The acute effect of beta-guanidinopropionic acid versus creatine or placebo in healthy men (ABC-Trial): A randomized controlled first-in-human trial

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AIMS
Increasing evidence indicates that the ATP-generating enzyme creatine kinase (CK) is involved in hypertension. CK rapidly regenerates ATP from creatine phosphate and ADP. Recently, it has been shown that beta-guanidinopropionic acid (GPA), a kidney-synthesized creatine analogue and competitive CK inhibitor, reduced blood pressure in spontaneously hypertensive rats. To further develop the substance as a potential blood pressure-lowering agent, we assessed the tolerability of a sub-therapeutic GPA dose in healthy men.

METHODS
In this active and placebo-controlled, triple-blind, single-centre trial, we recruited 24 healthy men (18–50 years old, BMI 18.5–29.9 kg m⁻²) in the Netherlands. Participants were randomized (1:1:1) to one week daily oral administration of GPA 100 mg, creatine 5 g, or matching placebo. The primary outcome was the tolerability of GPA, in an intent-to-treat analysis.

RESULTS
Twenty-four randomized participants received the allocated intervention and 23 completed the study. One participant in the placebo arm dropped out for personal reasons. GPA was well tolerated, without serious or severe adverse events. No abnormalities were reported with GPA use in clinical safety parameters, including physical examination, laboratory studies, or 12-Lead ECG. At day 8, mean plasma GPA was 213.88 (SE 0.07) in the GPA arm vs. 32.75 (0.00) nmol l⁻¹ in the placebo arm, a mean difference of 181.13 (95% CI 26.53–335.72).

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CONCLUSION
In this first-in-human trial, low-dose GPA was safe and well-tolerated when used during 1 week in healthy men. Subsequent studies should focus on human pharmacokinetic and pharmacodynamic assessments with different doses.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT
• Plasma activity of the creatine kinase (CK), the energy enzyme that rapidly regenerates ATP from phosphocreatine, is associated with blood pressure in the general population; a crude increase of 14 mm Hg in systolic pressure per log CK increase.
• High CK activity precedes the development of hypertension in animal and human studies.
• CK inhibition with beta-guanidinopropionic acid (GPA), a kidney-synthesized creatine analogue, lowers blood pressure in an animal hypertension without apparent side effects.

WHAT THIS STUDY ADDS
• This first-in-human study in healthy men, who orally ingested a daily dose of 100 mg GPA for 1 week raised no safety or tolerability concerns, including no adverse effects reported and no significant differences detected compared to baseline or placebo in physical examination, biochemistry or cardiovascular function, including blood pressure, cardiac contractility and QT interval.

Introduction
There is increasing evidence that creatine kinase (CK, EC 2.7.3.2) is intimately involved in the generation of blood pressure [1–6]. CK catalyses the rapid and reversible transfer of a phosphoryl group from creatine phosphate to ADP, thereby forming creatine and ATP: [1–6]

\[
\text{Creatine phosphate} + \text{MgADP} \leftrightarrow \text{Creatine} + \text{MgATP}
\]

Cytosolic CK is tightly bound in the immediate proximity of ATP-utilizing enzymes such as Na⁺/K⁺-ATPase, Ca²⁺-ATPase and myosin ATPase. Here, ATP synthesized by CK is preferentially used to fuel highly energy-demanding processes such as sodium retention, cardiovascular contractility, as well as remodelling of arteries, promoting high blood pressure [1–6]. We have shown in a random, multi-ethnic population sample that plasma CK activity after rest, a surrogate measure of tissue CK, is a main predictor of blood pressure, with a crude increase in blood pressure of 14 mm Hg systolic per log CK increase [2]. Although plasma and tissue CK activity were found to be higher in men, persons of African ancestry and obese patients [2, 7], the association was independent of sex, body mass index (BMI), or ethnicity, and has been replicated by others [2, 4].

Importantly, in accordance with a causal relationship, evidence indicates that high tissue or resting plasma CK precedes hypertension in animal models and in humans [6, 8, 9]. In addition, intracellular CK inhibition substantially reduces contractility of human resistance arteries ex vivo [3]. Furthermore, vascular CK gene expression is strongly associated with clinical blood pressure in humans [5], and high resting plasma CK is found to be a main predictor of failure of antihypertensive therapy in the general population [10]. Thus, CK inhibition might lower blood pressure. Recently, we showed in a randomized control trial that the creatine analogue and competitive CK inhibitor beta-guanidinopropionic acid (GPA) significantly reduced blood pressure in spontaneously hypertensive rats [11]. GPA is synthesized in the kidney in vivo, and elaborate studies in animals of different species and human cell lines indicate the safety profile [12, 13] Although the substance is used by sportspersons to increase stamina and lose weight, to our knowledge, there are no human data available for this potential blood pressure lowering agent. Therefore, we assessed the tolerability of a sub-therapeutic dose of GPA in healthy men for 1 week.

