Creatine uptake in brain and skeletal muscle of mice lacking guanidinoacetate methyltransferase assessed by magnetic resonance spectroscopy

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Kan HE, Meeuwissen E, van Asten JJ, Veltien A, Isbrandt D, Heerschap A. Creatine uptake in brain and skeletal muscle of mice lacking guanidinoacetate methyltransferase assessed by magnetic resonance spectroscopy. J Appl Physiol 102: 2121–2127, 2007. First published March 8, 2007; doi:10.1152/japplphysiol.01327.2006.—Creatine (Cr) levels in skeletal muscle and brain of a mouse model of Cr deficiency caused by guanidinoacetate methyltransferase absence (GAMT−/−) were studied after Cr supplementation with 2 g·kg body wt−1·day−1 Cr for 35 days. Localized 1H magnetic resonance spectroscopy (MRS) was performed in brain (cerebellum and thalamus/hippocampus) and in hind leg muscle of GAMT−/− mice before and after Cr supplementation and in control (Con) mice. As expected, a signal for Cr was hardly detectable in MR spectra of GAMT−/− mice before Cr supplementation. In the thalamus/hippocampus region of these mice, an increase in N-acetylaspartate (NAA) was observed. During Cr administration, Cr levels increased faster in skeletal muscle compared with brain, but this occurred only during the first day of supplementation. Thereafter, Cr levels increased by 0.8 mM/day in all studied locations. After 35 days of Cr supplementation, Cr levels in all locations were higher compared with Con mice on a Cr-free diet and NAA levels normalized. Only because of the repeated MRS measurements performed in this longitudinal Cr supplementation study on GAMT−/− mice were we able to discover the initial faster uptake of Cr in skeletal muscle compared with brain, which may represent muscular Cr uptake independent of Cr transporter expression. Our results can provide the basis for additional experiments to optimize Cr supplementation in GAMT deficiency, as increases in brain Cr are slow in patients after Cr supplementation.

Magnetic resonance spectroscopy (MRS) allows noninvasive assessment of various compounds central in the study of energy metabolism, and localized MRS has played an important role in the elucidation of the pathophysiology of GAMT deficiency. Localized 1H MRS of the brain of patients showed a strongly reduced Cr signal along with a broad new signal at 3.8 ppm (e.g., Ref. 40), probably arising from guanidinoacetate (Gua), the immediate precursor of Cr in the biosynthesis. 31P MRS of brain and skeletal muscle showed a strong reduction in phosphorylated Cr (Pcr) along with a high signal of phosphorylated Gua (PGua; e.g., Refs. 8, 32, 40).

Therapeutic approaches to alleviate symptoms in GAMT deficiency include Cr supplementation, as uptake of Cr from the blood can result in increases in Cr concentration in several tissues such as skeletal muscle and brain (44). The biosynthesis of Cr is assumed to take place mainly in the pancreas and liver, and Cr is exported to the blood and taken up in Cr-requiring tissues by a Cr transporter (CrT) against a large concentration gradient (44). The presence of biosynthetic enzymes in both brain (1, 2, 24, 41) and skeletal muscle (2, 6, 31), however, suggests that these organs can, at least partly, synthesize their own Cr.

Cr supplementation in GAMT-deficient patients leads to partial improvement of clinical symptoms, and 1H MRS studies of the brain of GAMT-deficient patients revealed that the increase in Cr concentration during long-term Cr supplementation is slow and still not complete after 2 yr (39). Methods to increase the rate of uptake of Cr in the brain in GAMT deficiency could therefore be beneficial for a more rapid decrease of symptoms. Recently, neonatal supplementation of Cr has been suggested to prevent the development of clinical symptoms in GAMT deficiency (35). However, as the majority of cases of GAMT deficiency is diagnosed after the neonatal period, a rapid increase in Cr could possibly prevent the development of severe clinical symptoms in young patients.

