the research adhering to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants.

2.2. Participants

Fifteen young adults (mean age [23.9 ± 2.9] years, eight females) provided informed written consent to participate voluntarily in this study. Participants were required to meet specific inclusion criteria, including an age range of 18.0–29.9 years, a body mass index (BMI) between 18.5 and 24.9 $kg{\cdot}m^{-2}$, a non-vegetarian diet, and a minimum of three years of training experience as collegiate athletes, engaging in at least 5 h of structured exercise per week. Exclusion criteria encompassed the presence of major chronic diseases or acute medical conditions, recent use of dietary supplements (within four weeks prior to study enrollment), unwillingness to comply with follow-up assessments, and concurrent participation in other clinical trials.

2.3. Experimental protocol

During the laboratory visit, participants were subjected to an incremental running-to-exhaustion treadmill test. The test protocol began with a 3-min warm-up walk at 5 $km{\cdot}h^{-1}$, followed by running at 8 $km{\cdot}h^{-1}$. The workload was progressively increased by 1.5 $km{\cdot}h^{-1}$ every 60 s until the point of exhaustion was reached. A specimen of unstimulated whole saliva (approximately 0.5 mL) was collected from beneath the tongue using a disposable Pasteur pipette. Saliva collection took place ~30 min before exercise after mouth cleansing, and immediately after exercise. After protein precipitation with methanol, the saliva sample was centrifuged at 6 000 rpm. The supernatant was dissolved in the mobile phase (80% acetonitrile and 20% 25 mM ammonium formate, pH = 3.3) and analyzed at a flow rate of 0.3 $mL{\cdot}min^{-1}$. Blood samples were collected from the antecubital vein using a gel vacutainer both before and after exercise. The samples were then centrifuged within 10 min at 3 000 *g* to isolate the serum. The serum and salivary samples were stored at −80 °C and subsequently analyzed for biochemical markers following the completion of the trial. Both samples underwent analysis for guanidinoacetic acid (GAA, a precursor of creatine), creatine, and creatinine (an end-product of creatine) utilizing a modified liquid chromatography–tandem mass spectrometry method (Agilent 1200 Series LC System, Agilent Technologies Inc., Santa Clara, CA), as previously described.[7] The coefficients of variation for the salivary and serum creatine assays were 14.0% and 3.6%, respectively. All measurements were performed between 07:00 and 10:00 following an overnight fasting period, and participants refrained from vigorous exercise within the preceding 24 h. All participants completed a familiarization session with the testing procedures prior to assessments, adhering to a standardized sequence of tests.

2.4. Statistical analyses

Differences in salivary and serum creatine and creatinine levels at baseline and post-exercise, as well as the slopes of the two regression lines (saliva *vs.* serum), were compared using paired two-tailed *t*-tests. Pearson's correlation test was utilized to evaluate the linear relationship between serum and salivary biomarkers at both time points of assessment. A significance level of $p < 0.05$ was applied. All statistical analyses were performed using SPSS version 24.0 for Mac (IBM SPSS Statistics, Chicago, IL).

3. Results

The average duration of running until exhaustion was (668 ± 64) s. A bout of high-intensity exercise resulted in a notable rise in salivary biomarkers related to creatine metabolism. Specifically, creatine levels demonstrated a significant elevation from (17.5 ± 14.2) $\mu mol{\cdot}L^{-1}$ at baseline to (43.6 ± 30.4) $\mu mol{\cdot}L^{-1}$ post-exercise ($p < 0.001$). This coincided with a notable alteration in salivary creatinine, which rose from (11.3 ± 5.8) $\mu mol{\cdot}L^{-1}$ to (17.0 ± 9.3) $\mu mol{\cdot}L^{-1}$ ($p = 0.04$). The levels of salivary GAA remained undetectable (below the limit of detection, 0.1 $\mu mol{\cdot}L^{-1}$) both before and after the exercise session. In contrast, serum creatine levels were unaffected by exercise ($p = 0.80$), while creatinine levels exhibited a strong tendency to decrease post-exercise (from [81.8 ± 17.5] $\mu mol{\cdot}L^{-1}$ to [73.1 ± 11.6] $\mu mol{\cdot}L^{-1}$; $p = 0.06$). Furthermore, the levels of creatine and creatinine in saliva were significantly lower compared to the corresponding serum levels, both at baseline and after exercise ($p < 0.05$). Fig. 1 illustrates the individual variations in salivary and serum levels of creatine and creatinine.

