Lower force and impaired performance during high-intensity electrical stimulation in skeletal muscle of GAMT-deficient knockout mice

H. E. Kan, T. E. Buse-Pot, R. Peco, D. Isbrandt, A. Heerschap, and A. de Haan. Lower force and impaired performance during high-intensity electrical stimulation in skeletal muscle of GAMT-deficient knockout mice. Am J Physiol Cell Physiol 289: C113–C119, 2005. First published March 2, 2005; doi:10.1152/ajpcell.00040.2005.—Force characteristics of skeletal muscle of knockout mice lacking creatine (Cr) due to a deletion of guanidinoacetate methyltransferase (GAMT) were studied in situ. Medial gastrocnemius muscles of anesthetized GAMT-deficient (GAMT−/−) and control (Con) littermates were stimulated at optimum length via the sciatic nerve at different stimulation frequencies (60–250 Hz). GAMT−/− mice showed reduced maximal twitch and twitch force, reduced relative force at 60 Hz, and increased relaxation times. High-intensity fatigue protocols consisting of 30 successive isometric or dynamic contractions showed a strong reduction in force at the beginning of the series in GAMT−/− mice, followed by a smaller reduction compared with Con littermates toward the end of the series. Cr supplementation for 2 days in GAMT−/− animals (GAMT−/−) resulted in normalization to Con values for relaxation times, relative force at lower stimulation frequencies, and relative force during 30 isometric contractions. Force per muscle mass, however, remained decreased. Furthermore, GAMT−/− mice showed differences compared with both Con and unsupplemented animals in maximal rates of force rise and relaxation times during the isometric protocol as well as in force during the dynamic protocol. Our results show that the absence of Cr plays a direct role in relaxation times, maximal rate of force rise, and force production during high-intensity fatigue protocols. The lower force per muscle mass, however, is probably caused by other factors; i.e., high intracellular guanidinoacetate concentrations.

The creatine kinase (CK) reaction, where creatine (Cr) and ATP are reversibly catalyzed to form phosphorylcreatine (PCr), ADP, and a hydrogen ion, is considered to be important for energy metabolism during muscular exercise (e.g., 15, 32). This so-called PCr-CK system is thought to play a key role in keeping ATP-to-ADP ratios balanced and possibly also in the transport of high-energy phosphates through the cytosol (2, 33, 35). In skeletal muscle, two major isoforms of CK are present: mouse muscle (MMCK) in the cytosol and skeletal (ScCKmit) in the intermembrane space of the mitochondria (33).

In the past decade, the significance of the PCr-CK system on muscle performance and energy metabolism has been studied in muscles of mice lacking either one or both of the isoforms of CK that are present in muscle (7, 9, 16, 22, 25, 28, 30), and even earlier in rats fed analogs of Cr (e.g., 19, 24). These measurements showed that in skeletal muscle of animals with an impaired PCr-CK system, twitch force production was normal (9, 19, 22, 24, 28, 30), whereas tetanic force was reduced at submaximal stimulation frequencies in animals lacking both isoforms (7). During repeated contractions at high intensity, a dramatic decrease in force was already observed in the second contraction of animals lacking MMCK or both isoforms of CK during twitch and tetanic contractions, whereas force increased slightly after that in animals lacking only MMCK (9, 22, 25, 28, 30). In general, it can be stated that differences between control animals and animals with an impaired PCr-CK system can be observed in particular during exercise with high metabolic demand.

Apart from studies in whole muscles in situ, investigations have been performed using single fibers and whole muscles of animals with an impaired PCr-CK system in vitro (e.g., 4–6). In these studies, it was highlighted that during high-intensity exercise, muscles with an impaired PCr-CK system lacking both isoforms of CK show a substantial decline in force and cytosolic Ca2+ concentration, whereas during less-demanding exercise, the resistance to fatigue is increased compared with controls (6).