High Gua concentrations in brain could play a role in both the incomplete improvement of the clinical symptoms (33) and the slow uptake of Cr in brain due to competitive binding to the CrT at the blood-brain barrier (25). Furthermore, the low permeability of the blood-brain barrier for Cr could also play a role (1). Strategies to improve clinical symptoms by restricting arginine and supplementing ornithine to reduce Gua concentrations led to a further improvement of clinical symptoms, but there was still no complete normalization (33). This same

GUANIDINOACETATE METHYLTRANSFERASE (GAMT) deficiency is an autosomal recessive disorder described for the first time as a new inborn error of metabolism over a decade ago (40). Its incidence is unknown (~30 patients have been described so far), and it is probable that many patients remain undiagnosed (21). GAMT is a key enzyme in the biosynthesis of creatine (Cr), an important molecule in energy metabolism of highly excitable tissues such as skeletal muscle and brain. Deficiency in GAMT leads to severe clinical symptoms, including mental retardation, muscle hypotonia, extrapyramidal movement abnormalities, and epileptic seizures (43). These symptoms highlight the importance of intact Cr metabolism in humans.

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strategy led to a faster increase in Cr concentration in skeletal muscle of a GAMT-deficient patient compared with brain, resulting in complete normalization of the Cr concentration in skeletal muscle within 3 mo (8).

As a model for the GAMT deficiency in humans, GAMT-deficient knockout mice (GAMT$^{-/-}$) were generated (31) that completely lack the essential enzyme GAMT and thus cannot form Cr. Our earlier studies on these mice validated their use as an animal model for GAMT deficiency (30, 31) and showed that, like patients, they accumulate Gua, the immediate precursor of Cr. Detailed studies on skeletal muscle revealed that the mice show hypotonia and that they can reversibly phosphorylate and use Gua as a substitute for PCR under mild metabolic demands. Short-term Cr supplementation relieved some but not all of the differences observed compared with control animals in skeletal muscle (17, 18).

A detailed study of increases in Cr during Cr supplementation in patients in various tissues is not available. A study in GAMT-deficient patients would require considerable resources and organization as GAMT deficiency is rare and the level of mental retardation of patients requires sedation for MRS acquisition. GAMT$^{-/-}$ mice, in contrast, provide an unique opportunity to study Cr increases in several tissues in a controlled manner. Localized $^1$H MRS enables simple longitudinal assessment of Cr increases in various tissues. Therefore, in the present study, we studied $^1$H MRS the increases in Cr concentration in skeletal muscle and brain during long-term Cr supplementation. It was hypothesized that the increase in Cr concentration in brain would be slower than in skeletal muscle. Our results could elucidate the differences in Cr uptake observed between skeletal muscle and brain as well as provide clues for therapeutic improvements to Cr uptake in brain.

MATERIALS AND METHODS

Animals. Adult male and female GAMT$^{-/-}$ mice (>6 mo old), generated by homologous recombination in embryonic stem cells (31), and homozygous and heterozygous wild-type (Con) littermates of the GAMT$^{-/-}$ animals were measured in this study (31). Animals were given free access to standard chow based on vegetable protein to eliminate possible Cr content (Ssniff Spezialdiäten, Soest, Germany). Animals were housed according to phenotype for at least 3 mo before the start of the study to prevent Cr intake resulting from coprophagia from control animals (18).

After a baseline MR measurement, all GAMT$^{-/-}$ mice were supplemented for 35 days with 2 g·kg$^{-1}$ body wt·day$^{-1}$ Cr monohydrate (Sigma C0780) dissolved in the drinking water and housed individually. A dose of 2 g·kg$^{-1}$ body wt·day$^{-1}$ in a mouse is equivalent to the common dose of 400–600 mg·kg$^{-1}$ body wt·day$^{-1}$ in humans according to methods of animal extrapolation (15). Saccharose (1.6 g·kg$^{-1}$ body wt·day$^{-1}$) was added to the drinking water to mask the bitter taste caused by the Cr monohydrate (15). Control animals received normal water.

