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# Single-dose oral guanidinoacetic acid exhibits dose-dependent pharmacokinetics in healthy volunteers

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ABSTRACT

Guanidinoacetic acid (GAA), the natural precursor of creatine, has potential as a dietary supplement for human nutrition, yet no data are available regarding its dose-dependent pharmacokinetic (PK) behavior. We hypothesized that a single dose of orally administered GAA exhibited dose-dependent PK behavior in healthy volunteers. Forty-eight young adults were enrolled in a randomized, placebo-controlled, double-blind, parallel-group trial to receive single oral doses of GAA (1.2, 2.4, and 4.8 g) or a placebo. Pharmacokinetic metrics for plasma GAA and creatine were assessed immediately before (0 hours) and at 1, 2, 4, 6, 8, 12, and 24 hours after GAA ingestion. The lag time appeared to be similar after the bolus ingestion of GAA (0.14 ± 0.17 hours for low-dose GAA, 0.31 ± 0.18 hours for medium-dose GAA, and 0.38 ± 0.32 hours for high-dose GAA; $P = .05$). An increase in the area under the concentration-time curve for plasma GAA was found for the dose range tested, with 2.4- and 9.3-fold increases in the area under the concentration-time curve for every 2-fold increase in the GAA dose ($P < .0001$). No differences were found for elimination half-time between the low-dose and medium-dose groups (<1.75 hours), whereas the elimination half-time was significantly longer (>2.1 hours) for the high-dose GAA regimen ($P = .001$). The volume of distribution was affected by the dosage of GAA applied (102.6 ± 17.3 L for low-dose GAA, 97.5 ± 15.7 L for medium-dose GAA, and 61.1 ± 12.7 L for high-dose GAA; $P < .0001$). Ingestion of GAA elevated plasma creatine by 80%, 116%, and 293% compared with the placebo for the 1.2, 2.4, and 4.8 g doses, respectively ($P < .0001$). Guanidinoacetic acid single-dose PK metrics were nonlinear with respect to dose size. Across the dose range of 1.2 to 4.8 g, systemic exposure to GAA increased in a greater than dose-proportional manner.



## 1. Introduction

Guanidinoacetic acid (GAA; also known as glycocyamine or guanidinoacetate) is a nitrogenous organic acid that occurs naturally in the human body. The primary biological role of GAA is to serve as a direct precursor of creatine, an energy carrier, and mediator in the cell [1]. Guanidinoacetic acid is synthesized endogenously from L-arginine and glycine, mainly in the kidney [2]. Under certain circumstances (eg, kidney failure, exercise-related GAA depletion, and deficient diet),

*Abbreviations:* AUC, area under the concentration-time curve; GAA, guanidinoacetic acid; PK, pharmacokinetic.

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supplemental GAA may be "semiessential," optimizing the body's energy use when the endogenous synthesis of GAA is limited [3]. Because GAA is more bioavailabile, stable, and cost-effective than creatine [4], it might be more suitable for use in human nutrition. In particular, GAA is more soluble in water then creatine (3600 mg/L for GAA vs 1330 mg/L for creatine). Guanidinoacetic acid is highly stable [5], whereas creatine is rapidly metabolized into creatinine when it comes into contact with water at elevated temperatures and low pH [6]. Finally, the cost of GAA powder is approximately 40% lower than the cost of creatine [7,8]. Although the first human use of supplemental GAA was reported more than 60 years ago, there is a little information available on its turnover, bioavailability, or elimination.

