Guanidinoacetic acid with creatine compared with creatine alone for tissue creatine content, hyperhomocysteinemia, and exercise performance

A randomized, double-blind superiority trial

Semeredi, Sasa; Stajer, Valdemar; Ostojic, Jelena; Vranes, Milan; Ostojic, Sergej M

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Author: Sasa Semeredi, Valdemar Stajer, Jelena Ostojic, Milan Vranes, Sergej M. Ostojic

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ORIGINAL ARTICLE

Guanidinoacetic acid with creatine compared with creatine alone for tissue creatine content, hyperhomocysteinemia and exercise performance: a randomized double-blind superiority trial

Sasa Semeredi [1], Valdemar Stajer [1], Jelena Ostojic [2], Milan Vranes [3], Sergej M. Ostojic [1, 4 *]

[a] Faculty of Sport and Physical Education, University of Novi Sad, Novi Sad, Serbia

[b] Faculty of Medicine, University of Southern Denmark, Ostende, Denmark

[c] Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

[d] University of Belgrade School of Medicine, Belgrade, Serbia

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* **Correspondence:**

Professor Sergej M. Ostojic, MD, PhD

ORCID ID: http://orcid.org/0000-0002-7270-2541

FSPE Applied Bioenergetics Lab, University of Novi Sad

Lovcenska 16, Novi Sad 21000, Serbia.

Phone: (++381)-21-450-188 | Fax: (++381)-21-450-199

E-mail: sergej.ostojic@chess.edu.rs

Highlights

- Guanidinoacetic acid (GAA) appears as a novel energy-enhancing supplement for brain and muscle
- Creatine-GAA mixture results in more powerful elevation of tissue creatine as compared to equimolar creatine
- Better outcomes were reported for upper body muscular strength and less weight gain in GAA-creatine group
- GAA-creatine mixture is an effective and safe creatine-boosting alternative to creatine alone in healthy men

Abstract

Purpose

Co-administration of creatine and guanidinoacetic acid (GAA) has been recently put forward as an advanced dietary strategy to optimize tissue bioenergetics. We hypothesized that creatine-GAA mixture would result in more powerful rise in brain and skeletal muscle creatine, as compared to creatine supplementation alone.

Methods

A randomized, double-blinded, crossover superiority trial has been performed at the University of Novi Sad from December 2016 to November 2017. A total of 14 healthy young men were randomized to receive GAA-creatine mixture (1 grams of GAA and 3 grams of creatine per day) or equimolar creatine (4 grams per day) by oral administration for 4 weeks.

Results

Creatine-GAA mixture was superior to creatine alone to increase mean creatine levels in skeletal muscle (16.9 ± 20.2 *vs*. 2.0 ± 6.0%; $P = 0.02$) and grey matter (5.8 ± 5.3% *vs.* 1.5 ± 3.2%; $P = 0.02$), also for bench press performance (6.0% *vs.* 5.1%; $P < 0.01$). Compared with creatine administration alone, combined GAA and creatine resulted in less weight gain (1.6 ± 0.2 kg vs. 0.7 ± 0.2 kg; $P < 0.01$). No inter-group differences were observed in terms of cardiorespiratory endurance, serum biomarkers, or adverse events.

Conclusions

Creatine-GAA mixture appeared to be superior to sole creatine for up-swinging tissue creatine content and upper body strength, and resulted toward a lower risk of weight gain in healthy active men. The formulation might be considered as a novel energy-boosting alternative to creatine alone in weight-sensitive setups. Trial registration: ClinicalTrials.gov NCT03350282.

Keywords: guanidinoacetic acid; creatine; MR spectroscopy; skeletal muscle; brain; exercise performance

Abbreviations

1-RM	One-repetition maximum
CR	Creatine
CRN	Creatinine
CT1	Creatine transporter
GAA	Guanidinoacetic acid
GAT1	Gamma-amino butyric acid transporter
MRS	Magnetic resonance spectroscopy
TauT	Taurine transporter
tHcy	Total homocysteine
VO_{2max}	Maximal oxygen uptake
VT	Ventilatory threshold