During the MR experiments, animals were anesthetized with 1.5% isoflurane in a gas mixture of 50% O$_2$ and 50% N$_2$O delivered through a face mask. Rectal temperature was monitored using a fluorescent thermometer (Luxtron 712) and maintained at 37.0 ± 1°C using a warm water bed. Breathing frequency was monitored optically (Sirecust 401, Siemens). All experiments were approved by the local animal ethics committee, and animals were killed by cervical dislocation after termination of the study.

MR experiments. MRS measurements were performed on a 7.0-T magnet (Magnex Scientific, Abingdon, UK) interfaced to an SMIS spectrometer (Surrey Medical Systems, Surrey, UK) operating at 300.20 MHz for $^1$H. The magnet had a free bore size of 120 mm and was equipped with a 150 mT/m shielded gradient set. Localized $^1$H MRS was performed on skeletal muscle and brain using a STEAM sequence (4) with an echo time (TE) of 10 ms, a mixing time (TM) of 15 ms, and a repetition time (TR) of 5,000 ms, and VAPOR water suppression (26). For brain measurements, 256 averages were acquired (measurement time 22 min), and 128 averages were acquired for skeletal muscle measurements (measurement time 11 min).

For brain measurements, a 16 mm surface coil was used and voxels (areas of interest) were acquired of the cerebellum (1.6 × 1.6 × 2.3 mm) and thalamus/hippocampus region (2.0 × 2.0 × 2.2 mm). The location of the voxels was guided by gradient echo MR images acquired in three oblique, perpendicular directions (TE = 10 ms, TR = 4,000 ms, slice thickness 1 mm, bandwidth 100 kHz, matrix size 128 × 128, 14 slices). Voxels were field shimmed until a line width [full width half maximum (FWHM)] of 13 Hz (thalamus/hippocampus) or 17 Hz (cerebellum) or less was reached.

For skeletal muscle measurements, an Alderman-Grant type of coil was used with the leg positioned under the magic angle (~55°) with respect to the main magnetic field to reduce dipolar interactions (13). A voxel (2 × 2 × 4 mm) was positioned in the triceps surae muscles of the hind leg, guided by gradient echo MR images acquired in three oblique, perpendicular directions (TE = 5 ms, TR = 250 ms for coronal and sagittal slices, TR = 300 ms for transversal slices, slice thickness 0.5 mm, bandwidth 100 kHz, matrix size 256 × 256, 10 slices for coronal and sagittal directions, 15 for transversal). Field shimming on the voxel was performed until a line width (FWHM) of 25 Hz or less was reached. Outer volume suppression was applied to minimize contributions from fat or bone.

Of all GAMT$^{-/-}$ mice ($n = 25$), an $^1$H MR spectrum was acquired of brain ($n = 16$) or skeletal muscle ($n = 9$) before Cr supplementation. Thereafter, mice were randomly divided into six groups (4 for brain and 2 for skeletal muscle), and a localized $^1$H MR spectrum was acquired at different time points during the Cr supplementation period of 35 days. Each group of animals consisted of four animals. Mice in the brain group were measured three times: one time before and two times during the supplementation period. This resulted in $n = 4$ mice for each time point during Cr supplementation, except for days 9 and 35 ($n = 2$). Mice in the skeletal muscle group were measured four times: one time before and three times during the Cr supplementation period. As one mouse died during the Cr supplementation period, an extra mouse was added to complete all time points to $n = 4$. Localized $^1$H MR spectra of the same locations were recorded for Con animals on a Cr-free diet ($n = 4$).

Data analysis. Data processing of the $^1$H MR spectra was performed using the unsuppressed water signal for eddy current correction and normalization. MR spectra of brain were processed in the frequency domain using the LCModel 6.1-4F software package (28). The basis set of model used as input for LCModel was simulated using NMRSIM (Bruker Biospin, version 4.6a) with the same sequence and parameters as the in vivo measurements. For proton spin systems of total Cr, myo-inositol (Ins), taurine (Tau), total choline, Glx (glutamine and glutamate signal around 2 ppm), and N-acetylaspartate (NAA), known values for T1 and T2 relaxation times for rat brain were used (7). The spin systems of other metabolites were simulated with a line broadening of 4 Hz. For absolute quantification using the unsuppressed water signal, a mean tissue water content of 78% was assumed (14) and the signal for NAA was used as a reference.

