Plasma guanidino compounds are altered by oral creatine supplementation in healthy humans

Wim Derave, Bart Marescau, Els Vanden Eede, Bert O. Eijnde, Peter P. De Deyn, and Peter Hespel. Plasma guanidino compounds are altered by oral creatine supplementation in healthy humans. J Appl Physiol 97: 852–857, 2004. First published April 23, 2004; 10.1152/japplphysiol.00206.2004.—Although creatine is one of the most widely used nutritional supplements for athletes as well as for patients with neuromuscular disorders, the effects of oral creatine supplementation on endogenous creatine synthesis in humans remains largely unexplored. The aim of the present study was to investigate the metabolic consequences of a frequently used, long-term creatine ingestion protocol on the circulating creatine synthesis precursor molecules, guanidinoacetate and arginine, and their related guanidino compounds. For this purpose, 16 healthy young volunteers were randomly divided to ingest in a double-blind fashion either creatine monohydrate or placebo (maltodextrine) at a dosage of 20 g/day for the first week (loading phase) and 5 g/day for 19 subsequent wk (maintenance phase). Fasting plasma samples were taken at baseline and at 1, 10, and 20 wk of supplementation, and guanidino compounds were determined. Plasma guanidinoacetate levels were reduced by 50% after creatine loading and remained ~30% reduced throughout the maintenance phase. Several circulating guanidino compound levels were significantly altered after creatine loading but not during the maintenance phase: homoarginine (+35%), α-keto-γ-guanidinobutyric acid (+45%), and argininc acid (+75%) were increased, whereas guanidinosuccinate was reduced (−25%). The decrease in circulating guanidinoacetate levels suggests that exogenous supply of creatine chronically inhibits endogenous synthesis at the transamidinase step in humans, supporting earlier animal studies showing a powerful repressive effect of creatine on L-arginine/glycine amidotransferase. Furthermore, these data suggest that this leads to enhanced utilization of arginine as a substrate for secondary pathways of creatine synthesis; urea cycle; ergogenic supplement; neurotoxins.

OVER THE PAST 10 YR, CREATINE has become one of the most popular dietary supplements of the Western world. Whereas its use was initially confined to the athletic population, the clinical potential of creatine supplementation is now investigated for a variety of diseases (recently updated in Refs. 8a, 31). Despite the widespread use of oral creatine supplements by athletes and patients, little is known about its impact on the regulation of endogenous creatine synthesis.

The first step in the biosynthesis of creatine in mammals is the formation of guanidinoacetate (GAA) from arginine and glycine catalyzed by L-arginine-glycine amidotransferase (AGAT), alternatively called transaminidase (reviewed in Refs. 29, 30). The second step involves the methylation of GAA to form creatine by the enzymatic action of guanidinoacetate methyltransferase (GAMT). In mammals, the primary organs involved in these synthesis reactions are the kidney, liver, and pancreas, although the relative contribution of each of these organs is species dependent and is still debated (30). The important role of endogenous creatine synthesis is illustrated by the recent disclosure of creatine deficiency syndromes, caused by inborn AGAT or GAMT deficiency in humans (14, 26). Patients suffering from these deficiencies display markedly reduced brain creatine levels that go together with developmental delay and mental retardation, and these symptoms can be partially reversed by exogenous creatine supplementation (24).

Early studies in the rat and chicken have shown that down-regulation of endogenous creatine synthesis by the end product creatine occurs at the first step, i.e., the transamidination by AGAT (9, 28). As shown by Van Pilsum and coworkers (10, 20), this feedback repression by creatine at the rate-limiting step of synthesis probably occurs at the pretranslational level of AGAT expression, rather than by a direct effect on enzymatic activity. This is further supported by the time course of AGAT suppression, which occurs in the range of days or weeks rather than hours (9). To our knowledge, the effect of chronically elevated plasma creatine levels, due to creatine supplementation in humans, on GAA formation has hardly been studied. Over 50 yr ago, Hoberman et al. (12) showed that creatine ingestion in a human subject resulted in elevated GAA excretion (12). This corroborated the prevailing opinion that the methylation of GAA (the second step), rather than its formation (the first step), was rate determining for creatine synthesis (12). However, these data seem more puzzling presently because they could implicate a species difference indicating that creatine synthesis suppression in humans is not exclusively accomplished on AGAT as in rats but that it occurs also at the site of GAMT. Therefore, in the present study, we have examined the circulating GAA concentrations in humans, subjected to a 1-wk high-dose (20 g) and subsequent 19-wk low-dose (5 g) creatine supplementation protocol. This will allow us to indirectly register whether the transaminidase (decreased GAA levels would indicate AGAT inhibition) or methylation (elevated GAA levels would indicate GAMT inhibition) reaction is the main site of creatine biosynthesis regulation in humans.

