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BRIEF COMMUNICATION

A single session of exhaustive exercise markedly decreases circulating levels of guanidinoacetic acid in healthy men and women**Valdemar Stajer¹, Tatjana Trivic¹, Patrik Drid¹, Milan Vranes², Sergej M. Ostojic^{1,3*}**¹ Faculty of Sport and Physical Education, University of Novi Sad, Serbia² Faculty of Sciences, University of Novi Sad, Serbia³ University of Belgrade School of Medicine, Belgrade, Serbia

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Abstract

We evaluated the effects of exercise on circulating concentrations of guanidinoacetic acid (GAA) and creatine in 23 healthy volunteers subjected to running to exhaustion and free-weight bench-press to volitional failure. Blood was taken before and following each exercise session. Running induced a significant decrease in serum GAA by 20.1% ($P < 0.001$), while free-weight exercise reduced GAA by 11.7% ($P < 0.001$), suggesting the possible use of serum GAA as a novel biomarker of exhaustion.

Keywords Bioenergetics; Creatine; Creatinine; Running-to-Exhaustion; Bench press

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Introduction

Guanidinoacetic acid (GAA) is a naturally-occurring amino acid derivative of glycine, and the metabolic forerunner of creatine, a compound that helps to supply energy to all cells, including the skeletal muscle, brain and heart (Wallimann *et al.* 2011). GAA is metabolized to creatine in a reaction catalyzed by guanidinoacetate *N*-methyltransferase (GAMT), with creatine synthesis mainly occurring in the liver, pancreas and kidneys (Brosnan *et al.* 2011). Although GAA has been identified as an endogenous substance in humans a long time ago (Hoberman 1947), and a recent study recognized its specific effect on brain cell development (Hanna-El-Daher *et al.* 2015), GAA metabolism is rather poorly described in the scientific literature, particularly during different bioenergetic challenges. This is quite surprising compared to its derivative creatine, which is well described in terms of utilization during energy-demanding circumstances, such as exercise or tissue injury (Sotgia *et al.* 2007; Lindbom *et al.* 2008; Lygate *et al.* 2013). In particular, no study known to the authors evaluated serum GAA utilization during heavy exercise in humans. Since exhaustive exercise markedly alters kidney function (Bellinghieri *et al.* 2008), with the kidney being a major organ for GAA production (Brosnan *et al.* 2011), monitoring GAA levels during exercise might be a useful biomarker of heavy exercise-induced kidney stress. A possible exercise-induced GAA depletion may result in disrupted creatine production, and jeopardize transfer and utilization of cellular energy that consequently could compromise exercise performance that depends on a high turnover of phosphocreatine (such as high-intensity short duration activities or the beginning/end of long duration exhaustive exercise). Alternatively, GAA depletion might be caused by increased creatine synthesis rate during exercise. Therefore, the main aim of this observational study was to evaluate the effects of a single exhaustive exercise session on circulating concentrations of GAA, creatine and creatinine in healthy men and women.

Methods

Participants

Twenty-three young apparently healthy volunteers (12 men and 11 women) gave informed consent to participate in this study. Sample size ($n = 23$) was calculated using the power analysis (one-tailed, effect size 0.5, α 0.05, power 0.80) for the primary outcome measure, exercise-induced drop in serum GAA concentrations (G-Power 3, Heinrich Heine University Düsseldorf, Germany), with the effect size previously categorized as medium in a related study (Ostojic *et al.* 2013). The mean physical characteristics of participants were: age 24.7 ± 2.5 years, height $174.9 \pm$

10.0 cm, and weight 69.6 ± 13.2 kg. All participants were free from cardio-metabolic diseases and musculoskeletal disorders, moderately physically active (~ 5 hours per week), and were not taking any medication or dietary supplementation. The study was conducted in accordance with the Declaration of Helsinki, with study approved by the local IRB at the University of Novi Sad Faculty of Sport and Physical Education (Ref. ID 031/15-ASN:228).

Study design

All volunteers were assessed on two occasions separated by 7 days, with participants subjected to a single session of exhaustive exercise at each visit to the lab. Measurements were carried out between 08:00 and 11:00 after an overnight fast, and no heavy exercise over the previous 24 h. During the first visit, participants were assigned to incremental running-to-exhaustion test on an institutional treadmill (3-min warm-up walk at 6 km/h followed by running at 8 km/h with progressive workload increment rate of 1.5 km/h every 60 sec until exhaustion). Test was finished when participants were physical tired to continue running, with subjective rates of perceived exertion (RPE; Buckley and Borg 2011) and heart rates monitored during the test (Polar S 810, Oy Kempele, Finland). At second visit to the lab, participants performed free-weight bench press exhaustive exercise (5-repetition warm-up with 10 kg followed by bench press repetitions at workload equal to 25% of participants' weight until volitional failure). All participants were familiar to testing procedures as part of their training routine. Two different modes of exhaustive exercise were chosen due to possible mode-response interaction with renal function and metabolism, with heavy aerobic exercise particularly effective to compromise kidney function as compared to resistance exercise (Bellinghieri *et al.* 2008; Moinuddin and Leehey 2008).