MR spectra of skeletal muscle were processed in the time domain using the jMRUI software package (23a). The first-order phase was constrained to zero as the first acquired data point was acquired at the top of the echo. As prior knowledge in both Con and GAMT$^{-/-}$ animals, the line width of the Cr signal was constrained to 0.8 times the Tau signal (experimentally determined in Con animals) and the integral of the methylene signal of Cr was set at a ratio of 0.67 to that of the methyl signal of Cr. For absolute quantification, water content was assumed to be 76% (37) and T2 correction was applied as described before (30).
Statistics. Differences in final Cr concentration (i.e., the average Cr concentration from days 25 and 35 combined) between cerebellum and the thalamus/hippocampus locations and between Con and GAMT−/− animals were compared using a Student’s t-test.

Linear regression was used to compare the increase in Cr concentration between skeletal muscle and the two brain regions from day 1 to day 16 (brain) or 25 (skeletal muscle) of Cr supplementation. Differences were considered significant at P < 0.05 and are presented as means ± SD in the text.

RESULTS

Gradient echo MR images of the brain of Con and GAMT−/− mice ensured reproducible localization of the voxels for 1H MRS in both cerebellum and thalamus/hippocampus regions (Fig. 1). Corresponding spectra of the thalamus/hippocampus region in a Con and GAMT−/− animal before Cr supplementation are shown in Fig. 1, E and F. Because of the almost complete absence peaks at 3.0 and 3.9 ppm, Cr appears to be virtually absent in the brain of GAMT−/− animals.

During the 35 days of Cr supplementation to GAMT−/− animals, the Cr concentration in both the cerebellum and thalamus/hippocampus regions in brain increased (Fig. 2), showing that Cr is taken up into the brain from the blood in GAMT−/− mice. Linear regression analysis of the increase in Cr concentration from day 0 to day 16 showed a linear increase (r² > 0.94 for both regions) in Cr concentration in both cerebellum and thalamus/hippocampus (0.8 ± 0.11 and 0.8 ± 0.07 mM/day, respectively). Rates of increase in Cr levels did not differ significantly between the two regions. The increase in Cr concentration in thalamus/hippocampus stabilized already after 16 days, whereas it continued to increase at the same rate in cerebellum.

Both in GAMT−/− animals at the end of the supplementation period and in Con animals, the Cr concentration was significantly higher in the cerebellum compared with the thalamus/hippocampus region (Table 1). Comparison of the Cr concentration between GAMT−/− animals at the end of the supplementation period and Con animals on a Cr-free diet showed that the Cr concentration in thalamus/hippocampus and in cerebellum in GAMT−/− animals was significantly higher than in Con animals (Table 1).

1H MRS enables the detection of other important brain metabolites such as NAA and glutamine (Gln) and glutamate (Glu). Before the start of Cr supplementation in GAMT−/− mice, a difference in NAA in thalamus/hippocampus compared with Con animals was observed. All the other analyzed metabolites (Gln, Glu, Tau, and Ins) showed no significant differences. Cr supplementation to GAMT−/− animals for 35 days resulted in a normalization of NAA in the thalamus/

![Fig. 1](http://jap.physiology.org/)

![Fig. 2](http://jap.physiology.org/)

Table 1. Absolute metabolite concentrations in Con and GAMT−/− animals before and after 35 days of Cr supplementation