Recently, guanidinoacetate methyltransferase (GAMT) deficient knockout mice (GAMT−/−) became available (23) as a model for GAMT deficiency in humans (27). The enzyme GAMT catalyzes the final step in the biosynthesis of Cr, and if this enzyme is absent, no Cr can be formed, leaving dietary supplementation as the sole source of Cr. High guanidinoacetate (Gua), the immediate precursor of Cr in the biosynthesis, and low Cr concentrations in body fluids were observed in both patients and GAMT−/− mice (23, 27), and Cr supplementation has been shown to alleviate some of the effects of GAMT deficiency in patients (26), especially in skeletal muscle. 31P-nuclear magnetic resonance spectroscopy (MRS) measurements of GAMT−/− mice confirmed the lack of PCr and showed that the mice phosphorylate Gua to PGua (17, 21), which in turn can be used by these animals for high-energy phosphoryl transfer through the CK reaction. However, the flux through this system seems to be a rate-limiting factor, especially at higher energy demands (17). Finally, because (P)Cr is absent in the skeletal muscle of GAMT−/− mice, (P)Gua is present in high concentrations and the flux through the CK reaction is compromised, they provide an excellent opportunity to study the PCr-CK system from a different perspective.
The purpose of this study was to investigate the influence of this compromised flux, Cr absence, and the presence of high levels of Gua on force characteristics of skeletal muscle of GAMT<sup>−/−</sup> knockout mice during both single and repeated contractions. Furthermore, because stimulation frequency was also shown to differentially influence force characteristics of mice with an impaired PCR-CK system, the effect of stimulation frequency was studied as well. Finally, the effect of short-term Cr supplementation on these parameters was investigated.

Because the 31P-MRS experiments on GAMT<sup>−/−</sup> mice showed that energy metabolism was affected to a higher extent than in mice lacking either MM-CK or mitochondrial ScCKmit, but not as much as in mice lacking both isoforms of CK, similar results regarding force characteristics and fatigability were expected in the present study. In the end, a unique phenotype was uncovered for GAMT<sup>−/−</sup> mice that differed from all other models for an impaired PCR-CK system.

MATERIALS AND METHODS

Animals. GAMT<sup>−/−</sup> mice were generated by homologous recombination in embryonic stem cells (23). Homozygous (+/+) and heterozygous (+/−) littermates (Con) of the GAMT<sup>−/−</sup> animals were used as a reference (23). Animals were given free access to standard chow based on vegetable protein to eliminate possible Cr content (R/M-H; Ssniff Spezialdiäten, Soest, Germany).

Three groups of adult males and females (>3 mo old) were measured in this study: one group consisted of Con littermates, the second group consisted of GAMT<sup>−/−</sup> animals on a Cr-free diet, and the third group consisted of GAMT<sup>−/−</sup> animals that received Cr monohydrate (Sigma C0780) and saccharose in the drinking water (GAMT<sup>−/−</sup>) for 2 days, as described by Kan et al. (17). Because the mice have been observed to show coprophagia and therefore might get Cr from the feces of Con littermates, animals were housed according to genotype after being genotyped.

All experiments were approved by the local animal ethics committee of the Radboud University Nijmegen Medical Center, and animals were euthanized by cervical dislocation after termination of the study.

Preparation. Before the experiment, animals were anesthetized with nembutal (80 mg/kg) and urethane (1.5 g/kg) and kept under anesthesia with regular doses of nembutal (20 mg/kg). Anesthesia was maintained by an anesthesia with regular doses of nembutal (20 mg/kg), and body temperature during the experiment was kept at 33°C by a water-saturated airflow around the muscle.

For the surgical preparation of the in situ stimulation, the medial head of the gastrocnemius muscle was prepared free from the surrounding tissue, leaving the origin on the femur and the blood supply intact. Distally, the Achilles tendon was cut and attached to a measurement device as described before (8). The muscle-tendon complex was stimulated (0.3–0.4 mA, pulse width 50 ms) with rest intervals of 3 min. Muscle temperature during the experiment was kept at 33°C by a water-saturated airflow around the muscle.

Contraction. In all three groups (Con, n = 10; GAMT<sup>−/−</sup>, n = 10; GAMT<sup>−/−</sup>, n = 5) L<sub>0</sub> was determined as described above and subsequently force-frequency relations were measured at several stimulation frequencies, varying from 60 to 250 Hz (stimulation duration 250 ms). The stimulation frequencies were applied in a random order, and contractions were separated by 3-min rest intervals.

