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

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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 a 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.* $2.0 \pm 6.0\%$; $P = 0.02$) and grey matter ($5.8 \pm 5.3\%$ *vs.* $1.5 \pm 3.2\%$; $P = 0.02$), 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 creatine alone for up-swinging tissue creatine content and upper body strength, and tended 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.

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 is a novel energy-enhancing supplement, with GAA being superior to creatine in facilitating creatine concentrations

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in the human brain and skeletal muscle [5]. This perhaps is due to the interaction of GAA 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 than administration of each compound per se [9]. In addition to providing a competitive advantage for enhanced levels of tissue creatine, a GAA–creatine mixture also might diminish side effects related to isolated GAA administration. However, no human studies to date have evaluated the effects of this mixture. In the present study, the authors compared the effects of a 4-wk co-administration of GAA and creatine versus creatine administration alone on serum biomarkers, exercise performance, and tissue creatine content in healthy, active young men.

Methods

Participants

Fourteen healthy men (24.4 ± 4 y of age; 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 a GAA–creatine mixture on tissue bioenergetics and exercise performance. Appropriate sample size (N = 12) was calculated using the power analysis (effect size 0.5, α error probability 0.05, power 0.80) for the primary outcome measure, an intervention-induced increase in brain creatine for GAA–creatine mixture versus 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 the 4 wk before the study commenced. All procedures were approved by the local institutional review board, 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.

Experimental procedures

Participants were randomly assigned to receive either 1 g GAA combined with 3 g creatine daily or equimolar creatine (4 g/d) by oral administration for 4 wk. The amount of creatine used was chosen as a minimal dose that gives the desired effect (e.g., 3–5 g/d for ~30 d 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 d 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-wk follow-ups. Laboratory assessments were carried out between 0800 and 1200 after an overnight fast, and participants were asked to not participate in exhaustive exercise over the previous 24 h. All laboratory 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 the loading protocol. Body weight was measured with digital scale (Omron BF508, Tokyo, Japan). Venous blood was drawn, centrifuged within the next 10 min at 3000g, with serum separated and analyzed for GAA, creatine, and creatinine *using* modified liquid chromatography–tandem mass spectromety (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 was performed on a 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, non–water-suppressed two-dimensional chemical shift imaging (CSI) and single-voxel spectroscopy (SVS) 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 SVS 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, USA). 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, USA). Jump height and 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 instructed to report any adverse effects of the intervention through an open-ended questionnaire at the end of the 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 analysis of variance with repeated measures to establish whether any significant differences existed between participants' responses over time of intervention (baseline versus postadministration), 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 (IBM Corporation, Armonk, NY, USA).

Results

All participants completed the trial, with no subjective side effects reported during the intervention periods. Compliance was rather high and comparable in both groups (91.8% ± 17.2% for the GAA–creatine group, 88.8% ± 20.1% for the creatine-alone 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

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

	Baseline	At follow-up		*P*-value*
		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	0.99
Handgrip strength (kg)	112.7 ± 15.8	116.3 ± 18.2	116 ± 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 ± 0.8	15.7 ± 0.9	16 ± 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 ± 33	0.00
$VO_{2\ max}$ (mL•kg•/min^{-1})	45.9 ± 5.5	44.2 ± 5.2	45 ± 6.6	0.37
VT (% $VO_{2\ max}$)	80.6 ± 7.9	82.2 ± 9	81.1 ± 8.6	0.72
Peak velocity (km/h)	18 ± 1.8	18.0 ± 2.2	17.9 ± 2	0.70
Time to exhaustion (s)	546 ± 73	544 ± 80	544 ± 75	0.99

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

administration alone, the GAA–creatine mix resulted in less weight gain (1.6 ± 0.2 versus 0.7 ± 0.2 kg; P < 0.01). No intergroup differences were observed for serum GAA, creatine, and tHcy, yet creatine was superior to the GAA–creatine blend in elevating serum creatinine levels ($P = 0.01$). Additionally, none of the participants experienced hyperhomocysteinemia (tHcy ≥150 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 the GAA–creatine group than in the creatine-alone group ($P < 0.01$); whereas the mixture was inferior to creatine alone 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 postadministration in handgrip strength, anaerobic, or aerobic performance.