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>GAMT−/−</th>
<th>GAMT−/−cv</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Creatine</td>
<td>14.7±1.6</td>
<td>1.2±0.8*†</td>
<td>20.6±3.4*</td>
</tr>
<tr>
<td>N-acetylaspartate</td>
<td>9.6±1.2</td>
<td>11.7±5.8</td>
<td>10.4±1.6</td>
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<tr>
<td>Glutamate</td>
<td>11.7±1.5</td>
<td>11.3±6.0</td>
<td>10.0±2.4</td>
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<tr>
<td>Glutamine</td>
<td>4.5±1.0</td>
<td>5.0±3.6</td>
<td>3.9±1.6</td>
</tr>
<tr>
<td>Taurine</td>
<td>7.1±0.6</td>
<td>5.1±2.8</td>
<td>8.3±1.2</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>8.8±1.6</td>
<td>9.0±4.5</td>
<td>7.9±1.0</td>
</tr>
<tr>
<td>Thalamus/hippocampus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>10.7±0.5</td>
<td>0.8±0.6*†</td>
<td>13.7±0.8*</td>
</tr>
<tr>
<td>N-acetylaspartate</td>
<td>8.6±1.5</td>
<td>11.8±1.4*†</td>
<td>9.5±1.2</td>
</tr>
<tr>
<td>Glutamate</td>
<td>10.4±1.2</td>
<td>9.7±1.8</td>
<td>10.0±2.2</td>
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<tr>
<td>Glutamine</td>
<td>3.0±1.3</td>
<td>3.6±1.0</td>
<td>3.2±0.5</td>
</tr>
<tr>
<td>Taurine</td>
<td>7.8±1.6</td>
<td>6.2±1.4</td>
<td>5.6±1.8</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>5.1±0.8</td>
<td>5.7±1.1</td>
<td>6.8±1.5</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
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<td></td>
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<tr>
<td>Creatine</td>
<td>27.7±3.9</td>
<td>3.4±3.9*</td>
<td>34.9±3.7*</td>
</tr>
</tbody>
</table>

Values are in mM and presented as means ± SD. Con, control mouse; GAMT−/−, guanidinoacetate methyltransferase-deficient mouse on a creatine (Cr) free diet; GAMT−/−cv, GAMT-deficient mouse after 35 days of Cr supplementation. *Significantly different from Con at P < 0.05; †significantly different from GAMT−/−cv at P < 0.05.
hippocampus region (Fig. 3) and a slight increase in Tau.

cerebellum. All other metabolites remained constant (Table 1).

Reproducible placement of the voxel for $^1$H MRS in the
triceps surae muscle of Con and GAMT$^{-/-}$ mice was guided
by gradient echo images (Fig. 4). As was already observed for
the brain of GAMT$^{-/-}$ mice, the Cr content was minimal in
skeletal muscle as indicated by an almost complete absence
of signals at 3.0 and 3.9 ppm in the $^1$H MR spectrum (Fig. 4D).
In the skeletal muscle of Con animals, Cr showed clear singlets
at these positions (Fig. 4E).

During the first day of Cr supplementation, a rapid increase
in Cr concentration in skeletal muscle was observed (Fig. 5),
showing that also in skeletal muscle, uptake of Cr from the
blood is important. The increase in absolute Cr concentration
after 24 h was over 10-fold faster in skeletal muscle compared
with thalamus/hippocampus, showing an increase of 14 ± 5
mM for the first day. After this rapid increase, the rate of
increase in Cr concentration in skeletal muscle decreased to
0.8 ± 0.06 mM/day until day 25 ($r^2 = 0.99$). Interestingly, this
rate of increase was similar in both cerebellum and thalamus/
hippocampus. After 35 days of Cr supplementation, Cr con-
centrations in skeletal muscle of GAMT$^{-/-}$ mice were signifi-
cantly higher than in Con animals on a Cr-free diet.

Relative increases in Cr concentration, compared with the
Con concentration, were calculated for both skeletal muscle
and brain regions. In skeletal muscle, the relative increase for
the first day was higher than in brain, 49 ± 20% for skeletal
muscle and 2 ± 6% for thalamus/hippocampus. When linear
regression was applied to relative increases during the second
week, the increase in thalamus/hippocampus was higher com-
pared with skeletal muscle. Relative Cr increases in cerebellum
and thalamus/hippocampus did not differ significantly.