Introduction

Targeting energy-demanding tissues in health and disease continues to be a challenging task in human nutrition and biomedicine. Impaired bioenergetics accompanies many different conditions, including cardiometabolic diseases, neurodegenerative disorders or high-intensity exercise, with various dietary interventions developed to restore cellular energy [1, 2]. Creatine is recognized as a beneficial and safe energy-boosting agent in both athletic and clinical environments [3]. However, its effectiveness in specific conditions seems to be fairly restrained due to its limits in transportability and performance [4]. Guanidinoacetic acid (GAA), a metabolic precursor of creatine, appears as a novel energy-enhancing supplement, with GAA being superior to creatine in facilitating creatine concentrations in the human brain and skeletal muscle [5]. This perhaps happens due to GAA interaction with cellular transporters previously dismissed as untargetable carriers by other similar therapeutics [6]. On the other hand, GAA loading remains under scrutiny due to its hyperhomocysteinemia-inducing potential [7], and possible neurotoxic effects [8]. Co-administration of creatine and GAA has been recently proposed as a better strategy comparing to administration of each compound per se [9]. Besides providing a competitive advantage for enhanced levels of tissue creatine, GAA-creatine mixture might also diminish side effects related to isolated GAA administration. However, no human studies so far evaluated the effects of this mixture. In the present study, we compared the impact of 4-week co-administration of GAA and creatine *vs.* creatine administration alone on serum biomarkers, exercise performance and tissue creatine content in healthy young men.

Methods

Participants

Fourteen healthy young men (age 24.4 ± 4.0 years, weight 79.9 ± 13.9 kg, height 176.5 ± 7.3 cm) were recruited and signed informed consent to voluntarily participate in this double-blind, randomized, creatine-controlled, crossover trial examining the effects of GAA-creatine mixture on tissue bioenergetics and exercise performance. Appropriate sample size (n = 12) was calculated using the power analysis (effect size 0.5, alpha error probability 0.05, power 0.80) for the primary outcome measure, an intervention-induced increase in brain creatine for GAA-creatine mixture vs. creatine alone (G-Power 3, Heinrich Heine University Düsseldorf, Germany). This was adjusted to 14 participants to account for a predicted 20% dropout. All participants were non-vegetarian, free from acute or chronic diseases, and not using any medication or dietary supplements within four weeks prior to the study commencing. All procedures were approved by the local IRB, in accordance with the Declaration of Helsinki, with the study

conducted in FSPE Applied Bioenergetics Lab at the University of Novi Sad from December 2016 to November 2017. The trial has been registered at ClinicalTrials.gov (NCT03350282).

Experimental procedures

All participants were randomly assigned to receive either GAA-creatine mixture (1 grams of GAA and 3 grams of creatine per day) or equimolar creatine (4 grams per day) by oral administration for 4 weeks. The amount of creatine used was chosen as a minimal dose that gives the desired effect (e.g. 3-5 g/day for about 30 days to increase muscle creatine) [10]. Participants were instructed to take the intervention each day as a single dose administered ~ 15 min before breakfast, with powder provided by the research team stirred into 250 mL of lukewarm water and immediately consumed. GAA was purchased from Axenic Lab (Oak Park, Australia) and creatine monohydrate from Twinlab Corporation (Hauppauge, NY, USA). Washout period lasted for 28 days to prevent the residual effects of interventions across study periods. Participants were asked to maintain their usual lifestyle (including diet and physical activity) and to abstain from using other dietary supplements or medication during the trial. All participants were assessed on three occasions, at baseline and after each of 4-week follow-ups. Lab assessments were carried out between 08:00 and 12:00 after an overnight fast, and no exhaustive exercise over the previous 24 h. All lab sessions were conducted at the same time for each participant on consecutive days, with the last dosage of experimental intervention consumed ~ 24 h before assessment to complete loading protocol. Body weight was measured with digital scale (Omron BF508, Tokyo, Japan). The venous blood was drawn, centrifuged within the next 10 min at 3000 *g*, with serum separated and analyzed for GAA, creatine and creatinine using modified LC-MS/MS (Agilent 1200 Series LC System, Agilent Technologies Inc., Santa Clara, CA, USA), and total homocysteine (tHcy) by a standard fluorescence polarization immunoassay method. Proton magnetic resonance spectroscopy (^{1}H-MRS) was performed on 1.5 T Avanto scanner (Siemens, Erlangen, Germany) using matrix head coil in circularly polarized mode, with metabolite spectra in the specific brain regions (left centrum semiovale white matter and midline occipital gray matter) and right vastus medialis muscle processed as previously described [5]. In short, nonwater-suppressed 2D-chemical shift imaging (CSI) and single voxel spectroscopy data were obtained to provide an internal water reference for the absolute quantification of tissue creatine. Total creatine (creatine + phosphocreatine) was calculated using water-suppressed CSI and single voxel data sets were acquired with point-resolved spectroscopy with repetition time/echo time of 1500/135 ms. Mono-exponential spin-lattice and spin-spin relaxation was assumed, and standard values of T1 and T2 relaxation times of water and total creatine measured at 1.5 T were used for relaxation corrections. After initial measurements, isometric strength of forearm muscles was assessed by handgrip dynamometer (Jamar J00105; Lafayette Instrument Company, Lafayette, IN). Muscular strength in the upper and lower body was assessed through one-repetition maximum test (1-RM) for the supine free-weight bench press and front squat exercise, respectively. Single and repetitive maximal vertical jump performance was assessed using a contact mat (Just Jump System; Probotics, Huntsville, AL). Jump height as well as both peak and mean anaerobic power were recorded. Cardiorespiratory endurance was evaluated by a maximal endurance running test, with gas exchange data collected throughout the test using a breath-by-breath metabolic system (Quark CPET, COSMED, Rome, Italy). Participants were also instructed to report on adverse effects of intervention through open-ended