It could be hypothesized that arginine sparing, by creatine synthesis suppression, may result in upregulation of other

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metabolic fates of arginine, such as formation of urea and ornithine, guanidinosuccinate (22), α-keto-δ-guanidinovaleric acid (GVA), and argininc acid (16). We have therefore investigated a wide range of guanidino compounds, which are related by well-described or less clear links to arginine. Several of the guanidino compounds are epileptogenic and are described as probable neurotoxins in uremic disease (5, 6). Although the moderate use of creatine supplements has no consistently reported adverse effects (23), it remains important to investigate whether creatine supplementation causes elevation of guanidino compound concentrations in the physiological or pathological range, which could yield a topic of interest for the study of potential side effects of prolonged or high-dose creatine supplementation.

METHODS

Subjects. Sixteen young volunteers (age 18.8 ± 0.3 yr, 12 men and 4 women) gave their written, informed consent to participate in the study. The study protocol was approved by the local Ethics Committee (Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium). The subjects reported to not have taken creatine supplements for the 6 mo before the study. They were instructed to abstain from taking any medication and to avoid making any changes in their physical activity level and other living habits during the period of the study.

Study protocol. A double-blind study was performed over a 20-wk period. Before the subjects started the supplementation, baseline measurements were obtained. Then, the subjects were assigned to either the placebo group (Pl; receiving maltodextrine) or to the creatine group (Cr; receiving creatine monohydrate). Subjects ingested 20 g of creatine (divided over 4 portions to be taken at regular intervals throughout the day) or placebo during the first week, and one dose of 5 g in the morning for the following 20 wk. Measurements were performed at baseline, after 1 wk of loading, and after 10 and 20 wk of low-dose supplementation.

Plasma metabolites. Fasting plasma samples were deproteinized by adding an equal volume of a trichloroacetic acid solution (300 g/l) and subsequent centrifugation in a Beckman microfuge (Beckman Instruments International, Geneva, Switzerland). Two hundred microliters of supernatant were used for guanidino compound determination, using a Biotronic LC 5001 (Biotronik, Maintal, Germany) amino acid analyzer adapted for guanidino compound determination. The guanidino compounds were separated over a cation-exchange column using sodium citrate buffers, and they were detected with the fluorescence ninhydrin method as previously reported in detail (18). Detected compounds include creatine, creatinine, GVA, GAA, guanidinosuccinate, arginine, argininc acid, homoarginine, α-N-acetyl arginine, guanidine, and γ-guanidinobutyric acid. Plasma urea was determined with diacetylmonoxime as described by Ceriotti (3).

Creatine retention. One week before the start of the study and after 1, 10, and 20 wk of supplementation, the supplementation protocol was interrupted for 1 day for determination of creatine retention, where all subjects (Pl and Cr) received a single dose of 10 g of creatine monohydrate. The subjects reported to the laboratory in the morning after an overnight fast, emptied their bladder, and received an oral load of 10 g of creatine monohydrate dissolved in 250 ml of water. Subjects were instructed to collect all urine during the following 24 h. The total urine volume was measured, and the creatine content of an aliquot was determined by a standard enzymatic fluorometric assay. Creatine retention was calculated as the difference between the amounts of creatine ingested (10 g) and excreted over the 24-h period.

Statistical analysis. Statistical comparison between Pl and Cr groups was done by applying a two-way ANOVA for repeated measures with time as within-subject factor and treatment as between-subject factor. Significance was accepted when the P value for interaction between time and treatment was <0.05. Correlations were calculated by means of a product-moment test. All statistical procedures were conducted by using Statistica software (Statsoft, Tulsa, OK).