Clinical chemistry

At each lab session, blood samples were drawn from an antecubital vein into a gel vacutainer before (fasting) and immediately after exercise. Gel vacutainers were immediately centrifuged at 3000g for 15 min, with serum stored at -20°C and analyzed for GAA and creatine after the completion of the study using modified LC-MS/MS (Agilent 1200 Series LC System, Agilent Technologies Inc., Santa Clara, CA, USA). The separation of the analytes was achieved on a Kinetex®HILIC column (100 mm × 2.1 mm i.d., 2.6 µm) (Phenomenex Inc., Torrance, CA, USA) with a column temperature of 30°C. The mobile phase consisted of three eluents, methanol (10%), acetonitrile (80%) and ammonium formate (pH 3.7, 10%) delivered with isocratic elution at a flow rate of 0.5 mL/min.

Creatinine was analyzed by standard colorimetric method (Heinegård and Tiderström 1973).

Statistical analyses

When homogenous variances were verified for normally distributed data, baseline vs. follow-up measures were compared by two-way mixed model ANOVA with repeated measures to establish if any significant differences existed between participants' responses over time (baseline vs. post-exercise), with exercise mode (running to exhaustion or free-weight bench-press) included as a between-subjects factor. When non-homogenous variances were identified, values were compared using Kruskal-Wallis test. The relationship between changes in serum GAA and creatine levels was examined using Pearson's product-moment correlation coefficient. Significance level was set at $P \leq 0.05$. The data were analyzed using the statistical package SPSS, PC program, version 21.0 (SPSS Inc., Chicago, Illinois, USA).

Results

Participants successfully completed both sessions of exhaustive exercise, with running test ended in 371 ± 94 s, with end-exercise RPEs 9.9 ± 0.2 and peak heart rate 189 ± 8 beats per min ($98.5 \pm 3.7\%$ of age-predicted maximal heart rate), while bench press test was finished after 57 ± 34 repetitions (test ended in 199 ± 86 s), and end-exercise RPEs 9.9 ± 0.2 . Baseline levels of fasting serum biomarkers were: GAA 3.0 ± 0.8 $\mu\text{mol/L}$, creatine 22.5 ± 8.0 $\mu\text{mol/L}$, and creatinine 6.9 ± 2.0 mg/L before running-to-exhaustion test; and GAA 2.6 ± 0.8 $\mu\text{mol/L}$, creatine 20.6 ± 6.4 $\mu\text{mol/L}$, and creatinine 7.6 ± 1.0 mg/L before free-weight bench press test. We found that exercise markedly affected circulating GAA, with both modes of exhaustive exercise induced a significant drop in serum GAA (2.4 ± 0.7 $\mu\text{mol/L}$ after running test, and 2.3 ± 0.8 $\mu\text{mol/L}$ after bench press test) compared to the baseline concentrations ($P < 0.05$). The effects are markedly superior after incremental running-to-exhaustion test comparing to free-weight bench press to volitional failure ($P = 0.02$; Figure 1). The highest alteration was found in a female participant who experienced a drop in serum GAA from 4.26 $\mu\text{mol/L}$ (at baseline) to 2.14 $\mu\text{mol/L}$ (post-exercise) after running-to-exhaustion test. The least reduction in serum GAA during the study (0.7%) was reported in a man after free-weight bench press test, and four participants (one women after running-to-exhaustion test, and two women and a men after bench press test) experienced a rise in serum GAA (from 1.0% to 14.9%) post-exercise. In addition, serum creatine was elevated after running-to-exhaustion exercise by 3.2 $\mu\text{mol/L}$ (95% CI = $3.0 - 3.4$ $\mu\text{mol/L}$; $P = 0.004$), while

free-weight exercise had no apparent effect on serum creatine (post-exercise levels were $23.9 \pm 6.4 \mu\text{mol/L}$; $P = 0.07$), and no differences were found in creatine upsurge from baseline to follow-up between two modes of exhaustive exercise applied ($P = 0.22$). Finally, both modes of exhaustive exercise significantly elevated serum creatinine comparing to baseline levels ($9.7 \pm 1.2 \text{ mg/L}$ after running test, and $7.4 \pm 1.4 \text{ mg/L}$ after bench press test; $P < 0.05$), with running-to-exhaustion found to be more powerful to elevate serum creatinine as compared to bench press exercise ($P < 0.001$). A moderate-to-strong negative linear correlation was reported between post-exercise changes in GAA and creatine for both exhaustive running ($r = -0.67$; $P < 0.001$) and free-weight exercise ($r = -0.39$; $P = 0.03$) for one-tailed probabilities, respectively.

