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

Hermien E. Kan,¹,² Esther Meeuwissen,¹ Jack J. van Asten,¹ Andor Veltien,¹ Dirk Isbrandt,³ and Arend Heerschap¹,²

¹Department of Radiology, Radboud University Nijmegen Medical Center, Nijmegen; ²Institute for Fundamental and Clinical Human Movement Sciences, The Netherlands; and ³Center for Molecular Neurobiology, University of Hamburg, Hamburg, Germany

Submitted 22 November 2006; accepted in final form 7 March 2007


Creative (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 METHYLMTRANSFERASE (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.

Address for reprint requests and other correspondence: H. Kan, Dept of Radiology (667), Radboud Univ. Nijmegen Medical Center, Geert Grootplein 10, PO Box 9101, 6500 HB Nijmegen, The Netherlands (e-mail: h.kan@rad.umcn.nl).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.jap.org 8750-7587/07 $8.00 Copyright © 2007 the American Physiological Society
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<sup>−/−</sup>) 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 P Cr 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<sup>−/−</sup> mice, in contrast, provide an unique opportunity to study Cr increases in several tissues in a controlled manner. Localized <sup>1</sup>H MRS enables simple longitudinal assessment of Cr increases in various tissues. Therefore, in the present study, we studied <sup>1</sup>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<sup>−/−</sup> mice (>6 mo old), generated by homologous recombination in embryonic stem cells (31), and homozygous and heterozygous wild-type (Con) littermates of the GAMT<sup>−/−</sup> animals were measured in this study (31). Animals were given free access to standard chow based on vegetable protein to a common dose of 400–600 mg/kg body wt−1 day−1 (15). A voxel (2.2 × 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 [full width half maximum (FWHM)] of 13 Hz (thalamus/hippocampus) or 17 Hz (cerebrum) 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).

**MR experiments.** MRS measurements were performed on a 7.0-T magnet (Magnex Scientific, Abingdon, UK) interfaced to an SMIS magnet (Magnex Scientific, Abingdon, UK) interfaced to an SMIS (Bruker Biospin, version 4.6a) with the same sequence and parameters as the in vivo measurements. For proton spin systems of total Cr, a voxel (2.5 × 2.2 × 2.0 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 shinned until a line width [full width half maximum (FWHM)] of 13 Hz (thalamus/hippocampus) or 17 Hz (cerebrum) 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).

**Data analysis.** Data processing of the <sup>1</sup>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, GdX (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.

The 1H 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<sup>−/−</sup> 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).
**RESULTS**

Gradient echo MR images of the brain of Con and GAMT−/− animals showed 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/hippocampus region.

**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−/− Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
</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-acetyl aspartate</td>
<td>9.6 ± 1.2</td>
<td>11.7 ± 5.8</td>
<td>10.4 ± 1.6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>11.7 ± 1.5</td>
<td>11.3 ± 6.0</td>
<td>10.0 ± 2.4</td>
</tr>
<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-acetyl aspartate</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>
</tr>
<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>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; GAMT−/− Cr, GAMT-deficient mouse after 35 days of Cr supplementation. *Significantly different from Con at P < 0.05; †significantly different from GAMT−/− Cr at P < 0.05.
hippocampus region (Fig. 3) and a slight increase in Tau concentration in skeletal muscle of GAMT−/− mice. All other metabolites remained constant (Table 1).

Reproducible placement of the voxel for 1H 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 1H 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 concentrations in skeletal muscle of GAMT−/− mice were significantly 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 compared 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 1H 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 1H 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).

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 distribution 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 normalization 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 cerebral and thalamus/hippocampus region in GAMT−/− mice normalized to, and even exceeded, control levels after 35 days of Cr supplementation. 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 enzyme 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 previous study using 1H 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.](http://jap.physiology.org/)
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 31P 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 β-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 biosynthesis in skeletal muscle itself (2, 31). Finally, a nonsat-
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 µM (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 $^3$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 $^1$H MR spectra of skeletal muscle in GAMT−/− mice. This more apparent dephasing seen in vivo $^1$H MR spectra of skeletal muscle in GAMT−/− mice. This more apparent dephasing seen in vivo $^1$H MR spectra of skeletal muscle in GAMT−/− mice. This more apparent dephasing seen in vivo $^1$H MR spectra of skeletal muscle in GAMT−/− mice. However, 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 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.

ACKNOWLEDGMENTS

The authors thank Ruben Peco for genotyping.

GRANTS

Financial support for this study was obtained from Nederlandse Organisatie voor Wetenschappelijk Onderzoek Investment Grant 834.04.007.

REFERENCES