Methods

Trial design
The full study protocol with detailed study procedures has been published previously [12]. In brief, we conducted a randomized, placebo and active controlled, triple blind, parallel group, single-centre exploratory clinical trial, with three arms: GPA, creatine and placebo. Trial location was the Academic Medical Center of the University of Amsterdam, The Netherlands. The publication of this trial adheres to the Consolidated Standards of Reporting Trials (CONSORT) 2010 statement [14].

Participants
We included healthy, non-smoking, non-vegetarian men aged 18–50 years, with a normal, non-obese body mass (BMI 18.5–29.9 kg m⁻²). Inclusion period was from March 2014 to March 2015. Exclusion criteria included high blood pressure defined as systolic ≥140 mmHg or diastolic ≥90 mmHg or the use of antihypertensive drugs, (history of) cardiovascular disease including transient ischaemic attack and stroke; the use of plasma CK-increasing drugs including statins; use of acetylsalicylic acid or nonsteroidal anti-inflammatory drugs in the 2 weeks prior to the first visit; neuromuscular or endocrine disorders; vasculitis; HIV infection; infectious hepatitis; personal or family history of bleeding disorders; sickle cell anaemia or other hereditary anaemia; current use or use within 2 months prior to start of the trial of creatine or other guanidino compounds; and abnormalities in glucose, lipid spectrum, thyroid, kidney or liver biochemistry parameters in the plasma. To stabilize and standardize plasma CK activity during the trial, participants were instructed to...
refrain from intensive physical exercise 3 days prior to the baseline visit or during the intervention in the first week [2, 12]. All study participants gave written informed consent, and the full study protocol was approved by the AMC Amsterdam Medical Ethics Review Committee on 25 November 2013 (MERC reference number 38368.018.12) [12]. The study complies with the Declaration of Helsinki (64th World Medical Assembly, General Assembly, Fortaleza, Brazil, October 2013).

**Intervention**

Trial procedures are summarized in Figure 1 [12]. In brief, participants came to the hospital in the morning after an overnight fast, in six visits over a period of 8 days, when medical history was obtained and physical and laboratory examinations were performed. To ensure intervention adherence, trial supplements were ingested by the volunteer in the presence of the trial staff during the hospital visits. In addition, we used pill counts for the supplements ingested at home. At visit 1 (day 0), after baseline measurements (haemodynamic and laboratory assessments), participants were included and randomized. On the next day at visit 2 (day 1, first intake of trial supplements), participants ingested the randomized, blinded intervention of 100 mg GPA, 5 g creatine, or placebo during the visit. At visit 3 (day 2 of trial supplements), haemodynamic and laboratory tests were performed, trial supplements were ingested, and participants received trial supplements to ingest at home on days 3, 4, 5 and 6 after an overnight fast. The participants returned to the hospital for visit 4 at day 7 of the intervention, and ingested the final trial supplements during the visit. They returned for visit 5 on day 8 for haemodynamic measurements and laboratory tests. The sixth and final visit was at day 21, for the final assessment of tolerability.

**GPA**

GPA or N-(aminoiminomethyl)-beta-alanine; (C₄H₉N₃O₂), is a structural isomer of creatine [12, 13]. GPA is generated *in vivo* in the kidney via transamidination of β-alanine. The physiological concentration in human plasma is reported to range from trace amounts to 1.40 μmol l⁻¹. Clearance is probably renal, akin to creatine, creatinine, and other guanidino compounds. GPA acts as a competitive inhibitor of cellular creatine uptake, and attenuates the flux through the cytoplasmic creatine kinase reaction. The effect on animal models, using different species, and human cells has recently been summarized [13]. Briefly, GPA decreased intracellular creatine and phosphocreatine in all tissues studied. In skeletal muscle, this effect induced a shift from glycolytic to oxidative metabolism, increased cellular glucose uptake and increased fatigue tolerance. In heart tissue this shift to mitochondrial metabolism was less pronounced. Myocardial contractility was modestly reduced, including a decreased ventricular developed pressure, albeit with unchanged cardiac output. Brain tissue adaptations in energy metabolism resulted in enhanced ATP stability and survival during hypoxia [13]. Despite the lack of human data on efficacy and side effects, GPA is available as a food supplement, and is used by sportspersons to induce endurance capacity and promote weight loss [12, 13].