Thereafter, in all three groups (Con, n = 9; GAMT<sup>−/−</sup>, n = 9; GAMT<sup>−/−</sup>, n = 5) resistance to fatigue during dynamic contractions was examined by applying a series of 30 repeated dynamic contractions within 7.5 s (stimulation duration 55 ms, stimulation frequency 150 Hz, shortening velocity 20 mm/s, one contraction every 250 ms).

In a separate experiment (Con, n = 10; GAMT<sup>−/−</sup>, n = 10; GAMT<sup>−/−</sup>, n = 6), determination of L<sub>0</sub> was followed by a series of 30 repeated isometric contractions (at L<sub>0</sub>, stimulation duration 50 ms, stimulation frequency 150 Hz, one contraction every 250 ms) within 7.5 s to test the resistance to fatigue during isometric contractions. Immediately after the last contraction, the muscle was freeze clamped, weighted, and stored in liquid nitrogen until further analysis.

All force signals of the muscle were digitized (1,000 Hz) and analyzed for peak force, maximal rate of force rise (RFR<sub>max</sub>), and half-time of relaxation (HRT) (time for force to fall from one-half to one-fourth at the end of stimulation) (11). Because force signals were hardly fused at a stimulation frequency of 60 Hz, the force of the first pulse at this stimulation frequency was used to estimate twitch force.

Chemical analysis of metabolite concentrations. Muscles frozen in liquid nitrogen after the fatigue protocol with isometric contractions were pulverized in a mortar under constant addition of liquid nitrogen, freeze-dried overnight, and stored in liquid nitrogen until further analysis. Metabolites were separated and quantified using a high-performance liquid chromatography (HPLC) system using RP-18 columns (Hewlett Packard), as described before (18). The HPLC system consisted of a Binary LC pump (model 250, Perkin-Elmer), an auto sampler with cooling tray and automatic injector (Basic Marath, Spark Holland), and a variable wavelength ultraviolet spectrophotometric detector (model 795A, Applied Biosystems). The size of the injection loop was 20 μl and the ultraviolet absorption was measured at 254 nm. PCR and Cr peaks were identified and quantified by custom-designed software written in Matlab (MathWorks, Natick, MA) that compared the peak heights of samples with those of external standards (18), and was expressed in micromoles per gram of dry weight.

Statistics. Force and RFR<sub>max</sub> variables from the force frequency relationships were tested for significance between the three groups using two-way ANOVA (factors for group and stimulation frequency). Force characteristics at 250 Hz, twitch force parameters, muscle mass, and metabolite concentrations were compared using ANOVA. Relative force signals, HRT, and RFR<sub>max</sub> values of the isometric fatigue protocol and relative force signals of the dynamic fatigue protocol were compared using a two-way ANOVA (factors for genotype/group and contraction number). Finally, Pearson’s correlations were calculated between total Cr concentration obtained from the HPLC analysis and the reduction in force during the dynamic fatigue protocol.

Two-way ANOVA tests were post hoc tested with Bonferroni posttests and ANOVAs with Tukey’s posttests. All tests were performed with Prism software (GraphPad, San Diego, CA), and results were considered significant at P < 0.05 and shown as means ± SD in the text and as means ± SE in the figures (for clarity).

RESULTS

Control vs. GAMT<sup>−/−</sup> without Cr supplementation. To confirm the almost complete depletion of Cr in skeletal muscle of GAMT<sup>−/−</sup> animals, freeze-dried muscles were analyzed for Cr content with HPLC. As was expected, total Cr concentrations (PCR + Cr) were significantly different between GAMT<sup>−/−</sup> and Con animals (Table 1). Any residual Cr in GAMT<sup>−/−</sup> animals was probably due to coprophagia that occurred before the animals were genotyped (17).