questionnaire at the end of intervention. All participants were familiarized with testing procedures and were assessed on the same day with the tests performed in the same order.

Statistical analyses

When homogenous variances were verified for normally distributed data, measures were compared by two-way mixed model ANOVA with repeated measures to establish if any significant differences existed between participants' responses over time of intervention (baseline vs. post-administration), with the intervention (GAA-creatine mixture or creatine) included as between-subjects factor. In the event of a significant F ratio, post hoc analyses were performed with a Tukey honest significant difference test to identify the differences between individual sample pairs. When non-homogenous variances were identified, values were compared using Kruskal-Wallis test, with a Games-Howell post hoc test used to evaluate between-group differences. Significance level was set at $P \leq 0.05$. The data were analyzed using the statistical package IBM SPSS Statistics for Mac, version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

All participants completed the trial, with no subjective side effects reported during each intervention period. Compliance was rather high and comparable in both groups (91.8 ± 17.2% for GAA-creatine group, 88.8 ± 20.1% for creatine group), as evaluated by counting unconsumed sachets at follow up. Changes in physical, biochemical and physiological indices during the study are depicted in Table 1. Compared with creatine administration alone, GAA and creatine mix resulted in less weight gain (1.6 ± 0.2 kg *vs.* 0.7 ± 0.2 kg; $P < 0.01$). No inter-group differences were observed for serum GAA, creatine and tHcy, yet creatine was superior to GAA-creatine blend to elevate serum creatinine levels ($P = 0.01$). In addition, no participants experienced hyperhomocysteinemia (tHcy ≥ 15.0 µmol/L) or elevated serum creatinine above reference range (> 110 µmol/L) at follow up. Upper body strength expressed as the change from baseline in 1-RM for bench press exercise was significantly greater in GAA-creatine group compared with creatine group ($P < 0.01$), while the mixture was inferior to creatine to amplify lower body strength expressed as the change from baseline in front squat exercise ($P < 0.01$). No significant between-group changes were observed at post-administration in handgrip strength, anaerobic or aerobic performance.

- Table 1 about here -

Baseline creatine levels were 35.5 ± 5.9 mM in vastus medialis muscle; 7.7 ± 0.6 mM in left centrum semiovale white matter; and 8.9 ± 0.8 mM in midline occipital gray matter. Changes in tissue creatine concentrations from baseline to week 4 are presented in Figure 1. Co-administration of creatine and GAA was superior to creatine alone to increase mean creatine levels in grey matter (5.8 ± 5.3% *vs.* 1.5 ± 3.2%; $P = 0.02$) and skeletal muscle (16.9 ± 20.2% *vs.* 2.0 ± 6.0%; $P = 0.02$), also the mixture tended to result in elevated creatine in white matter as compared to sole creatine (13.9 ± 12.8 % *vs.* 7.3 ± 7.4%; $P = 0.18$). In addition, tissue levels of choline remained unaffected by either intervention (data not shown).

- Figure 1 about here -

Discussion

In the present study, we confirmed our prespecified hypothesis that the creatine-GAA mixture results in more powerful elevation of tissue creatine, as compared to equimolar creatine. Furthermore, better outcomes were also reported for upper body muscular strength and less weight gain in GAA-creatine group, with no side effects and clinically relevant disturbances in tHcy and serum creatinine. This perhaps advances GAA-creatine mixture as an effective and safe creatine-boosting and performance-enhancing alternative to creatine alone in weight-sensitive setups.