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 in increasing mean creatine levels in gray matter (5.8% ± 5.3% versus 1.5% ± 3.2%; $P = 0.02$) and skeletal muscle (16.9% ± 20.2% versus 2% ± 6%; $P = 0.02$). The mixture also tended to result in elevated creatine in white matter compared with creatine alone (13.9% ± 12.8% versus 7.3% ± 7.4%; $P = 0.18$). Additionally, tissue levels of choline remained unaffected by either intervention (data not shown).

Discussion

In the present study, the authors confirmed the prespecified hypothesis that the GAA–creatine mixture results in more powerful elevation of tissue creatine than equimolar creatine. Furthermore, better outcomes also were reported for upper body muscular strength and less weight gain in the GAA–creatine group, with no side effects and clinically relevant disturbances in tHcy and serum creatinine. This perhaps advances the 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 GAA for augmented tissue bioenergetics has been proposed as a possible novel dietary

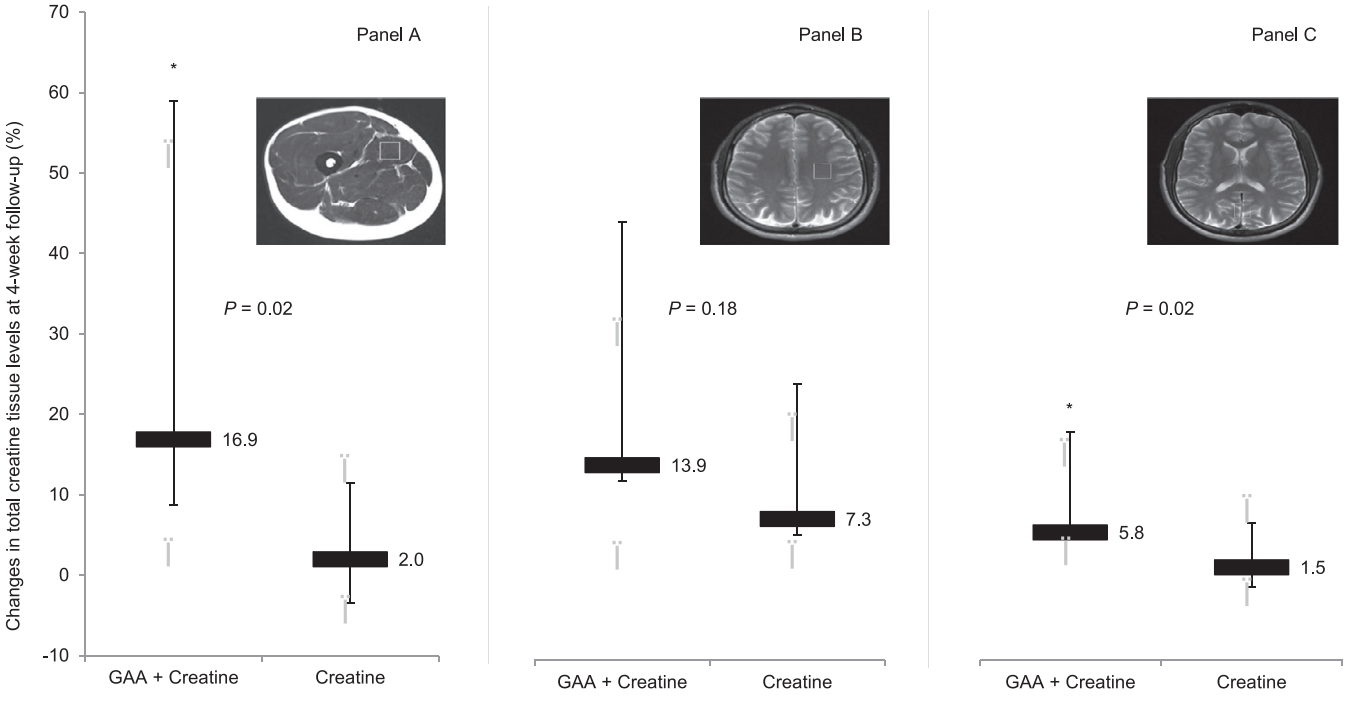

Fig. 1. Changes in the total creatine levels in vastus medials muscle (**A**), left centrum semiovale white matter (**B**), and midline occipital gray matter (**C**) after 4-wk co-administration with guanidinoacetic acid (GAA) and creatine, or creatine alone. White-line square in individual panel images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