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**Figure 1**

Trial overview. The duration of the intervention was 7 days. The trial supplements started at day 1, after baseline measurements, inclusion and randomization at day 0 (baseline). After 24 h and after 7 days of trial supplements (day 8), the baseline measurements were repeated. The last visit was at day 21, to assess potential side effects [12].
Manufacturing and testing

GPA and creatine were considered by the MERC to be food supplements as previously described [12]. GPA was ordered from Sequoia (Sequoia Research Products, Oxford, UK). GPA, creatine and identical placebo capsules were manufactured by the Pharmacy & Pharmacology Department of the Slotervaart Hospital, Amsterdam, The Netherlands. This department is GMP certified (ISO 9001:2001). The substance was tested for purity and for cyanide compounds. We established in our certified tests a purity of more than 99% (detection limit) and a cyanide level lower than 1 ppm (detection limit) [12].

Creatine

Creatine, which has an identical molecular formula to GPA, was chosen to assess the effect of the synergist. The average daily rate of creatine synthesis in healthy omnivorous males is estimated to be 1.3 g [12]. We used 5 g as recommended in studies on creatine supplementation. No side effects are apparent at this dose [12].

Dose calculation

We followed the Food and Drug Administration (FDA) ‘Guidance on Estimating the Maximum Safe Starting Dose in Initial Clinical Trials in Adult Healthy Volunteers’, to calculate the maximum recommended starting dose for this first-in-human clinical trial [15]. The purpose of this process is to ensure the safety of the human volunteers. Toxicity should be avoided at the initial clinical dose. However, doses should be chosen that allow reasonably rapid attainment of phase 1 trial objectives. The major elements of this process are as described previously: [12, 15]

- Determination of the no observed adverse effect levels (NOAELs) in the tested animal species
- Conversion of NOAELs to human equivalent doses (HED)
- Application of a safety factor

No observed adverse effect level (NOAEL) determination. In animal studies, GPA was administered through the diet in concentrations of 1% or more over 8 weeks without apparent adverse effects. In animals weighing 200 g and eating 20 g per day, we calculated a ‘no observed adverse effect level’ of 1000 mg kg⁻¹ day⁻¹. Furthermore, in a patent application, Meglasson et al. recommended a human dose of 1–500 mg kg⁻¹ day⁻¹ based on his research in mice and rhesus monkeys. In this paper, rhesus monkeys weighing 9 kg were treated with oral GPA 48 mg kg⁻¹ day⁻¹ (432 mg per monkey per day) over 2 weeks without apparent adverse events [12].

Conversion of the NOAEL to HED. We converted the oral NOAELs in rats and monkeys (1000 mg kg⁻¹ day⁻¹ and 48 mg kg⁻¹ day⁻¹, respectively) to oral HEDs based on an algorithm proposed by the FDA based on body surface area. This algorithm proposes a conversion factor from rat to human of 0.16 times the rat dose; and of monkey to men of 0.32 the monkey dose (in mg kg⁻¹ day⁻¹; for a man of 60 kg) resulting in HEDs of 160 mg kg⁻¹ day⁻¹ and 15 mg kg⁻¹ day⁻¹, respectively, for a man of 60 kg [12].

Application of a safety factor. A safety factor should be applied to the HED to increase assurance that the first dose in humans will not cause adverse effects. The use of the safety factor is based on the possibility that humans may be more sensitive to the toxic effects of a substance than predicted by the animal models, that bioavailability may vary across species, and that the models tested do not evaluate all possible human toxicities, or cannot be expressed by animals or easily measured, such as headache or nausea. We conservatively chose 15 mg kg⁻¹ day⁻¹ oral dose for our final calculations of the human dose, because this is the lowest dose, and because of the closer allometric relationship between monkey and man. The FDA advises a safety factor of at least 10. Based on an average weight of a male volunteer of 75 kg, we calculated a starting oral dose for this phase 1 study of 75 × 1.5 mg day⁻¹ = 112.5 mg day⁻¹; so we use 100 mg day⁻¹ [12].

Tolerability health questionnaire

The participants received a questionnaire to assess tolerability at home during the week of intervention and in the 2 weeks after the intervention. The questionnaire encompassed the perceived side effects of the trial supplements, using check boxes and free text space [12].