Force. To study the influence of the low muscular Cr concentrations found in GAMT<sup>−/−</sup> skeletal muscle on force characteristics in detail, three different stimulation protocols were applied. The influence of stimulation frequency was studied during single contractions. Furthermore, during repeated isometric and, energetically more challenging dynamic
contractions the resistance to fatigue was investigated. Forces were digitized and representative examples of these force tracings in a Con and GAMT−/− medial gastrocnemius muscle are shown in Fig. 1.

During single contractions, twitch force and absolute force at all studied stimulation frequencies were significantly lower in GAMT−/− muscle compared with Con muscle (Fig. 2A and Table 1). The force of a twitch compared with the force of a tetanus at a maximal stimulation frequency of 250 Hz (twitch-to-tetanus ratio), however, did not differ between mouse types (0.16 ± 0.06 for GAMT−/− and 0.19 ± 0.07 for Con animals). Average muscle mass was similar in the groups, and hence maximal force normalized to muscle mass also showed a significant difference between Con and GAMT−/− animals (Table 1). When the force at submaximal stimulation frequencies was expressed as a percentage of the force at maximal stimulation frequency (250 Hz), only at the lowest stimulation frequency (60 Hz) a significant difference was observed between Con and GAMT−/− mice (Fig. 2B).

Resistance to fatigue was studied in two different protocols, one consisting of isometric contractions and a second of dynamic contractions. During repeated isometric contractions, apart from the absolute difference in force production between GAMT−/− and Con mice that was already observed in the single contractions, a decrease in force was apparent in both mouse types. Normalized to the first isometric contraction, a stronger decrease of force was observed in GAMT−/− mice compared with Con mice after the second contraction, whereas after the sixth contraction, the force in the GAMT−/− mice stabilized to 63 ± 8% in the last contraction (Fig. 3A). In Con mice the force declined at a constant rate after the first contraction to end at 73 ± 3%.

A similar pattern was observed during repeated dynamic contractions in which, in GAMT−/− mice, a dramatic decrease in force to ~35% was observed within six contractions (Fig. 3B). After this initial decrease, the force remained constant throughout the remaining contractions. In contrast, in Con animals, the force was almost stable force during the first 10 contractions and subsequently decreased to similar levels compared with GAMT−/− mice (28 ± 11% for GAMT−/− and 38 ± 9% for Con mice in the last contraction).

Maximal rate of force rise. Apart from the maximal force of a contraction, the maximal rate at which this force is achieved, $R_{FIR}$, is another important parameter for muscular function. Absolute $R_{FIR}$ differed significantly between the mouse types at all stimulation frequencies during the single contractions (data not shown); however, when normalized to maximal force, the difference disappeared.

During repeated isometric contractions, relative $R_{FIR}$, values, normalized to the first contraction, showed a strong decrease during the first five contractions in GAMT−/− mice, and thereafter the value stabilized to 56 ± 8% in the last contraction (Fig. 3C). In contrast, in Con mice, the $R_{FIR}$ remained constant during the first half of the series and decreased slightly after that to 80 ± 3% in the last contraction.

Half relaxation time. The third parameter that was derived from the force tracings was the time for the force to fall from 50% to 25% of its maximal value (HRT) because increases in this parameter can indicate metabolic disturbances in the muscle cell. GAMT−/− animals showed a significantly slower relaxation than Con animals, as indicated by the higher HRT (Table 1). During the isometric protocol, there was a faster decrease during the initial five contractions in GAMT−/− mice, and thereafter the value stabilized to 56 ± 8% in the last contraction (Fig. 3C). In contrast, in Con mice, the $R_{FIR}$ remained constant during the first half of the series and decreased slightly after that to 80 ± 3% in the last contraction.