- Figure 1 about here -

Discussion

The present study showed a notable drop in serum GAA concentration in response to a single session of exhaustive exercise, with lowest values occurring immediately after endurance running exercise in healthy men and women. It seems that longer-duration exhaustive exercise that recruits large muscle groups (both legs and arms) generates a greater reduction in GAA availability, suggesting a mode-response interaction between an exhaustive workout and GAA utilization. Furthermore, serum creatine and creatinine are sensitive to different modes of exhaustive exercise.

GAA (also known as guanidinoacetate, glycocyamine or *N*-amidinoglycine) is a natural metabolite of glycine (aminoacetic acid) in which the amino group has been converted into a guanidine. The metabolic role of GAA in cellular bioenergetics, as a natural precursor of creatine and an alternative substrate for creatine kinase, has been well described (Ostojic 2015). After synthesized from glycine and *L*-arginine in the kidney and pancreas, GAA circulates to the liver (also kidney, pancreas, and reproductive organs) to yield creatine through the essential methylation reaction catalyzed by GAMT, using *S*-adenosyl methionine as the methyl donor (Brosnan *et al.* 2011). Normal endogenous creatine production from GAA in humans accounts for ~ 1 gram per day, with additional creatine provided by diet (about 1 g/day, mainly from meat) serves to maintain normal creatine homeostasis (Wyss and Kaddurah-Daouk 2000). Several clinical studies reported different disorders in endogenous GAA and creatine production, leading to severe impairments of cellular bioenergetics and clinical manifestations (Schulze 2013), however limited information is available regarding GAA physiology during energy-demanding circumstances in

healthy humans. In this study, we found an exhaustive exercise-induced diminution of GAA availability, as assessed by a notable acute drop in serum GAA post-exercise. Consequently, heavy exercise could be considered as a GAA-depleting intervention; this might be due to an exercise-induced reduction in GAA production, an increase in GAA utilization to creatine, or both. Since heavy exercise induces complex changes in the renal hemodynamics including a reduction in blood flow and metabolism (Bellinghieri *et al.* 2008), this might have an acute negative effect on GAA synthesis in the main GAA-producing organ by reducing the availability of glycine and *L*-arginine, or by suppressing the activity of *L*-arginine:glycine amidinotransferase, GAA synthesizing enzyme. Previous study by Japanese group reported low serum GAA in patients suffering from chronic kidney disease or diabetes mellitus (Tsubakihara *et al.* 2012), suggesting possible link between kidney dysfunction and GAA deficiency. Alternately, a bout of heavy exercise might acutely stimulate GAA methylation to creatine (and creatine to creatinine) which causes GAA depletion and creatine boosting, particularly during brief exercise where creatine is considered a main source of cellular energy. Here, we reported increased serum creatine levels after exercise, implying enhanced GAA utilization to creatine by exhaustive exercise, perhaps driven by enhanced GAMT activity. Further studies with isotopic GAA tracer along with enzyme activity/expression assays are needed to evaluate the contribution of each factor for GAA utilization during energy-demanding circumstances such as exercise. In addition, gender-related differences in GAA dynamics following exercise need to be addressed in future studies, since men and women seem to differ in creatine-related markers after high-intensity exercise (Willcocks *et al.* 2010). This was not addressed during the present trial due to small subsamples of men and women. Furthermore, we compared does two different modes of exhaustive exercise had a different impact on GAA utilization in healthy men and women. It seems that endurance running induced a more pronounced fall in serum GAA immediately post-exercise as compared to weight lifting (21.3 vs. 11.2%). This might illustrate a mode-response effect of heavy exercise on GAA availability, considering the fact that endurance running-to-exhaustion was of longer duration and engaged more skeletal muscle (a major organ of creatine utilization) that probably consumes and/or utilizes more GAA. Alternatively, heavy aerobic exercise might induce profound changes in the renal hemodynamics and metabolism that consequently reduces GAA synthesis in the kidney and its availability in the circulation. This hypothesis should be further evaluated since the current study did not test any other markers of kidney damage, and perhaps validate serum GAA as a biomarker of exercise-induced kidney stress. On the other hand, exhaustive resistance exercise seems to less aggressively affect GAA-creatine cycle, inducing rather moderate decrease in serum GAA while serum creatine

tended to increase at post-exercise. This might be due to either lower bioenergetic cost or minor kidney dysfunction imposed by this mode of exercise, as compared to aerobic exercise.

In conclusion, circulatory GAA seems to be responsive to acute exhaustive exercise, with serum GAA availability depleted from 3.4% up to 49.8% (maximum change) by extended and all-encompassing exercise. GAA should be further evaluated as a possible biomarker of exercise load, with additional studies needed to describe physiological background and possible consequences of exercise-induced GAA depletion.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Figure caption

Figure 1. Changes from baseline (%) for serum GAA, creatine and creatinine levels at post-exercise. Values are presented as means, with upper and lower bounds represent 95% confidence intervals. * Indicates significant difference baseline vs. post-exercise ($P < 0.05$). † Indicates significant difference between running-to-exhaustion and free-weight bench press exercise ($P < 0.05$).

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