Haemodynamics and electrocardiography

On day 0 (baseline), day 1 (in the first hour after intake trial supplements), day 2 (after 1 day of trial supplements), and day 8 (after 7 days of trial supplements) of the intervention period, we measured sitting brachial systolic and diastolic blood pressure with an Omron M4 oscillometric device (Omron Healthcare Europe BV, Hoofddorp, The Netherlands) after 5 min of rest with an adjusted cuff size on the left arm, at heart level. In addition, we performed electrocardiography (MAC 5000 Resting ECG System; GE Healthcare; Boston, MA, USA) and ambulatory 24-h blood pressure monitoring (Spacelabs 90217 Ambulatory Blood Pressure Monitor, Spacelabs Inc., Redmond, WA, USA). Furthermore, at baseline, day 2 and day 8, we estimated pulse wave velocity and the augmentation index of the aorta in duplicate after 10 min of supine rest (the mean of these two pulse wave velocity measurements was used for the analysis) with the Arteriograph (Tensiomed Kft, Budapest, Hungary). Finally, we monitored haemodynamics including heart rate, cardiac contractility, cardiac output and total peripheral resistance noninvasively during 5 min in the supine position, using a Nexfin BMEYE device (Amsterdam, The Netherlands).

Laboratory studies

At baseline, we assessed resting plasma CK (after 3 days of rest), glucose, lipid profile, creatinine, liver enzymes (ASAT, ALAT), gamma GT, cardiac troponin, TSH (to exclude subclinical hypothyroidism associated with high plasma CK), sodium, potassium, platelet count, coagulation tests (aPTT, PT) and ADP-induced platelet aggregation (area under curve at final concentrations ADP, see below). Furthermore, we assessed creatinine, sodium and potassium in 24-h urine.
Tests were repeated after 7 days of trial supplements at day 8. Plasma GPA was measured as described previously [16] in participants using GPA vs. placebo at baseline vs. day 8. With the focus on tolerability, further pharmacokinetic studies were considered an unnecessary burden for the participant by the local MERC in this ‘phase 0’ study [12].

**Platelet aggregation test**

We assessed ADP-induced platelet aggregation by light transmittance aggregometry (PAP-8E platelet aggregation profiler, Bio/Data Corporation, Horsham, PA, USA) at baseline and after 7 days of intervention. Citrate-anticoagulated blood (0.32%) was centrifuged (Rotina 420R, Hettich Lab Technology, Tuttingen, Germany) for 15 min at 180 g to obtain platelet-rich plasma. Platelet-poor plasma was prepared by 10 min centrifugation at 1550 g. Experiments were performed at 37°C under stirring conditions. Thrombin receptor-activated peptide (TRAP; final concentration 15 μmol l⁻¹, Bachem, Bubendorf, Switzerland) was used to induce maximum platelet aggregation (100%). ADP and arachidonic acid were used to initiate the platelet aggregation in the test, final concentrations of ADP (0.1, 0.2, 0.5, 1.0, 2.0 μmol l⁻¹, Sigma-Aldrich, St. Louis, MO, USA); arachidonic acid (2 mmol l⁻¹, Sigma-Aldrich, St. Louis, MO, USA). Aggregations were performed with and without the addition of phosphocreatine (CrP 5 mmol l⁻¹ final concentration; Sigma-Aldrich, St. Louis, MO, USA).

**Adverse and serious adverse events**

We did not expect a significant difference in adverse effects between sub-therapeutic GPA and placebo. Although used by sportspersons [12], there are no FDA or other reports in formal or informal sources on the side effects of ingestion of this dose of GPA in animals or humans. Adverse events were to be reported by the included subject spontaneously or through the questionnaire, or observed by the trial staff or health care worker, and when occurred, were to be recorded and judged by the study group and the independent physician. The trial staff was to convert the reported symptoms to a standard lexicon, the Common Terminology Criteria for Adverse Events (CTCAE) [17], to facilitate international scientific reporting. Adverse effects were to be classified based on the FDA guideline [15], as overt toxicity (e.g., clinical signs, macro- and microscopic lesions); surrogate markers of toxicity (e.g., plasma liver enzyme levels); or other adverse effects; and we planned to use the adverse drug reaction probability scale [18] to assess the causal relationship between trial supplement use and any reported adverse event.

**Outcomes**

The primary outcome was the tolerability of 1 week of 100 mg oral GPA daily, as compared to placebo. Secondary outcomes included the comparison of tolerability with creatine, and the effect of 1 week of oral GPA on haemodynamic parameters, including peripheral and central blood pressure, and cardiac contractility as compared to creatine and placebo.

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**Figure 2**

CONSORT flow diagram. Participant’s flow in the study. All eight participants in the placebo arm, including one drop out, were analysed for the primary outcome of tolerability of GPA vs. placebo.
The tertiary outcome was the effect of GPA on biochemical parameters, including ADP-induced platelet aggregation [19], compared to creatine and placebo.

**Sample size**

This is a first-in-human study with GPA, with allometric data available from other species [12]. According to the European Medicines Agency guidelines [20], we included eight subjects in each arm, to assess the tolerability of GPA vs. placebo and creatine over 1 week.