### Table 1. Force characteristics and other parameters of skeletal muscle of control, GAMT−/−, and GAMT−/− animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>GAMT−/−</th>
<th>GAMT−/−</th>
</tr>
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<tbody>
<tr>
<td>Twitch force, mN/mg</td>
<td>346 ± 121</td>
<td>194 ± 72*</td>
<td>271 ± 64</td>
</tr>
<tr>
<td>Maximal force, mN/mg</td>
<td>1,840 ± 266</td>
<td>1,221 ± 188*</td>
<td>1,590 ± 230</td>
</tr>
<tr>
<td>Force/muscle mass, mN/mg</td>
<td>30.8 ± 4.0</td>
<td>22.6 ± 4.2*</td>
<td>25.2 ± 2.6*</td>
</tr>
<tr>
<td>HRT, ms</td>
<td>7.1 ± 1.2</td>
<td>9.4 ± 1.7*</td>
<td>7.0 ± 0.7</td>
</tr>
<tr>
<td>Muscle mass, mg</td>
<td>60.2 ± 8.6</td>
<td>54.8 ± 8.8</td>
<td>63.0 ± 4.4</td>
</tr>
<tr>
<td>Total creatine, mmol/kg</td>
<td>90.0 ± 9.7</td>
<td>97.2 ± 2.8*</td>
<td>65.6 ± 19.9*</td>
</tr>
</tbody>
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Values are means ± 5D. GAMTCr guanidinoacetate methyltransferase (GAMT)-deficient animals supplemented with creatine (Cr) for 2 days; HRT, half time of relaxation. *P < 0.05, significantly different compared with control mice.
increase in HRT in the GAMT−/− animals, which became significant after 10 contractions (Fig. 3D). In the last contraction, relative HRT was 201 ± 50% in the GAMT−/− compared with 159 ± 24% in the control animals.

Creatine supplementation. Next, the effect of short-term Cr supplementation on the observed differences between Con and GAMT−/− animals was investigated. A protocol with only 2 days of Cr supplementation was selected because our previous study (17) of these mice indicated that after this period, considerable amounts of PCr and PGua are present without significantly reducing inorganic phosphate (Pi) concentrations.

After 2 days of Cr supplementation, the total Cr concentration had increased to 65.6 ± 19.9 μmol/g dry wt in the GAMT−/− group. Furthermore, some of the differences observed between GAMT−/− and Con animals disappeared. In contrast to GAMT−/− animals without Cr supplementation, absolute force and HRT at maximal stimulation frequency did not differ from Con values; however, normalized to muscle mass the difference in force between GAMT−/− and Con difference persisted in the GAMT−/− group (Table 1).

During repeated isometric contractions, the force in GAMT−/− animals declined at a similar rate as in Con mice (Fig. 4A). During the dynamic protocol, however, the decline in force was different compared with both other groups. The fast decrease in force during the first five contractions observed in GAMT−/− animals without Cr supplementation was absent in the GAMT−/− group (Fig. 4B). However, after these first five contractions, force decreased faster than in Con animals. During the isometric protocol changes in normalized RFRmax (Fig. 4C) and HRT values (Fig. 4D) were different from both Con and GAMT−/− animals.

Finally, the influence of total Cr concentration on force production during the dynamic fatigue protocol was investigated. For each contraction of the series, average force (as % of the first contraction) and total Cr concentration of GAMT−/− and Con animals, and the individual forces and total Cr concentrations of the GAMT−/− animals were plotted. This yielded significant Pearson’s correlations between total Cr concentration and force up to contraction 13 (r² ranging from 0.70 at the second to 0.72 at contraction 13), the highest correlation coefficient was found at contraction 10 (Fig. 5) (r² = 0.97). After contraction 13, the correlation coefficient decreased steadily.

DISCUSSION

In this study, we investigated skeletal muscle force and performance of creatine-deficient GAMT knockout mice. Skeletal muscle of GAMT−/− mice showed a lower absolute force, lower relative force (compared with maximum force) at lower stimulation frequencies, and attenuated resistance to fatigue during repeated high-intensity isometric and dynamic contractions. Two days of Cr supplementation in GAMT−/− mice (GAMT−/−) were sufficient to normalize relative force at lower stimulation frequencies during single contractions. Force normalized to muscle mass remained lower in GAMT−/− mice, as did the attenuation in the resistance to fatigue during the dynamic contraction fatigue protocol, although the reduction in force was postponed by ~8 contractions.

Single contractions. GAMT−/− mice showed a decreased absolute force of the medial head of the gastrocnemius muscle compared with Con mice during twitches and tetanic contractions at all stimulation frequencies. This lower force in skeletal muscle is in agreement with clinical findings in GAMT-deficient patients (31) as well as with a previous study (23) on GAMT−/− mice, where maximum grip force of male GAMT−/− mice was lower than in control animals.

