

Does Dietary Provision of Guanidinoacetic Acid Induce Global DNA Hypomethylation in Healthy Men and Women?

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Keywords

Guanidinoacetic acid · Supplementation · Creatine · DNA methylation · Homocysteine · 5-Methylcytosine

Abstract

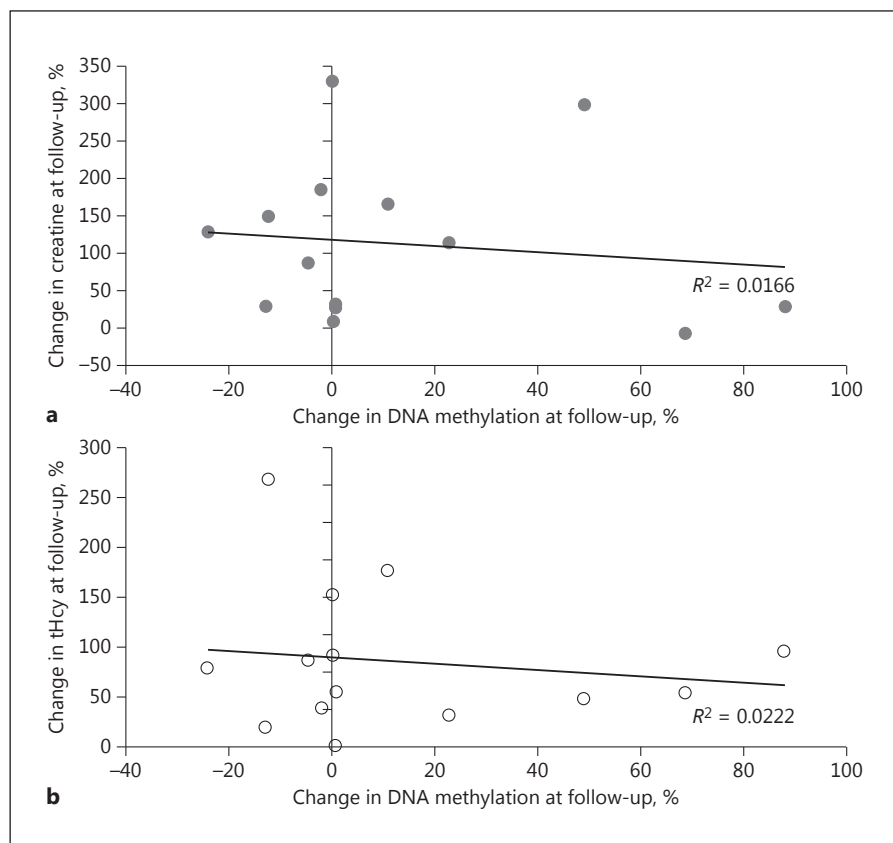
Background/Aims: Guanidinoacetic acid (GAA) is an experimental dietary additive and has been reported to induce methyl depletion when provided by the diet. However, no study evaluated whether supplemental GAA affects DNA methylation, a critical epigenetic process for genome regulation. **Methods:** In this open-label, repeated-measure interventional trial, we evaluated the impact of 12 weeks of GAA supplementation on global DNA methylation in 14 healthy participants (8 women and 6 men, age 22.2 ± 2.3 years, body mass index 24.8 ± 5.7). **Results:** Dietary provision of GAA had no effect on global DNA methylation, with 5-methylcytosine (m^5C) nonsignificantly increased by 13.4% at postadministration when averaged across participants (95% confidence interval -5.5 to 32.3 ; $p = 0.26$). Notable DNA hypomethylation (corresponding to a 5% drop in m^5C) was found in 3 of 14 participants at follow-up. **Conclusion:** Global DNA methylation seems to be unaltered by dietary provision of 3 g of GAA per day for 12 weeks in healthy men and women.

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Introduction

DNA methylation is a critical epigenetic process for genome regulation without changing the sequence, with abnormal hypomethylation of DNA having been implicated in many cardiometabolic diseases, cancer, and aging [1]. Studies to date suggest that certain dietary components may affect genomic and gene-specific DNA methylation levels in systemic and target tissues, affecting genomic stability and transcription of tumor suppressors and oncogenes [2]. It appears that DNA methylation can be modulated by dietary levels of methyl donor components (such as choline or folic acid), with low folate being associated with decreases in global DNA methylation and concomitant pathologies [3]. On the other hand, limited data are currently available concerning a link between methyl group acceptors provided by the diet and DNA methylation. Guanidinoacetic acid (GAA), an experimental dietary additive, is a natural precursor of creatine and a strong methyl group acceptor [4]. In this study, we evaluated the impact of a 12-week GAA supplementation on global DNA methylation in healthy men and women.

Fig. 1. Correlation between changes in blood global DNA methylation and changes in levels of plasma creatine (a) and homocysteine (tHcy) (b) during the study. Each point indicates 1 participant.