**Randomization**

Randomization was performed by an independent party, the Clinical Pharmacy Unit of the Academic Hospital of the University of Amsterdam, using a computer-generated, non-adaptive, restricted randomization scheme with a 1:1:1 allocation ratio. The pharmacy generated the random allocation list and did not release the randomization code until the data bank had been closed and all outcomes were analysed. Thus, randomization was conducted without any influence of the investigators, outcome assessors or participant characteristics. After assignment to interventions, trial participants, trial staff and the outcome assessor remained blinded to whether the participant was given a placebo or a supplement until after all outcome data had been assessed.

**Data analysis**

The primary outcome was the tolerability of GPA vs. placebo as a descriptive measure, in an intent-to-treat analysis. Because of the small sample size, the distribution of the data could not be formally tested. Since parametric analysis may not be accurate with small sample sizes, and nonparametric analysis may lack power to detect a significant difference, we used parametric statistics for our secondary analysis (i.e., arithmetic mean with SE, unpaired *t*-tests, and one-way ANOVA with the appropriate post-test with Bonferroni correction); and reanalysed the data in a sensitivity analysis with non-parametric methods (i.e., median with interquartile range, Mann–Whitney test, or Kruskal–Wallis test with a Dunn’s post-test). For the secondary outcome, a two-tailed *P*-value < 0.05 was considered statistically significant. We did not adjust the *P*-values for multiple outcomes, but limited

### Table 1

Physical examination and laboratory outcomes

| Parameter                      | Baseline GPA | Creatine | Placebo | Day 8 GPA | Creatine | Placebo |
|-------------------------------|--------------|----------|---------|-----------|----------|---------|
| **BMI, kg m⁻²**                | 24.5 (0.7)   | 22.1 (0.5) | 24.2 (0.6) | 24.4 (0.7) | 22.3 (0.5) | 23.9 (0.8) |
| Haemoglobin, mmol l⁻¹          | 8.5 (1.1)    | 7.6 (4.1) | 8.3 (3.0) | 9.5 (0.1) | 9.6 (0.3) | 9.2 (0.4) |
| Platelets, 10⁹ l⁻¹             | 241 (17.9)   | 261 (10.0) | 215 (22.0) | 246 (19.5) | 263 (7.0) | 258 (23.7) |
| Glucose, mmol l⁻¹              | 5.1 (0.1)    | 4.9 (0.1) | 5.0 (0.1) | 4.9 (0.1) | 4.5 (0.2) | 5.0 (0.1) |
| Creatinine, μmol l⁻¹           | 74.1 (3.4)   | 75.5 (3.2) | 80.6 (3.0) | 71.9 (3.4) | 80.6 (3.9) | 78.6 (4.1) |
| Creatine kinase, U l⁻¹         | 415.6 (198.4) | 178.5 (70.2) | 341.3 (149.5) | 159.9 (38.4) | 121.3 (7.8) | 152.7 (51.0) |
| Gamma GT, U l⁻¹                | 35.4 (9.0)   | 16.4 (1.6) | 24.8 (5.6) | 40.0 (9.7) | 16.1 (1.6) | 22.1 (5.3) |
| ASAT, U l⁻¹                    | 34.5 (10.1)  | 23.8 (1.7) | 30.6 (5.5) | 27.3 (3.4) | 23.0 (2.1) | 26.3 (2.3) |
| ALAT, U l⁻¹                    | 32.8 (8.9)   | 23.5 (3.9) | 30.3 (3.9) | 35.5 (8.9) | 19.1 (1.1) | 27.1 (3.9) |
| Cholesterol, mmol l⁻¹          | 4.6 (0.2)    | 4.0 (0.2) | 4.2 (0.1) | 4.7 (0.2) | 4.1 (0.2) | 4.3 (0.2) |
| HDL, mmol l⁻¹                  | 1.3 (0.1)    | 1.3 (0.1) | 1.4 (0.1) | 1.3 (0.1) | 1.3 (0.1) | 1.3 (0.2) |
| LDL, mmol l⁻¹                  | 2.7 (0.2)    | 2.3 (0.2) | 2.5 (0.2) | 2.8 (0.2) | 2.4 (0.2) | 2.5 (0.2) |
| Triglycerides, mmol l⁻¹        | 1.1 (0.2)    | 0.8 (0.2) | 0.9 (0.2) | 1.4 (0.4) | 1.0 (0.2) | 1.3 (0.3) |
| Troponin, μg l⁻¹               | 0.007 (0.0)  | 0.005 (0.0) | 0.009 (0.0) | 0.007 (0.0) | 0.007 (0.0) | 0.006 (0.0) |
| Urine Na, mmol 24 h⁻¹           | 140.3 (21.8) | 127.5 (18.9) | 182.4 (12.6) | 171.4 (27.2) | 109.5 (19.7) | 162.0 (20.2) |
| Urine K, mmol 24 h⁻¹            | 67.8 (10.3)  | 75.0 (11.1) | 82.0 (12.6) | 67.4 (9.6) | 58.4 (26.1) | 77.6 (14.3) |
| Urine creatinine, μmol kg⁻¹ 24 h⁻¹ | 118.0 (35.1) | 187.2 (44.6) | 134.1 (34.8) | 144.3 (32.7) | 135.7 (32.3) | 113.6 (37.6) |