Previous studies of dietary GAA reported its "creatine recovery effect" in both athletes and clinical patients. Studies have found that orally ingested GAA administered over the course of 6 weeks increases plasma GAA and creatine by up to 50% in male and female collegiate athletes, with no major adverse effects reported [9]. Studies have shown that supplemental GAA may increase homocysteine formation [10], although the effects of increased homocysteine on health status seem to be clinically insignificant [9], and can be corrected by homocysteine removal mechanisms [11]. Dietary GAA has been found to be beneficial for patients with heart disease, neuromuscular disorders, and chronic renal failure [12]. Scientific research has been limited with regard to the behavior of dietary GAA between the time of ingestion and subsequent elimination from the human body. A Japanese group was the first to describe the timeline of plasma concentrations and urinary excretions of GAA in humans after a single oral dose of 1 g of GAA [13]. The study suggested that GAA was absorbed well, with peak plasma GAA occurring after 2 hours (~600 μg/dL) and extra GAA excreted in the urine at a rate of up to 40 mg/h. We recently reported that an acute oral dose of GAA has a notable effect on plasma GAA, creatine, creatinine, total homocysteine, and excretion of urinary GAA and creatine in 20 healthy volunteers [3]. In our study, supplemental GAA was found to be readily bioavailable and rapidly transformed to creatine within the first 2 hours. Neither study described relevant pharmacokinetic (PK) metrics (eg, lag time, volume of distribution, elimination half-life, and clearance) after GAA ingestion, and no studies have examined the dose-dependent PK of oral GAA. Exploratory PK studies with increasing doses of GAA are needed to describe its biochemical behavior and to decide whether GAA has scientific merit for further development as a dietary supplement for humans.

This study tested the hypothesis that single-dose, orally administered GAA would exhibit dose-dependent PK in healthy men and women. To test this hypothesis, we conducted a double-blind, placebo-controlled, randomized, parallel-group intervention trial, and evaluated PK metrics for plasma GAA and creatine to assess the acute effects of 1.2, 2.4, and 4.8 g GAA loads in healthy men and women. The study was designed as a phase I, first-in-humans trial to establish whether oral GAA behaves in human subjects as preclinical studies would suggest. Assessment of oral GAA dose escalation and its absorption, distribution, biotransformation, and elimination is a necessary step to clinically evaluate GAA as a potentially useful health intervention.

## 2. Methods and materials

### 2.1. Study population

Candidates for inclusion in the study were moderately physically active men and women between 20 and 25 years old. Potential participants were not admitted to the study if they met any of the following criteria: (1) a history of metabolic disease, (2) known heart disease, (3) use of any performance-enhancing drugs or dietary supplements within the 60 days before the study commenced, (4) smoking, and (5) pregnancy. Participants were fully informed both verbally and in writing about the nature and demands of the study and the known health risks, and they provided informed consent regarding their voluntary participation in the study. The participants completed a health history questionnaire covering cardiovascular disease risk factors, history and present status regarding any signs and symptoms suggestive of cardiovascular disease, history of chronic illnesses, history of surgeries and hospitalizations, history of any musculoskeletal or joint injuries, past and present habits that could affect health, current medication use, family health history, and other health history information [14]. All participants completed routine prescreening including blood and urine profiling, and they received a general medical examination during the initial recruitment. If any specific markers (eg, liver and muscle enzymes, kidney function) were above the reference values, subjects were excluded from the study. Upon initial recruitment, 48 participants (n = 48; 24 men and 24 women) met the criteria to participate in the study; the total number of participants fulfilled the optimal sample size (see below). The mean physical characteristics of participants were as follows: age 22 ± 2 years, weight 71 ± 14 kg, and height 176 ± 10 cm. Participants' nutrition was monitored before the experiment began using a standardized questionnaire [15] and computed using NutriBase software (CyberSoft Inc, Phoenix, AZ, USA). Approval of the local institutional review board (No. 05/A-2010/014) was obtained, and all the procedures performed were in accordance with the Declaration of Helsinki and the principles set forth in the Guidelines for Good Clinical Practice [16].

### 2.2. Experimental protocol

Participants were randomized according to a computer-generated list in a double-blind, parallel-group design to receive single oral doses of GAA (1.2, 2.4, and 4.8 g) or a placebo (inulin). The GAA dosages chosen were the equimolar equivalent to dietary creatine amounts that appear to be therapeutically effective [17]. The placebo was administered as a control treatment to monitor the changes in GAA and creatine plasma concentrations during the day, especially the effects of creatine-containing foods on plasma concentrations. Twelve participants were allocated to each intervention group, with women having an equal probability of assignment to the groups. Groups were matched for participants' age, weight, and daily energy intakes. The participants received standardized meals on the day before the experiment. In the 24 hours before the tests, the subjects did not participate in any prolonged exercise and were not permitted to drink alcoholic or caffeinated beverages. On the day of the experiment, each participant arrived at the laboratory at 9:00 AM