Methods

Fourteen apparently healthy young volunteers (8 women and 6 men, age 22.2 ± 2.3 years, body mass index 24.8 ± 5.7 , baseline plasma creatine 30.2 ± 9.7 $\mu\text{mol/L}$, total homocysteine [tHcy] 6.9 ± 1.6 $\mu\text{mol/L}$) were assigned to receive 3 g per day of oral GAA for 12 weeks in this open-label, repeated-measure interventional trial. The primary outcome was the change in blood global DNA methylation assessed at baseline and at 12 weeks using MethylFlash™ Methylated DNA Quantification Kit (Epigentek, New York, NY, USA); the input DNA for 5-methylcytosine (m^5C) assay was 100 ng. Secondary outcomes were plasma creatine and tHcy.

Creatine was measured by high-performance liquid chromatography with fluorimetric detection (Hewlett-Packard, Palo Alto, CA, USA), and tHcy was determined by chemiluminescent immunoassay method (DPC Immulite 2000, Siemens, Germany). All volunteers were required to maintain their usual diets (apart from GAA intervention) and physical activity levels during the trial. All participants gave written informed consent before study inclusion, and the study was approved by the local internal review board in accordance with the Declaration of Helsinki.

A two-tailed paired *t* test was used to examine significant differences in participant response during the intervention (baseline vs. 12 weeks). The data were analyzed using the statistical software SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was established at $p < 0.05$.

Results

All participants completed the trial; compliance was determined to be $86.3 \pm 11.3\%$ based on counting unused sachets at 12 weeks. Dietary provision of GAA had no effect on global DNA methylation, with m^5C being nonsignificantly increased by 13.4% at postadministration when averaged across participants ($0.26 \pm 0.07\%$ at baseline vs. $0.28 \pm 0.04\%$ at follow-up, $p = 0.26$; 95% confidence interval [CI] for percent change -5.5 to 32.3). Notable DNA hypomethylation (corresponding to a 5% drop in m^5C) was found in 3 of 14 participants at follow-up. Plasma creatine and tHcy levels significantly increased from before to after GAA ingestion by 112.9% (95% CI 51.9 to 173.9; $p = 0.001$) and 85.8% (95% CI 44.7 to 126.9; $p < 0.001$), respectively. No significant correlation was found between changes in DNA methylation and changes in creatine ($r = 0.13$) or tHcy levels ($r = 0.15$) during the study ($p > 0.05$) (Fig. 1).

Discussion

Supplying a methyl group acceptor, such as GAA, induces methyl depletion during its conversion to creatine [5]. This dietary GAA-driven consumption of methyl groups can alter many physiological methylation pathways [4]. A lack of methyl groups may hypothetically decrease global DNA methylation, a stable epigenetic attribute of gene regulation.

The results of the present study support a link between dietary GAA and increased methylation, as evidenced by augmented creatine and tHcy output. However, global DNA methylation seems to be unaltered by dietary provision of 3 g of GAA per day for 12 weeks in healthy men and women. Although GAA induced a notable rise (85.8% corresponding to 5.3 mmol/L) in plasma tHcy, an indicator of methylation pathway alteration, it appears that the DNA methylation pattern remained stable. This indicates that there is no direct utilization of methyl groups from DNA for creatine synthesis and no consequent DNA hypomethylation-related gene silencing induced by this nutrient. A poor correlation between changes in m⁵C and elevation in tHcy (also creatine) additionally implies a distinction between the effect of GAA on creatine methylation and on DNA methylation, with approximately 1 in 5 participants developing global DNA hypomethylation during the intervention. Nevertheless, increased methylation demand caused by dietary GAA perhaps affects other biological methylation pathways, including amino acid and protein methylations, or polysaccharide methylation. This requires further investigation, employing randomized double-blind, placebo-controlled trials,

to elucidate metabolic consequences of an exposure to methyl group-consuming compounds such as GAA.

For this study, volunteers were required to maintain their usual diets, yet no direct control for GAA content in food was employed here. However, the uptake of GAA through the diet was considered negligible (e.g., meat GAA <24 mg/kg) [6]. Nevertheless, the high interindividual variability in DNA methylation found at follow-up indicates a possible effect of dietary GAA in a subset of individuals. Future intervention studies examining GAA should account for personal characteristics of participants, including age, gender, body mass index, and acute and chronic diseases status.

In conclusion, dietary provision of GAA did not result in a significantly lower DNA methylation at 12 weeks among healthy young men and women. Further research is necessary to assess metabolic consequences of increased methylation after GAA intake, as manifested by elevated serum homocysteine levels.

Acknowledgements

This project was partly supported by the Serbian Ministry of Education, Science and Technological Development (grant No. 175037, 173051), the Provincial Secretariat for Higher Education and Scientific Research (grant No. 114-451-710), the Faculty of Sport and Physical Education, University of Novi Sad, and the Center for Health, Exercise and Sport Sciences, Belgrade.

Disclosure Statement

There are no conflicts of interest related to this study.

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