Data are mean (SE) for baseline and after 7 days of treatment. Biochemistry data are plasma unless stated otherwise. Data concern *n* = 8 men in each treatment arm, except for placebo, which was *n* = 7 at day 8. Mean age in the three treatment arms was 27.4, 22.8 and 25.8 years, respectively. Participants had significant lower BMI and cholesterol in the creatine treatment arm from baseline compared to the GPA arm. There were no significant differences at day 8 between treatment arms. BMI, Body mass index.
formal statistical testing on non-primary outcomes. The nature of missing data was to be analysed and addressed accordingly, using single imputation with unconditional means for data missing completely at random, as assessed through inspection and with the Little's test in SPSS. A sensitivity analysis was to be performed for imputed outcomes. All analyses were performed with SPSS statistical software package for Windows, version 22.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± SE, unless indicated otherwise.

Results

The first participant was randomized in March 2014, and the last follow-up visit was performed in March 2015, as the target population of 24 participants was achieved. Trial flow is depicted in Figure 2. Of 29 volunteers assessed for eligibility, five did not meet the inclusion criteria, because of vegetarianism (n = 1), high blood pressure at baseline screening (n = 1), use of prescription drugs (n = 2), and tobacco use (n = 1). Twenty-four randomized participants received the allocated intervention, and 23 completed the study. One participant dropped out on day 4 in the placebo treatment arm because of an external event in his family. This participant experienced no side effects, including during a re-challenge with the assigned drug. Baseline and day 8 characteristics of all randomized study participants are shown in Table 1. At baseline, there was no significant difference in mean plasma GPA concentration of participants using GPA vs. placebo, respectively 26.88 (SE 0.07) vs. 40.63 (0.01) nmol l⁻¹, probably reflecting endogenous synthesis. At day 8, mean plasma GPA was significantly higher in the GPA arm compared to placebo as expected, respectively 213.88 (SE 0.07) vs. 32.75 (0.00) nmol l⁻¹, a mean difference of 181.13, 95% confidence interval of the difference 26.53–335.72 nmol l⁻¹, P = 0.025.

Tolerability

Low dose GPA was well tolerated. Adverse events, reported in all treatment arms, were minor and mild, and mostly present at baseline, except for an unpleasant taste in the mouth without change in the diet reported by one participant in the placebo arm at day 21 (Table 2). There were no unexpected serious adverse reactions or serious adverse events. No significant changes were found compared to placebo in clinical safety parameters, physical examination including blood pressure, or laboratory measurements. In addition, there were no significant differences in 12-lead ECG parameters after treatment including an unchanged QT interval.

Haemodynamics

The haemodynamic parameters are presented in Table 3. At baseline participants had significant higher aorta augmentation index in the GPA treatment arm compared to the creatine and placebo arm (P = 0.015). There were no significant differences between treatment arms after 7 days of active treatment.

| Day GPA (n = 8) | Creatine (n = 8) | Placebo (n = 8) |
|---------------|----------------|----------------|
| 0 n = 4 participants | Cough (3) | Cough (2) | Cough (3) |
|               | Fatigue (2) | Fatigue (5) | Fatigue (3) |
|               | Insomnia (1) | Dizziness (1) | Insomnia (1) |
|               | Headache (1) | Headache (2) | |
|               | Hyperhidrosis (1) | Hyperhidrosis (2) | |
|               | Myalgia (1) | | |
| 1 n = 3 participants | Cough (2) | Cough (2) | Cough (2) |
|               | Restlessness (1) | Fatigue (1) | Restlessness (1) |
|               | | Dizziness (1) | |
|               | | Headache (2) | |
|               | | Hyperhidrosis (2) | |
| 3 n = 1 participant | Cough (1) | Fatigue | Cough (3) |
|               | Restlessness (1) | Cough (3) | Fatigue (1) |
| 7 n = 1 participant | Cough (1) | Fatigue | Cough (2) |
|               | Restlessness | Cough | |
|               | | Hyperhidrosis | |
|               | | Headache | |
|               | | Myalgia (1) | |
| 21 n = 1 participant | Cough (1) | Fatigue | Cough (1) |
|               | Restlessness | Fatigue (2) | |
|               | | Headache | Insomnia* (1) |
|               | | | Dyseusia (1) |

Table 2
Reported adverse events at baseline and during follow up

Treatment allocation, data collection and data analysis were blinded. Day 0, baseline; 1, 3, 7, day of active treatment; 21, 2 weeks after active treatment. Data between brackets are number of participants with adverse effects. All reported adverse events were CTCAE [14] classification (mild), except insomnia

In the placebo treatment arm, which was moderate.