**DISCUSSION**

This study shows that Cr supplementation for 1 mo in
GAMT$^{-/-}$ mice results in a compensation of Cr levels in both
skeletal muscle and brain, despite very low Cr levels in these
tissues before Cr supplementation. Due to a large increase
during the first day of Cr supplementation, the increase in Cr
concentration in skeletal muscle was faster compared with the
increase in Cr levels in brain. Thereafter, the rate of increase in
Cr levels was similar in two regions in brain and in skeletal
muscle.

Before Cr supplementation, localized $^1$H MRS showed very
low Cr levels in brain of GAMT$^{-/-}$ mice, in agreement with
our previous studies on these animals (30, 31). Very low Cr
levels in brain were also observed in GAMT-deficient patients
(see e.g., Ref. 40), supporting the use of the mouse model as a
model for the disease in patients. Other studies on adult mouse
brain using short TE localized $^1$H MRS have observed similar
Cr concentrations in mouse brain of the same strain compared
with Con animals in the present study for both regions in brain
(30, 36, 42). Higher Cr levels in cerebellum compared with
other regions in brain, as observed in Con mice in the present
study, have also been observed in healthy volunteers (16, 27).

Curiously, one study using long TE localized $^1$H MRS in
3-wk-old mice found higher Cr concentrations in thalamus
compared with cerebellum and higher values of Cr in thalamus
compared with the present study (20). The reason for this
discrepancy is unclear. Tissue levels of NAA, Gln, and Glu in
both thalamus/hippocampus and cerebellum in Con mice were
in agreement with a recent study on mouse brain (42).

In GAMT$^{-/-}$ animals before Cr supplementation, a small
elevation was observed in NAA in the thalamus/hippocampus
region. Interestingly, an increase in NAA levels has also been
observed in mice lacking both brain isoforms of creatine kinase, the
enzyme catalyzing the phosphorylation of Cr (14). Increased NAA levels could result from several factors, including
adaptations in intracellular pathways, a redistribution of
cerebral osmolytes or changes in cell size or cell type distrib-
ution across brain regions (Ref. 14 and references therein). In
both creatine kinase-deficient mice and GAMT$^{-/-}$ mice, the
increased NAA levels are accompanied by a decreased Cr level
and as the increased NAA level disappeared after normaliza-
tion of Cr levels in the present study, a redistribution of
cerebral metabolites may play a role in this observation.

The Cr concentration in both the cerebellum and thalamus/
hippocampus region in GAMT$^{-/-}$ mice normalized to, and
even exceeded, control levels after 35 days of Cr supplemen-
tation. If these results in GAMT$^{-/-}$ mice can be extrapolated
to normal brain, this large uptake of Cr from the blood could
indicate that the novo synthesis of Cr is not of large importance
in the brain. The strong reduction of Cr in the brain of
CrT-deficient patients (5) agrees with this suggestion. On the
other hand, it is well possible that GAMT$^{-/-}$ mice have
adapted to the absence of Cr biosynthesis in the brain, for
instance by upregulating the activity and/or level of the CrT
(22). Furthermore, a strict separation of cells that synthesize Cr
and cells that accumulate the compound could exist in the brain
(23, 32), thereby causing the absence of Cr in CrT-deficient
patients. Recent studies where expression of the GAMT en-
zyme was observed mainly in glial cells and astrocytes, with
only minimal expression in neurons, supports this hypothesis
(31, 41). However, results on the expression of GAMT are not
conclusive, as another study found ubiquitous expression of
this enzyme in the brain (1).

As in brain, also in skeletal muscle of GAMT$^{-/-}$ mice Cr
levels before Cr supplementation were very low, in accordance
with previous studies (17, 18, 30, 31). Cr levels in skeletal
muscle determined in Con animals agree well with our previ-
ous study using $^1$H MRS on mouse hind leg of the same strain.