The salivary indicators of creatine metabolism showed a non-significant correlation with the corresponding serum indicators. The Pearson two-tailed correlation coefficients for creatine concentrations at baseline and post-exercise were $r = 0.18$ ($p = 0.51$) and $r = 0.33$ ($p = 0.21$), respectively. The corresponding correlation coefficients for creatinine were $r < 0.01$ ($p = 0.99$) for baseline values and $r = 0.40$ ($p = 0.13$) at post-exercise. However, a comparison of the slopes of the two regression lines (saliva *vs.* serum) revealed significant differences for both creatine ($p = 0.01$) and creatinine ($p = 0.03$).

4. Discussion

The present study potentially represents the pioneering demonstration of exercise-induced alterations in salivary biomarkers related to creatine metabolism. We observed that high-intensity exercise led to an escalation in both salivary creatine and creatinine levels among young, healthy adults. A rise in salivary creatine was consistently observed in all fifteen participants (100%), whereas an increase in salivary creatinine was evident in eleven out of fifteen participants (73.3%). The elevated concentrations of creatine and creatinine in saliva might be attributed to an exercise-induced concentration gradient, potentially facilitating greater diffusion of these compounds from the serum into the saliva. The specific impact likely depends on factors such as the intensity and duration of exercise, individual metabolism, hydration status, and other physiological factors,[4,8] which require further investigation. Interestingly, the correlation between salivary biomarkers and concentrations measured in serum was weak, indicating potentially distinct biodynamics of creatine metabolites in these two bodily fluids, both before and after exercise. Given that serum creatine levels reportedly remain largely unchanged following acute exercise,[9] the utilization of salivary creatine might be suggested as a more responsive biomarker to exercise and perhaps illustrate changes in creatine metabolism outside of the muscle tissue. This may be attributed to the bioenergetics specific to salivary glands during exercise and/or inflammation induced by exercise. Still, factors like individual differences in metabolism, the route of creatine excretion, and the influence of other components in saliva may affect the reliability of using saliva as a proxy for creatine turnover. Our results partially corroborate findings from a study demonstrating a difference between GAA and creatine concentrations in the saliva and plasma of healthy subjects.[5] Still, the authors provided no correlation indices