The observed decrease in force could be attributed to several factors, both biochemical and ultrastructural. However, because no marked morphological changes were observed in skeletal muscles of these mice (23), it is unlikely that cytoarchitectural abnormalities caused the reduced force. Furthermore, as besides absolute force, the force divided by muscle mass was also reduced compared with Con animals, it is unlikely that a decreased muscle mass caused the observed difference.

Local ATP-to-ADP ratios. As mentioned earlier, the PCr-CK system is considered important for maintaining local ATP-to-ADP ratios. It would seem logical that in GAMT−/− mice, in which this system is impaired due to a reduced flux and slightly reduced ATP concentrations (17), local myocellular disturbances in this ratio are present. This is supported by the longer relaxation times observed in the GAMT−/− animals as local myocellular disturbances of ATP-to-ADP ratios could lead to an increase in this parameter (1).

At submaximal stimulation frequencies, local shortage of ATP near the Ca²⁺ channels at the sarcoplasmic reticulum could lead to a reduced Ca²⁺ release, which would lead to lower force production (34). However, at maximal stimulation frequencies and therefore saturating Ca²⁺ levels, no such effect can be expected (7). As in GAMT−/− mice force compared...
with the maximal force differed from Con animals only at 60-Hz stimulation, it is unlikely that a lower Ca\(^{2+}\) release caused the observed reduction in force at maximal contractions. At 60-Hz stimulation, however, it is possible that less Ca\(^{2+}\) is available in the sarcoplasm of GAMT\(^{-/-}\) animals compared with Con animals, leading to a lower force production (7).

Interestingly, after supplementing GAMT\(^{-/-}\) animals with Cr for 2 days, the difference in relaxation times as well as the reduction in relative force at 60-Hz stimulation normalized to Con values. This indicates that Cr plays a direct role in the observed differences and suggests that the ATP-to-ADP ratio buffering by the PCr-CK reaction is important. In contrast, force divided by muscle mass still differed between GAMT\(^{-/-}\) and Con mice. Obviously short-term Cr supplementation was not sufficient to overcome the observed differences. Furthermore, it indicates that local perturbations of ATP-to-ADP ratios that might underlie the longer relaxation times probably did not cause the persistent force differences.

**P, and pH.** In our previous study on GAMT\(^{-/-}\) mice (17), \(^{31}\)P-MRS measurements showed normal resting tissue pH but higher P\(_i\) levels in their hindleg musculature. Increased concentrations of P\(_i\) could lead to a depression in muscle force, as is commonly seen in fatigue (1, 14). However, most of the experiments in which the influence of P\(_i\) was investigated have been performed on single muscle fibers at subphysiological temperatures, and temperature has been shown to influence the effect of P\(_i\) on muscle force (10). Furthermore, mice lacking both isoforms of CK also showed higher levels of P\(_i\); however, they did not show lower maximal tetanic and twitch forces (7, 25).

**Influence of Gua.** Finally, it is also possible that Gua has a toxic effect and therefore plays a role in the reduction in absolute force. In patients, Cr supplementation has been shown to alleviate some but not all of the symptoms of GAMT deficiency, and this was partly attributed to the toxic effects of Gua in the brain (31), where it interacts with the GABA\(_A\) receptor (20) and Na\(^+-\)K\(^+\) ATPase activity (36). In a previous study (17) of these mice, \(^{31}\)P-MRS experiments showed that PGua levels after 2 days of Cr supplementation had not decreased to Con levels. This suggests that Gua levels in the GAMTCr group in the present study were probably still elevated compared with Con mice.

**Repeated contractions.** During 30 repeated isometric contractions, force declined rapidly in GAMT\(^{-/-}\) mice and stabilized after several contractions. The isometric protocol was performed at a stimulation frequency of 150 Hz, and during the single contractions no differences were observed between Con and GAMT\(^{-/-}\) mice at this stimulation frequency. Therefore, possible suboptimal stimulation frequency is unlikely to have caused the reduction. Apparently, GAMT\(^{-/-}\)-skeletal muscle is less fatigue resistant than Con muscle during this high-intensity isometric exercise. After Cr supplementation, relative force in GAMTCr\(^{-/-}\) mice was similar to that in Con mice. This indicates that the absence of Cr played a role in the attenuated relative force in GAMT\(^{-/-}\) mice.