![Fig. 3. The changes in Cr and NAA levels in thalamus/hippocampus of
GAMT$^{-/-}$ mice. Cr levels (■) and NAA levels (○) in GAMT$^{-/-}$ mice before,
during, and after 35 days of Cr supplementation. Corresponding metabolite
levels in Con animals are shown in gray symbols at day 0. Data are shown as
means ± SE.](image-url)
as well as with biochemical determination of the Cr concentration in mixed fiber type skeletal muscles of rodents (12, 44). The rapid increase in Cr after only 1 day of Cr supplementation observed in the present study (to 61 ± 14% of normal concentrations) is in agreement with our previous study on these mice. In that study, an increase to 61 ± 4% of normal levels was observed in the phosphorylated form of Cr (PCr) after 1 day of Cr supplementation, measured by \(^{31}\)P MRS (18). This indicates that the phosphorylated fraction of Cr remains the same after Cr supplementation, in contrast to observations in some studies using muscle biopsies (9) but in agreement with our previous MRS study on human skeletal muscle (19).

Repeated MRS measurements enabled an accurate determination of the time course of the increase in Cr levels in GAMT\(^{-/-}\) mice. This revealed that after the rapid increase of Cr in skeletal muscle during the first day of Cr supplementation, the increase in Cr decreased to a constant rate thereafter. A recent study in rat skeletal muscle after Cr depletion by \(--\)guanidinopropionic acid feeding showed a significant elevation in Cr uptake rates during the first few days of Cr supplementation after Cr depletion (3), but this occurred only in the high oxidative fibers. Interestingly, this increased uptake was not accompanied by an increased CrT expression. This indicates that other mechanisms are responsible for the regulation of Cr uptake at low Cr concentrations in skeletal muscle, like, for instance, the regulation of the activity of the transporter (3). Alternatively, it has been suggested that, apart from the CrT, alternative Cr uptake mechanisms exist in skeletal muscle, as Cr is present in skeletal muscle of CrT-deficient patients (29). In contrast to the presence of Cr in GAMT-deficient muscle, however, Cr in these patients could also result from Cr bio-synthesis in skeletal muscle itself (2, 31). Finally, a nonsat-

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**Fig. 4.** Gradient echo MR images and \(^1\)H MR spectra of the mouse hind leg in vivo. Gradient echo MR image of the mouse hind leg in 3 perpendicular directions (A, B, C). The location of the voxel is shown by the white box. Corresponding \(^1\)H MR spectra of a GAMT\(^{-/-}\) mouse (D) and Con mouse (E) are shown on the right. As in brain, note the severely decreased signal for TCr at 3.0 and 3.9 ppm in the Cr-deficient animal.

**Fig. 5.** The increase in Cr in skeletal muscle of GAMT\(^{-/-}\) mice. Absolute Cr levels in skeletal muscle of GAMT\(^{-/-}\) mice before, during, and after 35 days of Cr supplementation (●). The Cr level of Con mice of a corresponding region in skeletal muscle is at day 0 (○; n = 4). Data are shown as means ± SE.
urable component of Cr transport, likely diffusion of Cr over the cell membrane, has been reported, but only at unphysiological extracellular Cr concentrations (>1.5 mM; Ref. 44).

In brain, the rapid initial increase in Cr as observed in skeletal muscle was absent and, therefore, the increase in Cr levels in skeletal muscle was faster. These results are in agreement with a study on a GAMT-deficient patient (8) and a study using cloned cells (25). It is possible that an incomplete saturation of the CrT plays a role in the difference between skeletal muscle and brain. However, normal plasma concentrations of Cr in the mouse well exceed the \( K_m \) of the CrT of 16 \( \mu M \) (25), and it is therefore working near saturating levels in vivo (38). Since the present Cr supplementation protocol has been shown to elevate plasma Cr concentration levels about fivefold in the mouse (15), the CrT is probably saturated with this Cr dose. Alternatively, the limited permeability of the blood-brain barrier for Cr could play a role in the observed difference. While the microcapillary endothelial cells have been shown to express the CrT, the astrocytes contacting them have not (1, 25, 41) and it is unclear how this influences Cr uptake. Interestingly, the absolute increase in Cr in both tissues is similar after the first day of Cr supplementation, despite large differences in the final Cr concentration. This suggests that, after the first day of Cr supplementation, 0.8 mM/day represents an upper limit for Cr uptake by the CrT in both tissues in these mice. Apparently, the combined effect of both specific activity and number of CrTs is similar between the tissues during this period.