RESULTS

Plasma guanidino compounds. As shown in Fig. 1A, plasma creatine levels at baseline were 20–30 μM and remained in this range throughout the study in Pl. In Cr, fasting plasma creatine levels were 10-fold higher after the 1-wk high-dose supplementation and remained ~5-fold higher in the 19-wk low-dose regimen. Plasma GAA levels (Fig. 1B) remained at baseline level (±2.4 μM) in Pl, but they decreased by 50% and 20%–30% in Cr after high-dose and low-dose creatine supplementation, respectively (P < 0.05). When all plasma samples of Cr are considered, the GAA levels correlated negatively (R = −0.59;
over time (Table 1). At significantly different between treatment groups fiConcentrations were signifi-
tenn low-dose supplements. Plasma creatinine was slightly con-
centration on guanidino compound levels. After 1 wk of supple-
mentation, homoarginine, GVA, and argininic acid levels in Cr tration in healthy humans. GAA is an intermediate between the rst and second reaction of creatine biosynthesis, and this tion in accordance with the findings of several studies performed in animals (10, 20, 28). Although one cannot rule out the possibility that the decrease in circulating GAA levels is caused by alternative mechanisms (excretion, degration, transport), the negative correlation between plasma creatine and GAA levels (Fig. 1C) demonstrates that creatine is possibly a powerful regulator of AGAT in vivo in humans. However, because the rst blood sample was only taken after 1 wk of creatine supplementation, our results do not allow discriminating whether the negative feedback is obtained by rapid catalytic activity inhibition or by reduced enzyme expression. Analogy with animal studies would suggest that the latter phenomenon is predominant because reduction of AGAT activity is observed not within hours but only within days after the start of creatine supplementation (9). Therefore, future studies will have to determine the exact time course of downregulation of creatine biosynthesis in humans. The creatine supplementation regimen employed in the present study (1-wk loading phase with 20 g/day and a subsequent maintenance dose of 5 g/day) is routinely used by athletes and patients, on the basis of scientifi evidence (13, 27). We now suggest that such creatine supplementation regimen downregulates endogenous creatine biosynthesis within 1 wk and as long as supplementation is continued.

A second aim of the present study was to explore the impact of creatine supplementation on the circulating levels of related guanidino compounds, assuming that arginine sparing would stimulate alternative pathways of arginine catabolism. Interestingly, several of the metabolites were sensitive to creatine

### Table 1. Plasma concentrations of the guanidino compounds that were influenced by creatine supplementation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 10</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homoarginine, μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>1.72±0.14</td>
<td>1.89±0.15</td>
<td>1.90±0.18</td>
<td>1.75±0.17</td>
</tr>
<tr>
<td>Cr</td>
<td>1.68±0.22</td>
<td>2.27±0.32*</td>
<td>1.58±0.19</td>
<td>1.48±0.11</td>
</tr>
<tr>
<td>α-Keto-δ-guanidinvaleric acid, nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>47.6±3.8</td>
<td>48.6±6.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cr</td>
<td>47.4±6.3</td>
<td>69.2±6.5*</td>
<td>ND</td>
<td>49.6±4.12</td>
</tr>
<tr>
<td>Argininic acid, nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>94±8</td>
<td>94±8</td>
<td>121±20</td>
<td>98±18</td>
</tr>
<tr>
<td>Cr</td>
<td>89±10</td>
<td>156±18*</td>
<td>110±19</td>
<td>108±16</td>
</tr>
<tr>
<td>Creatinine, μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>78.4±2.8</td>
<td>79.3±3.3</td>
<td>76.3±2.7</td>
<td>78.6±4.4</td>
</tr>
<tr>
<td>Cr</td>
<td>78.6±3.1</td>
<td>89.4±3.8*</td>
<td>81.7±4.1</td>
<td>89.2±4.5*</td>
</tr>
<tr>
<td>Guanidinosuccinate, nM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>252±21</td>
<td>262±30</td>
<td>278±25</td>
<td>268±19</td>
</tr>
<tr>
<td>Cr</td>
<td>290±64</td>
<td>214±34*</td>
<td>278±58</td>
<td>270±79</td>
</tr>
<tr>
<td>Arginine, μM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>102.9±8.1</td>
<td>105.4±7.7</td>
<td>98.9±6.7</td>
<td>88.8±12.8</td>
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<tr>
<td>Cr</td>
<td>90.4±8.2</td>
<td>86.7±7.2</td>
<td>89.4±8.5</td>
<td>98.3±9.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Pl, placebo group; Cr, creatine-supplemented group; ND, not determined (because of analytic problems). *Significant difference compared with Pl, P < 0.05.