Interestingly, RFR\(_{\text{max}}\) and HRT values were not different from those of Con animals in the Cr-supplemented group of GAMT\(^{-/-}\) animals. This indicates that there still could be local disturbances in the ATP-to-ADP ratio that are bigger than those in Con animals. However, these disturbances are apparently not big enough to cause force impairment, possibly due to the short duration of the contraction.

During the dynamic protocol, the differences between GAMT\(^{-/-}\) mice and Con mice were even more dramatic than those during the isometric protocol. Furthermore, in contrast to the isometric protocol, in the dynamic protocol relative force of the GAMTCr\(^{-/-}\) group showed a different pattern compared with both Con and GAMT\(^{-/-}\) mice without Cr supplementation. Force was maintained longer than in nonsupplemented...
GAMT \(^{--/-}\) mice but still declined earlier than in Con animals. These differences compared with the results obtained with the isometric protocol could be attributed to a higher metabolic demand as during dynamic exercise metabolic fluxes are higher than during isometric exercise (12). As was mentioned above, the flux through the CK reaction with PGua as a substrate is lower than with PCr and could well be rate limiting during this exercise (17). In turn, this could cause the reduction in force by a decrease in ATP-to-ADP ratios. Interestingly, the total Cr concentration obtained in the HPLC measurements highly correlated with the relative force produced during the first half of the series of contractions during the dynamic protocol. This indicates that the total Cr concentration plays a direct role in the reduction of force during the first half of this protocol.

Comparison to other animals with an impaired PCr-CK system. Because previous studies (e.g., 3, 13, 29) have shown that the cytosolic isoform of CK can play a role in phosphorylating Cr analogs, it is likely that CK activity is responsible for the phosphorylation of Gua. Therefore, it is interesting to compare the results of the present study with those from studies on mice lacking muscle CK or other animals fed Cr analogs.

Experiments similar to those as described in the present study were performed on skeletal muscle of mice lacking either one or both isoforms of muscle CK (7, 9, 25). As was mentioned earlier, these and other studies showed that in all mice lacking one or both isoforms of CK and also in rats fed the Cr analog \(\beta\)-guanidinopropionic acid, twitch force production was normal. Interestingly, in contrast to those animals, twitch force was lower in GAMT \(^{--/-}\) animals compared with Con mice in the present study. This suggests that impairment of the PCr-CK system itself does not necessarily result in reduction of twitch force and, as was argued above, the reduction in force in GAMT deficiency has another origin. The results of the GAMT \(^{--/-}\) group corroborate this hypothesis as (twich) force normalized to muscle mass still differed from Con values.

The fatigue protocol, both dynamically and isometrically, showed that GAMT \(^{--/-}\) animals have a reduced capacity to maintain force during high-intensity exercise. This was also observed in other animals with an impaired PCr-CK system, although the effect varied in intensity (5, 9, 25). Interestingly, in vitro experiments on single fibers and whole muscles of animals with an impaired PCr-CK system (e.g., 4-6) and a study on intact muscle (16) showed that these effects are observed only during high-intensity stimulation protocols and the animals perform better than Con animals if the intensity is reduced (5). It is conceivable that this would also occur in GAMT \(^{--/-}\) animals; however, this remains to be investigated.
In summary, our results indicate that the absence of Cr caused by GAMT deficiency results in a lower ability to maintain force during high-intensity stimulation in skeletal muscle and in slightly lower relative forces at lower stimulation frequencies. Cr supplementation for 2 days resulted in an increase of total Cr in muscle and alleviated some of the consequences of GAMT deficiency, indicating that (the absence of) Cr plays a direct role in those processes. However, the absence of Cr itself probably does not cause the reduction in force per muscle mass observed in these animals.

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GRANTS

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