Tissue creatine content

Co-administration of creatine and guanidinoacetic acid for augmented tissue bioenergetics has been recently proposed as a possible novel dietary strategy to improve cellular levels of creatine in clinical medicine and nutritional science [9]. It has been suggested that favorable aspects of each component could enable a GAA-creatine mixture to perhaps tackle energy-demanding tissues difficult to reach by other agents, in safe and convenient manner. This is particularly vital for the brain since previous studies reported limited applicability of dietary creatine to improve reduced creatine levels in neurodegenerative diseases (for review see Ref. 4). On the other hand, GAA reaches the human brain superiorly than creatine [5], yet its individual use might be limited due to several safety constraints [11]. We found that the addition of GAA to creatine superiorly raises creatine levels in the grey matter and skeletal muscle, with additional gains versus creatine alone were 14.9% for muscle creatine (95% CI -6.8 to 36.6%), and 4.4% for brain creatine concentrations (95% CI -2.1 to 10.7%). A trend for enhanced tissue creatine has been reported for the white matter as well, with GAA-creatine improved creatine levels for extra 6.6% versus pure creatine (95% CI -8.7 to 21.9%). This perhaps happen due to better transport capacity of the mixture containing GAA, with added GAA being able to reach target tissues via different membrane transport proteins, besides creatine transporter (CT1). While creatine is mainly transported via CT1, GAA might be transported via CT1, gamma-amino butyric acid transporter (GAT1), taurine transporter (TauT), or even via passive diffusion [12], with absorbed GAA methylated to creatine in the target cell [13]. It appeared that the addition of 1 gram of GAA to daily dosage of creatine traditionally used in interventional studies (3 grams) was a better strategy for augmenting creatine content comparing to further increase of a creatine dosage in the supplement. This might be particularly true for tissues with high density of GAT1 and TauT, such as cerebral cortex, intestine, liver and gonads, yet no human studies so far revealed transporter-specific delivery kinetics of GAA *in vivo*. Furthermore, no information is currently available concerning possible side effects of exogenous GAA interaction with above transporters. In addition, creatine supplementation alone promoted very modest elevation in total creatine content (up to 7.3% on average) which is not in accordance with previous studies that demonstrated ~ 20% elevation in skeletal muscle creatine content after creatine supplementation [14, 15]. This might be due to a specific physiological profile of participants to respond to creatine supplementation [16], including high initial levels of muscle creatine reported here (35.5 mM on average).

Apparently, 4 grams of creatine during 4 weeks might be enough to promote creatine saturation in the skeletal muscle of the study population, and addition of GAA improves creatine saturability. Nevertheless, more studies comparing GAA plus creatine versus creatine alone are needed in participants with different initial creatine content to evaluate is the mixture more effective than sole creatine above of the saturated levels. In particular, GAA should be evaluated as a follow-up supplementation strategy in participants who completed a traditional muscle creatine loading protocol [14].

Side effects

Referring to doses used in this trial, no subjectively reported adverse events have been described in either intervention. GAA alone induces a drop in brain choline levels [5], a possible side effect that turns up as a consequence of amplified methylation to creatine through guanidinoacetate N-methyltransferase-controlled reaction. Our study found no disturbances in tissue choline levels, either due to a low dose of GAA used in the mixture or a protective effect of creatine. Other safety biomarkers, including serum tHcy, appeared to be principally unaltered by the intervention. Combination of creatine and GAA seemed to inhibit hyperhomocysteinemia, an undesired harmful effect of GAA supplementation [7], and a well-known individual risk factor for cardiometabolic diseases. This either occurs due to positive effect of creatine on lowering tHcy [17], low dose of GAA used in the present study, or both. Well-powered, longitudinal studies are highly warranted to evaluate the safety of GAA-creatine mixtures in humans before recommending the optimal proportion of GAA to creatine, dosage and duration of treatment, and possible interactions.