### Table 2. Amount of creatine and creatine excreted in the 24 h urine after oral ingestion of 10 g of creatine monohydrate (=8.79 g creatine)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 10</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine creatine, g/24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>3.70±0.78</td>
<td>3.36±0.46</td>
<td>3.47±0.59</td>
<td>2.42±0.54</td>
</tr>
<tr>
<td>Cr</td>
<td>5.09±0.82</td>
<td>7.70±1.03*</td>
<td>8.94±0.83*</td>
<td>7.07±1.34*</td>
</tr>
<tr>
<td>Urine creatine, g/24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pl</td>
<td>1.51±0.12</td>
<td>1.55±0.11</td>
<td>1.43±0.08</td>
<td>1.53±0.22</td>
</tr>
<tr>
<td>Cr</td>
<td>1.57±0.10</td>
<td>1.73±0.13</td>
<td>1.71±0.15</td>
<td>1.64±0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant difference compared with Pl, P < 0.05.

P < 0.05) with creatine levels (Fig. 1C). Plasma arginine concentrations were ~80–100 μM and remained unchanged over time (Table 1). At week 20, however, arginine concentrations were significantly different between treatment groups because of a nonsignificant fall and rise in Pl and Cr, respectively.

Table 1 shows the significant effects of creatine supplementation on guanidino compound levels. After 1 wk of supplementation, homoarginine, GVA, and argininic acid levels in Cr were increased by 35, 45, and 75%, respectively, compared with baseline (P < 0.05), whereas they remained unchanged in Pl. These changes were not present during subsequent long-term low-dose supplements. Plasma creatinine was slightly increased throughout the study in Cr (Table 1), the difference reaching statistical significance at 1 and 20 wk. In Cr, guanidinosuccinate concentrations decreased from 290 ± 64 nM at baseline to 214 ± 34 nM after 1 wk (P < 0.05), yet they returned to baseline levels in the subsequent phase of the study.

Other plasma guanidino compounds were unaffected by treatment: α-N-acetyl arginine, guanidin, and urea. The concentration of γ-guanidinobutyric acid was below the detection limit in most samples.

**Creatine retention.** At baseline, 3.7 ± 0.8 and 5.1 ± 0.8 g of creatine were excreted in the 24 h after 8.8 g creatine (10 g creatine monohydrate) ingestion in Pl and Cr (not significant), respectively (Table 2). Creatine excretion remained unchanged in Pl throughout the study and increased (creatine retention decreased) at 1, 10, and 20 wk in Cr.

**DISCUSSION**

A major finding of the present study is that plasma GAA concentration is reduced by exogenous creatine supplementation in healthy humans. GAA is an intermediate between the first and second reaction of creatine biosynthesis, and this suggests that hypercreatinemia suppresses creatine synthesis at the first committed step, catalyzed by AGAT. This finding is in accordance with the findings of several studies performed in animals (10, 20, 28). Although one cannot rule out the possibility that the decrease in circulating GAA levels is caused by alternative mechanisms (excretion, degradation, transport), the negative correlation between plasma creatine and GAA levels (Fig. 1C) demonstrates that creatine is possibly a powerful regulator of AGAT in vivo in humans. However, because the first blood sample was only taken after 1 wk of creatine supplementation, our results do not allow discriminating whether the negative feedback is obtained by rapid catalytic activity inhibition or by reduced enzyme expression. Analogy with animal studies would suggest that the latter phenomenon is predominant because reduction of AGAT activity is observed not within hours but only within days after the start of creatine supplementation (9). Therefore, future studies will have to determine the exact time course of downregulation of creatine biosynthesis in humans. The creatine supplementation regimen employed in the present study (1-wk loading phase with 20 g/day and a subsequent maintenance dose of 5 g/day) is routinely used by athletes and patients, on the basis of scientific evidence (13, 27). We now suggest that such creatine supplementation regimen downregulates endogenous creatine biosynthesis within 1 wk and as long as supplementation is continued.

A second aim of the present study was to explore the impact of creatine supplementation on the circulating levels of related guanidino compounds, assuming that arginine sparing would stimulate alternative pathways of arginine catabolism. Interestingly, several of the metabolites were sensitive to creatine
supplementation. Although modest in magnitude, L-homoarginine levels were elevated by 1-wk high-dose creatine supplementation (Table 1). Homoarginine may be formed by a homologous urea cycle (Fig. 2) in which ornithine is replaced by lysine (25). In the light of the possible physiological effects of elevated homoarginine levels by creatine supplementation, it is important to note that homoarginine can replace arginine as a substrate for nitric oxide (NO) synthase in the formation of NO (15).