Platelet aggregation test

There was no significant difference between GPA, creatine and placebo in platelet aggregation parameters at baseline or at day 8.

Discussion

The main finding of this first-in-human study is that GPA, given for 1 week in a sub-therapeutic dose as recommended by the FDA [15], is safe and well tolerated in healthy men. There were no serious or severe adverse events reported with the use of GPA, and no significant differences with placebo in safety measures including self-reported data obtained with unstructured and structured questionnaires, physical examination, laboratory tests including kidney and liver parameters, or cardiovascular safety including the QT interval.
This study was conducted because incremental data indicate that the ATP regenerating enzyme CK enhances the energy-demanding processes involved in hypertension [1–6]. The CK enzyme system is thought to promote hypertension through rapid regeneration of ATP from phospho-creatine near ATPases involved in resistance artery contractility and salt retention [1–6, 10]. Animal and human studies found that plasma and tissue CK activity was a main determinant of blood pressure and of resistance artery contractility, while hypertension was found to be more severe in individuals with high plasma CK activity [1–6, 10]. In addition, the creatine analogue and competitive CK inhibitor GPA safely and reversibly reduced blood pressure in spontaneously hypertensive rats [11]. Therefore, the CK enzyme system is a potential target for lowering blood pressure in humans with high plasma CK activity.

Currently, there is a need for new conservative options to treat resistant hypertension [10, 21–24]. A substantial proportion of treated hypertensive patients do not achieve blood pressure control, even with multiple drugs. Risk factors for poor control include obesity, age, African ancestry, the presence of diabetes or end organ damage; but non-adherence of the patient, the white-coat effect, therapeutic inertia of the physician, dietary factors, or the concomitant use of blood pressure increasing drugs may also contribute [10, 21–24]. Importantly, resting plasma CK was the main predictor of failure of hypertension treatment in the general population [10]. Therefore, antihypertensive agents acting through CK inhibition might aid in achieving better control in patients with difficult-to-treat hypertension and high plasma CK activity [10].

Data from more than 120 animal studies in different species indicate that creatine is not indispensable, as recently summarized [13, 25]. Taking into account the wide variation in CK activity found in humans [1, 2, 6, 26–28], we suggest that moderate CK inhibition should be feasible in humans with high CK to reduce blood pressure without major side effects [12].

The main strength of this study is that we provide first-in-human data on the safety and tolerability of the specific small molecule CK inhibitor GPA, in a sub-therapeutic dose given for 1 week, in a triple blinded randomized, placebo and active controlled trial. We collected these data in close adherence to the US FDA and European guidelines [15, 20]. Limitations are the obligatory sub-therapeutic dosing and the use for 1 week only, aimed at preventing toxicity, which limited efficacy assessments. Another limitation is that we did not assess pharmacokinetics of GPA, following the imperative advice of our local medical ethical committee to focus on safety and tolerability in this first-in-human data collection. Finally, although tolerability studies are part of the formal assessment of new drugs, the relevance of such studies for clinical safety is