Exercise performance

The superiority of GAA-creatine mixture was accompanied by a more powerful rise in upper body muscular strength, as compared to creatine alone (6.0% *vs.* 5.1%; 95% CI -6.8 to 8.6%), while the mixture was inferior to creatine to amplify lower body strength, and equivalent to creatine for affecting other physical fitness attributes. In addition, compared with creatine administration alone, combined GAA and creatine resulted in less weight gain. Increase exposure to creatine causes weight gain [3], with this aspect sometimes recognized as an side effect of creatine intervention. GAA-creatine mixture appears to offset this event, while maintaining most performance-enhancing effects of creatine supplementation. No clear mechanism explains this phenomenon, yet additional GAA in the mixture might possess less water-bonding capacity due to lower polarizability of GAA molecule versus creatine (10.5 vs. 12.2 $Å^3$), with more polar molecules considered hydrophilic [18]. Our study confirms previous trial on ergogenic effects of GAA, where GAA alone provides performance-enhancing benefits in young men and women, with emphasis on specific muscles [19]. GAA-creatine mixture appeared to be more effective than creatine alone for improving strength in muscle groups with lower level of strength (e.g. upper body for general population), with no mechanistic explanation for this outcome. Hypothetically, GAA component of the mixture might be favorably absorbed by specific muscle groups (e.g. chest, shoulders and arms) that have lower initial level of GAA (and creatine) as exercise-naïve tissues. This theory warrants further investigation evaluating GAA levels at specific muscles pre- and post-administration. GAA-creatine mixture might be particularly effective for clinical populations

suffering from muscle weakness, elderly or healthy actives focused to improve muscular performance in specific muscle groups with lower initial levels of strength in weight-sensitive setups. Our participants presented increased performance on 1RM, instead of repeated sprints and jumps, while most of the studies on creatine supplementation demonstrated elevated repeated jumping performance [10]. This perhaps happened due to relatively low dose of creatine used in the present study.

Limitations

Several limitations must be pointed out when this pilot trial findings are interpreted. The trial recruited only fourteen participants, with study population included moderately active young healthy men: it remains unknown whether the effects of GAA-creatine mixture change with age, gender, level of physical activity, or clinical pathology. Specifically, since sex hormones strongly affect creatine biosynthesis from GAA [20], future studies should at least account for sexual dimorphism of GAA-creatine intervention. We employed here only a limited number of clinical tests; an extensive safety profiling, including laboratory enzymes, biomarkers of genetic toxicity or patient-reported outcomes, is highly warranted to address the risk of GAA-creatine exposure. In addition, the use of an advanced tissue profiling via ^{31}P-MRS in future studies could help to measure phosphocreatine levels (also adenosine triphosphate and other related nucleotides), and investigate muscle and brain bioenergetics after GAA-creatine intervention in more detail. Although we asked participants to maintain their usual diet, no nutrition was controlled for creatine containing foods while the uptake of GAA through the diet was considered negligible [21]. Furthermore, the lack of non-target analyzes by mass spectrometry weakness the study. Together with the tandem mass spectrometry, non-target analyzes using qualitative mass spectrometer would be very important for further research, accompanied by muscle biopsy to correlate medical imaging data, blood biomarkers and tissue metabolites. Confirmatory trials and future studies of longer duration (> 4 weeks) that controls for food-obtained creatine are required to watch the long-term safety and efficacy of GAA-creatine mixture. Finally, other GAA-creatine formulations (besides 1-to-3 ratio used in the present trial) should be developed, along with comparison with GAA intervention alone, and their effects determined in well-powered trials.

Conclusion

GAA-creatine mixture (1:3) was superior to pure creatine for improved brain and muscle creatine levels, and upper body muscular strength, accompanied by less weight gain after 4-week intervention in healthy physically active men. This mixture, referring to dosage used in this study, has been found harmless, concerning the risk of hyperhomocysteinemia or brain choline depletion. Nevertheless, more randomized controlled trials are mandatory before recommending GAA-creatine mixture as a safe and effective dietary additive for general use.

Statement of authorship

S.M. Ostojic contributed to the conception of the study. All authors contributed to the design of the research. S. Semeredi, V. Stajer, J. Ostojic and M. Vranes contributed to the acquisition and analysis of the data. S. Semeredi

and S.M. Ostojic contributed to the interpretation of the data. S.M. Ostojic drafted the manuscript. All authors critically revised the manuscript, and read and approved the final manuscript.

Conflict of Interest

The authors report no conflict of interest.

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Fig 1. Changes in the total creatine levels in vastus medials muscle (Panel A), left centrum semiovale white matter (Panel B), and midline occipital gray matter (Panel C) after 4-week co-administration with guanidinoacetic acid (GAA) and creatine, or creatine alone. White-line square in individual panel images indicates the segment of tissue processed with MRS. Gray dots indicate individual data for maximum and minimum values. Values are shown as mean change ± 95% confidence intervals from baseline to post-administration. Asterisk (*) indicates significant interaction effect (treatment *vs.* time) at $P < 0.05$.