The present data show that creatine supplementation clearly induces elevated GVA and argininic acid levels. It has been suggested that GVA could be synthesized by transamination from arginine and that it can be further metabolized to argininic acid (4, 16). GVA and argininic acid are metabolically related and are elevated in states of hyperargininemia (19). The present findings suggest for the first time that even in the absence of hyperargininemia, the supposed creatine synthesis suppression can lead to upregulation of a secondary pathway of arginine metabolism, i.e., synthesis of GVA and argininic acid. The latter guanidino compounds are convulsants and can inhibit GABA responses in neurons (7, 8), although it should be emphasized that the concentrations reached in the present study are far below the pathological range. Despite the popularity of creatine as a nutritional supplement for >10 yr, no such side effects on the central nervous system amino acid responses have been reported or suspected. Therefore, it is unlikely that the increased circulating concentrations of GVA and argininic acid are a potential source of harmful side effects at the studied dosage (5–20 g) of creatine supplementation.

Some theories exist about the biosynthesis pathway of guanidinosuccinate, of which the transamination of arginine to aspartate (22) (theory 1), the transformation of urea to guanidinosuccinate through the so-called “guanidine cycle” (21) (theory 2), and the cleavage of argininosuccinate by the hydroxyl radical (2) (theory 3) received most attention. Although a tight coupling between guanidinosuccinate and urea is well established, the metabolic relationship between arginine and guanidinosuccinate is controversial. Some investigators have shown that increased availability of arginine gives rise to guanidinosuccinate formation through transamination (22) (theory 1), whereas others believe that arginine, a NO precursor, by elevating NO production, leads to scavenging of the hydroxyl radical, necessary for cleavage of argininosuccinate to guanidinosuccinate (1) and consequently decreased guanidinosuccinate synthesis (theory 3). Our present data show that guanidinosuccinate levels decrease with the increased arginine

![Diagram](image-url) fig. 2. Putative scheme of biochemical reactions of guanidino group-containing metabolites around arginine and the influence of exogenous creatine supplementation (Cr suppl; 1 wk at 20 g/day) hereon in humans.

=, No change; ↓, decrease; ↑, increase.
provides a valuable way to spare amino acids and energy in the and nearly complete excretion of ingested creatine, the mech-
creatine content. In light of this abundance of body creatine indicating no further increase in and a saturation of the body
humans that oral creatine supplementation in doses of 5
g/day is related to a reduction in circulating GAA levels,
additional, these data suggest the creatine biosynthesis repression leads to enhanced utiliza-
tion of arginine as a substrate for secondary guanidino com-
pound pathways.

ACKNOWLEDGMENTS

We gratefully acknowledge the practical contribution by Monique Ramae-
kers and Ilse Possemiers. Degussa (Freising, Germany) kindly donated the
available, which likely results from suppressed creatine syn-
thesis. Thus our data are more in agreement with theory 3 than
with theory 1 and 2. Theory 3 suggests that increased arginine availability leads to an inhibition of guanidinosuccinate syn-
thesis from argininosuccinate because increased arginine leads
to increased NO production and a lesser availability of hy-
droxy radicals necessary for the cleavage of argininosuccinate.
A similar pattern of decreased guanidinosuccinate levels and
increased GVA and argininc acid levels is present in hyper-
argininemic patients (17), which further supports the idea that
the altered guanidino compound pattern in the present study is
secondory to elevated arginine availability.

In Fig. 2, we attempt to summarize the effects of oral
creatine supplementation on the metabolic pathways around
arginine. Following this proposed scheme, downregulation of
AGAT expression stimulates flux through secondary pathways
leading to formation of GVA, argininc acid, homoarginine, and
possibly NO but not of urea. These effects occur after 1 wk of
20 g/day supplementation but not after 10–20 wk of 5 g/day.

The urinary excretion of creatine and creatinine is shown in
table 2. The total ingested amount of creatine is 8.8 g (by oral
challenge) plus a presumed dietary intake of ~0.5–2 g (11) and
an unknown amount of endogenously synthesized creatine.
Thus, at baseline, a minimum of 9.3 g of creatine is added to the
total creatine pool, and between 5.2 g (PI) and 6.7 g (Cr) are
excreted (as creatine and creatinine), leaving a net increase in
body creatine content of minimum 2.6 to 4.1 g. After 1, 10, and
20 wk of creatine supplementation, the fractional excretion increased to ~100% of the total amount of dietary creatine,
indicating no further increase in and a saturation of the body
creatine content. In light of this abundance of body creatine and
nearby complete excretion of ingested creatine, the mech-
anism of downregulation of endogenous creatine synthesis
provides a valuable way to spare amino acids and energy in the human body.

In summary, the present results for the first time confirm in
humans that oral creatine supplementation in doses of 5–20
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