Dietary creatine-guanidinoacetate acutely improves circulating creatine levels and running performance in healthy men and women

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To the editor,

Creatine is considered a conditionally essential nutrient that plays a critical role in sustaining high-energy phosphate bioenergetics across the human body (1). Several recent studies demonstrated an exercise-induced impairment in creatine status, with circulating levels of guanidinoacetate (GAA, a direct biosynthetic precursor of creatine) particularly sensitive to heavy exercise (2,3). Interestingly, females appear more prone to creatine and GAA depletion due to lower endogenous creatine stores compared to males, and higher excretion of those compounds by the kidneys (4), making this subpopulation especially eligible for a replenishment. Supplying dietary GAA and creatine before exercise has been anecdotally suggested as a viable strategy to prevent creatine reduction. However, no studies to date have evaluated the potency and gender-specific response of this combination in humans. In this pilot trial, we assessed the effects of a single-dose creatine-GAA intake on creatine biomarkers during a running-to-exhaustion test in young men and women.

A total of sixteen (n = 16) young men and women (age 22.8 ± 1.9 years, body mass index 23.8 ± 2.8 kg/m^2; eight females) signed an informed consent to voluntarily participate in this open-label interventional trial. All participants were non-vegetarian, free from acute and chronic disorders, and had not consumed any dietary supplements for at least four weeks prior to the study. During the trial, all participants were assessed on two occasions separated by a 7-day interval. At the first laboratory visit, participants were subjected to an incremental running-to-exhaustion treadmill test, with breath-to-breath metabolic assessment conducted during the test (Quark CPET, Cosmed, Rome, Italy). The test commenced with a 3-minute warm-up walk at 5 km/h, followed by running at 8 km/h with a workload increment rate of 1.5 km/h every 60 seconds until reaching the point of exhaustion. For a second visit, a mixture containing 1 gram of creatine monohydrate and 1 gram of creatine (CreGAAtine^{TM}, Carnomed, Novi Sad, Serbia) was consumed 30 minutes prior to the exercise test. All measurements were conducted between 07:00 and 10:00 after an overnight fast, and participants refrained from engaging in heavy exercise within the previous 24 hours. Blood samples were collected from an antecubital vein into a gel vacutainer before and immediately after exercise at each lab session. The blood samples were analyzed for GAA, creatine, and creatinine using a modified liquid chromatography–tandem mass spectrometry method (Agilent 1200 Series LC System, Agilent Technologies Inc., Santa Clara, CA, USA), as described previously (5).

Exercise itself induced no statistically significant drop in creatine biomarkers across the sample (P > 0.05), yet a strong trend was reported for reduced serum GAA (8.1%) and creatine levels (30.3%) after exercise in a female and male subsample, respectively. Dietary intake of creatine-GAA mixture instigated a significant elevation in serum GAA by 62.4% (95% confidence interval [CI], from 37.0 to 87.8) (P < 0.0001). This was accompanied by a remarkable rise (660.3%) in serum creatine levels at post-exercise follow-up (95% CI, from 383.9 to 936.7) (P < 0.0001). Serum

creatinine remained unaffected by the intervention ($P > 0.05$). The creatine-GAA mixture non-significantly affected the running performance across the whole sample by extending the time to exhaustion by 4 sec (95% CI, from -7 to 15), raising anaerobic threshold for 1.2 units (95% CI, from -0.5 to 2.9), and improving end-test oxygen uptake by 0.8 ml/kg/min (95% CI, from -0.5 to 2.1) ($P > 0.05$). Still, the magnitude of improvement after creatine-GAA intake was more pronounced in a female subsample ($P < 0.05$) for time to exhaustion (7 sec, 95% CI, from -7 to 15), anaerobic threshold (2.7 units, 95% CI, from 0.9 to 4.5), and oxygen uptake by 1.6 ml/kg/min (95% CI, from 0.4 to 2.8). In a subsample of men, no significant changes for running performance indices were demonstrated after creatine-GAA intake ($P > 0.05$), with time to exhaustion increased by 1 sec (95% CI, from - 66 to 68), and anaerobic threshold and oxygen uptake decreased for 0.3 units (95% CI, from - 4.1 to 3.6) and 0.1 ml/kg/min (95% CI, from - 5.8 to 5.6), respectively. No participants reported any side effects of the intervention.

The present trial demonstrated a significant impact of supplemental creatine-GAA consumed immediately before exercise on circulating GAA and creatine levels in young adults, with women benefiting more from the mixture to acutely improve running-to-exhaustion indices. A single-dose supplementation with GAA and creatine could be suggested as a safe pre-exercise ergogenic formulation for upholding creatine status. This trial corroborates previous research where medium-term co-administration of GAA and creatine positively affected creatine metabolism and exercise performance in young men (6). Our findings extend previous research by using a more brief protocol of supplementation while addressing gender-specific responses in creatine biodynamics. Interestingly, a comparatively low dosage of creatine and GAA used here (one gram of each component) appears capable to acutely augment creatine status perhaps due to favorable bioavailability and transport kinetics of this nutritional combination (7). Further randomized controlled trials are required to validate our findings by assessing short-term uptake and transfer of exogenous GAA and creatine across target organs (*e.g.*, the kidneys, liver, skeletal muscle, brain) while accounting for gender-specific impact(s) of sex hormones on creatine metabolism. Whether the mixture triggers creatine-independent mechanisms to improve performance, such as insulin stimulation, arginine sparing and antioxidant support (8), also necessitates scientific inquiry. In addition, investigating the effects of medium- to long-term supplementation is warranted to assess the pharmacovigilance of GAA-creatine combination, and ergogenic capacity for other components of physical fitness and exercise performance (*e.g.*, metabolic fitness, body composition, muscular strength and endurance, flexibility, skill-related fitness components).

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