In control mice with normal Cr concentrations, the rate of increase of Cr levels in both skeletal muscle and brain during Cr supplementation is considerably lower than in GAMT\(^{-/-}\) mice in the present study (15). The low intracellular Cr concentration at the start of the Cr supplementation protocol in GAMT\(^{-/-}\) probably influences this (22, 38). Furthermore, elevated serum Gua concentrations in GAMT\(^{-/-}\) mice apparently do not severely limit the uptake of Cr. This could be due to serum concentrations of Gua in GAMT\(^{-/-}\) animals below concentrations where considerable limitations on Cr uptake have been observed (10, 25, 31).

While \(^{31}P\) MRS of skeletal muscle shows a clear signal for PGua (e.g., Refs. 8, 18, 30, 32, 34), the signal for Gua (representing PGua and Gua) was hardly detectable in vivo \(^1H\) MR spectra of skeletal muscle in GAMT\(^{-/-}\) mice. While very low Cr concentrations, when Gua concentrations are expected to be high (18, 30), the Gua signal at best appeared as a broad line. In contrast, using high-resolution magic angle spinning methods, Gua appears as a singlet at 3.78 ppm in freeze-clamped skeletal muscle of GAMT\(^{-/-}\) mice (30). In our previous study on these mice, this curious result was ascribed to possible influences of conformational or environmental changes in vivo (30). In brain, Gua is hard to detect in \(^1H\) MRS as well, possibly due to the line shape but also because of many overlapping peaks at the region where Gua is resonating (like Glu, Ins) and the low expected concentration of Gua (~1.3 mM; Ref. 30). Although it remains a broad resonance, the signal for Gua is usually somewhat more clear in \(^1H\) MR spectra of the brain of patients compared with the mouse model in the present study (8, 40, 43). This could be due to conformational changes in Gua, making the observation of its \(^1H\) signal less favorable at a field strength of 7 T.

As was mentioned in the introduction, the increase in Cr levels in brain in GAMT-deficient patients is slow and incomplete after 2 yr. This long time course not only hampers rapid improvement of clinical symptoms in patients, it also makes optimization of Cr supplementation strategies time consuming. Furthermore, a more rapid increase in Cr levels may prevent the development of clinical symptoms when Cr supplementation is started at a young age. The measurements obtained in the present study could provide a basis for the optimization of Cr supplementation strategies in humans. Measurements in mice are more rapid compared with humans, and therapeutic interventions can be applied easily. Despite the absence in the mouse model of the apparent biphasic pattern of the increase in Cr level in the brain of a GAMT-deficient patient (39) and the difference in time frame, we believe that the model can be used to optimize Cr supplementation strategies to be applied in humans. Any deviation from the uptake kinetics obtained in the present study on GAMT\(^{-/-}\) mice on a change in the Cr supplementation protocol would be a valuable starting point for a similar protocol in humans. Changes in the Cr supplementation protocol could include an increase in the administered Cr dose. However, as the CrT is likely to be saturated at the current dose and previous attempts in humans have been unsuccessful (39), a higher dose of Cr is unlikely to increase the Cr uptake in brain. Intermittent Cr supplementation to avoid possible saturation of the CrT or supplementation or restriction of other metabolites, as has been attempted before with arginine and ornithine (8, 33), however, could provide a useful strategy.

In conclusion, our results show that the faster increase in skeletal muscle Cr levels is only apparent at the beginning of the Cr supplementation period in GAMT\(^{-/-}\) mice. This more rapid increase in skeletal muscle Cr levels compared with brain is probably not caused by an increased expression of the CrT in skeletal muscle in this period. Finally, these results can provide the basis for further experiments to optimize Cr supplementation strategies in human GAMT deficiency.

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