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CASE REPORT

Human skeletal muscle contains no detectable guanidinoacetic acid

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Abstract

We analyzed data from previously completed trials to determine the effects of supplemental guanidinoacetic acid (GAA) on markers of muscle bioenergetics in healthy men using 1.5 T magnetic resonance spectroscopy. No detectable GAA ($< 0.1 \mu\text{M}$) was found in the vastus medialis muscle at baseline nor at follow-up. This implies deficient GAA availability in the human skeletal muscle, suggesting absent or negligible potential for creatine synthesis from GAA inside this tissue, even after GAA loading.

Keywords: guanidinoacetic acid; magnetic resonance spectroscopy; vastus medialis

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Creatine synthesis in tissues with high-energy demands remains somewhat puzzling, concerning the availability of guanidinoacetic acid (GAA), a direct natural precursor of creatine and an intermediate in cellular bioenergetics. The liver, kidney and pancreas are recognized as the main organs of creatine synthesis, with GAA concentrations varied in the range of 0.5 and 1 mM across specific tissues (Wyss & Kaddurah-Daouk 2000). Creatine-synthesizing forerunner is also available in the brain, with GAA detectable in the grey and white matter of healthy humans at levels of ~ 0.2 mM (Ostojic & Ostojic 2018). Another energy-consuming organ, the skeletal muscle, has also been suggested as a possible site of GAA-to-creatine synthesis (Daly 1985). However, specific studies are rather rare, with no evidence available concerning the GAA availability in the human skeletal muscle.

In this report, we conducted a secondary analysis of previously completed trials (Ostojic *et al.* 2016a; Ostojic *et al.* 2016b) aimed to determine the effects of GAA loading on markers of muscle bioenergetics in healthy men using 1.5 T proton magnetic resonance spectroscopy (MRS) studies. For this communication, MRS spectra from 10 healthy men (age 23.5 ± 3.1 years) were additionally analyzed for GAA concentrations in the vastus medialis muscle using TARQUIN 4.3.10 software package at the baseline and following GAA supplementation. We evaluated quantitatively 30 spectra for presence of GAA peak at 3.8 ppm. The TARQUIN algorithm performs a fully automated fit to the data using a predefined basis set, with a detection limit for GAA *in vivo* was ~ 0.1 μ M (Ostojic & Ostojic 2018). A detailed information of the methods used, including participants details, ethical standards, and GAA dosages administered, has been described previously (Ostojic *et al.* 2016a; Ostojic *et al.* 2016b). In short, volunteers were supplemented with up to 4.8 grams of GAA daily for 8 weeks, with metabolic profiles monitored at baseline, 4-wk and 8-wk intervals.

No detectable GAA was found in the vastus medialis muscle of healthy men at baseline nor at follow-up. An algorithm employed revealed average GAA levels < 0.1 μ M at the baseline, with no relevant peaks were noted in MR spectra at 3.8 ppm corresponding to GAA in any participant (Figure 1). Exogenous GAA administration demonstrated no effect on muscle GAA levels, with GAA remained below detection limit in our cohort of healthy men.

- Figure 1 about here -

The present analysis implies deficient GAA availability in the human skeletal muscle, suggesting an absent or negligible potential for creatine synthesis from GAA inside this tissue, even after GAA loading. GAA drives creatine synthesis, with muscle creatine improved for $\sim 8.4\%$ after oral GAA intake (Ostojic *et al.* 2016b), yet it appears that organs other than the skeletal muscle play a role in this process. A limited GAA transport from the circulation to the muscle cell due to transporter specificity might account for poor GAA availability (Joncquel-Chevalier Curt *et al.* 2015), rather than a shortage of guanidinoacetate *N*-methyltransferase (GAMT), an enzyme that catalyzes the methylation of GAA to creatine. The specific activity of GAMT in the muscle appears to be low (Wyss & Kaddurah-Daouk 2000) yet enough to synthesize all creatine needed in this tissue (Daly 1985). Therefore, a low GAA bioavailability might be due to the possible competition between circulating GAA and creatine for mutual membrane transporter (SLC6A8) (Tachikawa *et al.* 2009), with creatine as a high-affinity substrate fends off GAA

from the muscle cell. Muscle GAA levels also appeared to be immune to GAA supplementation, with creatine has been rather synthesized elsewhere and delivered to the muscle afterwards. Further well-powered confirmatory trials are highly warranted, but also studies with labeled GAA to characterize its kinetics in physiological conditions and during interventional trials. In addition, the use of more powerful imaging systems in future studies (e.g. 3.0 T proton MRS) might provide a sufficient resolution to determine GAA levels in the skeletal muscle (also brain), specifically for mild-to-moderate changes in tissue GAA concentrations after dietary interventions. Finally, supplemental GAA must be carefully scrutinized in the subsequent bioavailability trials due to its secondary effects, including methyl group consumption and homocysteine overproduction (Ostojic *et al.* 2016b)

Conflict of Interest

The authors report no conflicts of interest associated with this manuscript.

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FIGURE CAPTION

Fig 1. An example of magnetic resonance spectra for vastus medialis muscle metabolites illustrates no detectable peak for guanidinoacetic acid (GAA) at 3.8 ppm (red arrow) at baseline (Panel A), also at 4-week (Panel B), and 8-week follow-up (Panel C) in a 23-year old young men. Cho – choline, Cr- creatine, Lip – intramyocellular lipids.

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