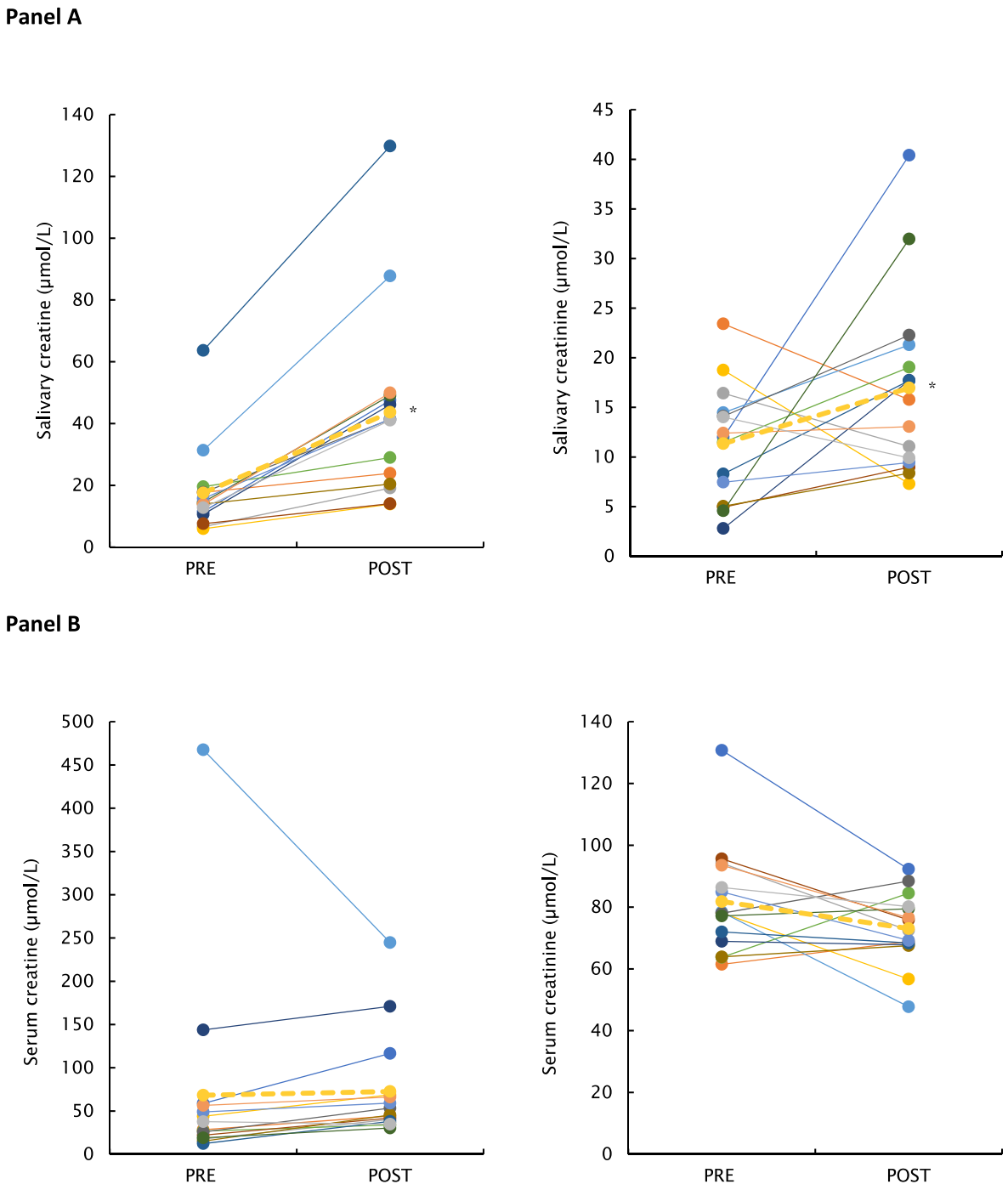

Fig. 1. Variations in individual levels of salivary (panel A) and serum (Panel B) creatine and creatinine before (PRE) and after exercise (POST). The dashed lines represent the mean values. The asterisk (*) denotes statistical significance between pre- and post-measurements at $p < 0.05$.

between creatine levels in two fluids and concluded that salivary biomarkers could be used as a noninvasive, safe, and inexpensive tool for diagnosing creatine disturbances across various conditions. Based on our findings, there is no observed association between salivary and serum biomarkers, indicating that they should not be utilized interchangeably.

Despite its interesting findings, our study has several limitations that impact its conclusions. The small sample size (15 participants) reduces statistical power and limits generalizability, especially given the homogeneous population of young, physically active adults. A broader study including individuals of varying fitness levels, ages, and health statuses would be necessary to validate these results. Additionally, the study does not account for potential confounding factors such as hydration status, diet, and individual differences in creatine metabolism, all of which could influence biomarker fluctuations. Methodologically, while saliva offers a non-invasive alternative to blood sampling, its reliability as a biomarker source remains uncertain. The study does not clarify whether salivary creatine increases due to diffusion from the blood or whether it is influenced by exercise-induced changes in salivary gland metabolism. Furthermore, the storage conditions, sample processing, and potential degradation of creatine in saliva over time could introduce variability in the results. Given these concerns, more research with larger sample sizes, improved methodological rigor, and varied exercise protocols is necessary before saliva can be considered a reliable biomarker for creatine metabolism in response to physical exertion.

CRediT authorship contribution statement

Bogdan Andjelic: Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Tijana Lainovic:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Nikola Todorovic:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Jovana Panic:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Milan Vranes:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation. **Valdemar Stajer:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis. **Sergej M. Ostojic:** Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethical approval statement

Ethical clearance for conducting the study was obtained from the local Institutional Review Board at the University of Novi Sad (Approval # 49-03-14/2023-1), with the research adhering to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants.

Declaration of competing interest

The authors declare there are no competing interests.

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