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 as previous studies reported limited applicability of dietary creatine to improve reduced creatine levels in neurodegenerative diseases (for review see [4]). On the other hand, GAA reaches the human brain more superiorly than creatine [5], yet its individual use might be limited due to several safety constraints [11]. The authors found that the addition of GAA to creatine superiorly raises creatine levels in the gray matter and skeletal muscle. Additional gains versus creatine alone were 14.9% for muscle creatine (95% confidence interval [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 improving creatine levels for an extra 6.6% versus pure creatine (95% CI, −8.7% to 21.9%). This perhaps may happen due to the 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). Although creatine is mainly transported via CT1, GAA might be transported via CT1, γ-aminobutyric 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 g of GAA to a daily dosage of creatine traditionally used in interventional studies (3 g) was a better strategy for augmenting creatine content to further increase a creatine dosage in the supplement. This might be particularly true for tissues with a 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, to the authors' knowledge, no information is currently available concerning possible side effects of exogenous GAA interaction with the above transporters. Additionally, creatine supplementation alone promoted very modest elevation in total creatine content (≤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 g of creatine over a 4-wk period might be enough to promote creatine saturation in the skeletal muscle of the study population, and the 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 whether the mixture is more effective than creatine alone to increase the saturated levels. In particular, GAA should be evaluated as a follow-up supplementation strategy in participants who complete a traditional muscle creatine-loading protocol [14].

Side effects

Referring to doses used in this trial, no subjectively reported adverse events were 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. The present 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], the 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 the GAA–creatine mixture was accompanied by a more powerful rise in upper body muscular strength than creatine alone (6% versus 5.1%; 95% CI, −6.8% to 8.6%), whereas the mixture was inferior to creatine alone in amplifying lower body strength, and equivalent to creatine for affecting other physical fitness attributes. Additionally, compared with creatine administration alone, combined GAA and creatine resulted in less weight gain. Increased exposure to creatine causes weight gain [3], with this aspect sometimes recognized as a side effect of creatine intervention. The GAA–creatine mixture appears to offset this event, while maintaining most of the 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 versus 12.2 $Å^3$), with more polar molecules considered hydrophilic [18]. The present study confirms findings from a previous trial on ergogenic effects of GAA, where GAA alone provided performance-enhancing benefits in young men and women, with emphasis on specific muscles [19]. The GAA–creatine mixture appeared to be more effective than creatine alone for improving strength in muscle groups with lower levels of strength (e.g., upper body for general population), with no mechanistic explanation for this outcome. Hypothetically, the 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-naive tissues. This theory warrants further investigation evaluating GAA levels at specific muscles pre- and post-administration. The GAA–creatine mixture might be particularly effective for clinical populations suffering from muscle weakness, the elderly, or healthy active people focused on improving muscular performance in specific muscle groups with lower initial levels of strength in weight-sensitive setups. The participants presented increased performance on 1-RM, instead of repeated sprints and jumps, whereas 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.

Study limitations

Several limitations must be pointed out when the findings from this pilot trial are interpreted. The trial recruited only 14 participants, with the study population including moderately active young healthy men. It remains unknown whether the effects of a GAA–creatine mixture change with age, sex, level of physical activity, or clinical pathology. Specifically, because sex hormones strongly affect creatine biosynthesis from GAA [20], future studies should at least account for sexual dimorphism of the 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 for GAA–creatine exposure.

Additionally, the use of an advanced tissue profiling via ^{31}P-MRS in future studies could help 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 nontarget analyzes by mass spectrometry weakens the study. Together with the tandem mass spectrometry, nontarget 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 wk) that controls for food-obtained creatine are required to watch the long-term safety and efficacy of a GAA–creatine mixture. Finally, other GAA–creatine formulations (in addition to the 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

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

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