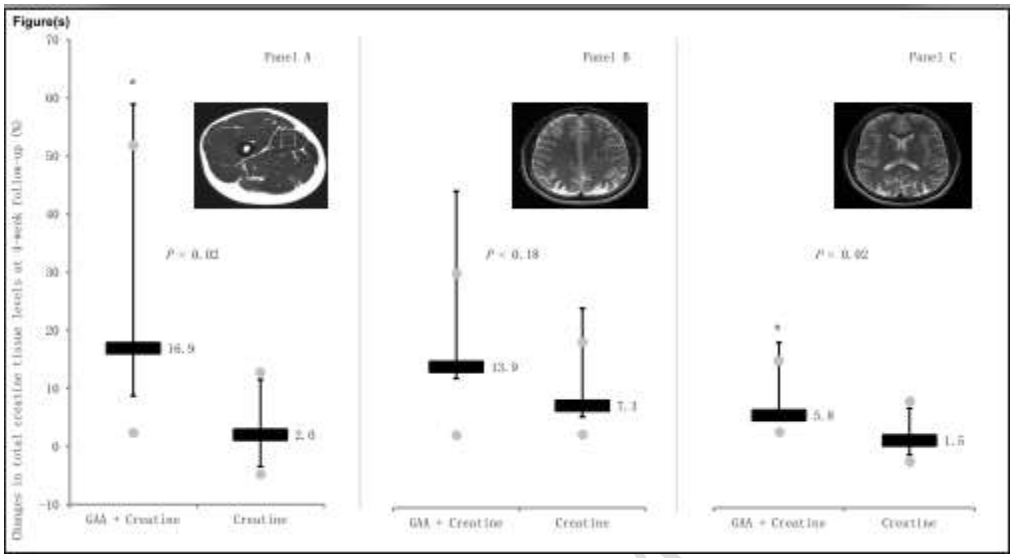

Table 1. Physical, biochemical and exercise performance variables during the study. Values are mean ± SD.

	Baseline	At follow-up		*P* *
		CR	GAA + CR	
Weight (kg)	79.9 ± 13.9	81.5 ± 14.2	80.6 ± 13.7	0.00
GAA (µmol/L)	2.0 ± 0.4	1.8 ± 0.2	1.9 ± 0.5	0.26
CR (µmol/L)	20.9 ± 5.3	44.5 ± 16.5	39.8 ± 12.4	0.39
CRN (mg/L)	84.8 ± 8.7	90.5 ± 12.6	88.5 ± 6.4	0.01
tHcy (µmol/L)	10.2 ± 2.1	10.5 ± 1.9	10.7 ± 2.0	0.99
Handgrip strength (kg)	112.7 ± 15.8	116.3 ± 18.2	116.0 ± 16.9	0.92
Vertical jump (cm)	54.2 ± 5.5	52.6 ± 5.6	52.4 ± 4.3	0.86
Peak anaerobic power (W/kg)	16.0 ± 0.8	15.7 ± 0.9	16.0 ± 0.8	0.89
Mean anaerobic power (W/kg)	12.7 ± 0.8	12.7 ± 1.2	12.7 ± 0.8	0.87
1-RM Bench press (kg)	97.1 ± 23.7	101.7 ± 22.7	102.9 ± 25.7	0.00
1-RM Front squat (kg)	107.5 ± 15.3	122.5 ± 10.6	113.0 ± 33.0	0.00
VO_{2max} (ml/kg/min)	45.9 ± 5.5	44.2 ± 5.2	45.0 ± 6.6	0.37
VT (% VO_{2max})	80.6 ± 7.9	82.2 ± 9.0	81.1 ± 8.6	0.72
Peak velocity (km/h)	18.0 ± 1.8	18.0 ± 2.2	17.9 ± 2.0	0.70
Time to exhaustion (s)	546 ± 73	544 ± 80	544 ± 75	0.99

Abbreviations: GAA - guanidinoacetic acid, CR - creatine, CRN - creatinine, tHcy - total homocysteine, 1-RM - one repetition maximum, VO_{2max} - maximal oxygen uptake, VT - ventilatory threshold. * *P* value from two-way mixed ANOVA or Kruskal-Wallis test (treatment *vs.* time interaction).