| Parameter | Baseline GPA | Baseline Creatine | Baseline Placebo | Day 8 GPA | Day 8 Creatine | Day 8 Placebo |
|-----------|--------------|------------------|----------------|---------|--------------|--------------|
| 24-h SBP, mmHg | 124 (2.2) | 122 (2.9) | 123 (3.0) | 127 (2.9) | 126 (2.5) | 120 (2.3) |
| 24-h DBP, mmHg | 72 (1.3) | 69 (2.1) | 71 (2.4) | 73 (1.7) | 72 (3.0) | 71 (1.9) |
| 24-h HR, bpm | 74 (3.8) | 72 (4.4) | 68 (2.7) | 75 (3.7) | 75 (4.7) | 66 (3.3) |
| Sitting SBP, mmHg | 127 (1.8) | 125 (2.0) | 122 (3.9) | 128 (2.8) | 120 (4.5) | 124 (3.7) |
| Sitting DBP, mmHg | 72 (6.4) | 72 (2.3) | 73 (3.5) | 72 (2.5) | 71 (3.5) | 72 (3.3) |
| Sitting HR, bpm | 72 (4.1) | 65 (13.2) | 61 (4.4) | 77 (5.2) | 73 (5.8) | 62 (4.2) |
| Supine SBP, mmHg | 116 (8.3) | 117 (3.9) | 113 (3.4) | 106 (3.6) | 104 (7.8) | 109 (5.4) |
| Supine DBP, mmHg | 66 (5.2) | 64 (1.7) | 61 (3.4) | 62 (2.6) | 59 (3.6) | 62 (2.6) |
| Supine HR, bpm | 68 (4.3) | 61 (4.6) | 61 (3.3) | 70.5 (4.8) | 68 (4.3) | 61 (3.2) |
| Stroke volume, ml | 111.1 (5.8) | 106 (12.7) | 115.8 (5.6) | 108 (3.8) | 108.4 (6.8) | 118 (4.1) |
| Cardiac output, l min⁻¹ | 7.4 (0.3) | 15.1 (9.0) | 7.1 (0.5) | 7.5 (0.4) | 7.2 (0.4) | 7.2 (0.4) |
| LVC (dP/dt), mmHg s⁻¹ | 819.6 (117.5) | 1068.4 (147.9) | 757.4 (89.3) | 697.3 (35.4) | 761 (88.0) | 820.4 (71.4) |
| SVR, dyn·s cm⁻⁵ | 956.6 (84.3) | 1407.9 (463.1) | 921.3 (60.2) | 831.1 (68.8) | 869.3 (57) | 911.6 (72) |
| PWV, m s⁻¹ | 5.9 (0.5) | 6.0 (0.4) | 6.3 (1.0) | 5.9 (0.4) | 6.4 (0.6) | 5.3 (0.3) |
| AIX aorta, % | 14.5 (4.9) | 1.2 (1.5) | 4.3 (2.3) | 4.1 (2.1) | 5.1 (5.5) | 6.9 (2.4) |
| QT-interval, ms | 382 (8.5) | 398 (15.2) | 403 (6.9) | 380 (10.5) | 398 (11.5) | 405 (9.4) |

Data are mean (SE) for baseline and after 7 days of treatment. n = 8 in each treatment arm, except for placebo n = 7 at day 8. Aix, Augmentation index of aorta. DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; LVC, left ventricular contractility; PWV, Pulse wave velocity; SBP, systolic blood pressure; SVR, Systemic vascular resistance.

P W n = 6–8; AIX n = 5–8. Participants assigned to GPA had significant higher Aix from baseline compared to creatine and placebo treatment arm

*P = 0.015. There were no significant differences at day 8 between treatment arms.
limited, mainly because of the small sample sizes. Thus, the value of tolerability studies for drug development has been questioned [29]. However, even with larger studies, no drug can be universally acclaimed to be well tolerated and safe [29]. We perceive that the data on GPA that has been presented should be looked upon within the context of the existing evidence: the molecule is not novel but kidney-synthesized, has a large body of data collected in different animal species and human cells, is on the market as a food supplement [12], and was tested in this study for 1 week only in a sub-therapeutic dose in healthy men. We observed an increase in plasma GPA concentration, without evidence of any desirable or undesirable effect. Further clinical studies are needed to establish early in the process of further drug development the safety, tolerability and pharmacokinetics of GPA doses or plasma concentrations likely to produce a pharmacological effect.

Competing Interests

L.B. is an inventor on NL patent WO/2012/138226 (filed). L.B. is a recipient of a VENI fellowship (grant number 916.10.156) awarded by the Netherlands Organization for Scientific Research (NWO) as part of its Innovational Research Incentives Scheme. The funders had no role in study design, data collection and analysis, preparation of the manuscript, or decision to publish.

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Contributors

F.K. co-designed and conducted the clinical trial, conducted the primary statistical analysis, and drafted the manuscript. D.H. co-designed the platelet tests and participated in conducting the clinical studies. Y.H. co-designed and conducted the clinical trial. L.W. co-designed and conducted the clinical trial. M.S. conducted the platelet tests. I.O. co-designed and supervised the clinical trial. G.M. co-designed and supervised the clinical trial. R.N. co-designed and supervised the platelet tests. G.S. co-designed the clinical trial and assessed GPA. J.C. co-designed the clinical trial. L.B., the grant holder, designed the clinical trial and platelet studies, provided statistical expertise, supervised the clinical and laboratory studies, conducted and supervised the statistical analyses, and drafted the manuscript. All authors contributed to the writing of the manuscript for important intellectual content, and read and approved the final manuscript.

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