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Effects of the creatine analogue β-guanidinopropionic acid on skeletal muscles of mice deficient in muscle creatine kinase

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Abstract

To evaluate the effects of phosphocreatine (PCr) and creatine (Cr) depletion on skeletal muscles of mice deficient in muscle creatine kinase (M-CK), we have fed mutant mice a diet containing the creatine analogue β-guanidinopropionic acid (βGPA). After 8–10 weeks of feeding, accumulation of the creatine analogue in M-CK-deficient muscles was comparable to that observed in muscles of wild-type mice. Strikingly, and unlike wild types, mutants did not accumulate phosphorylated βGPA, indicating that MM-CK is the only muscle CK isoform which can phosphorylate βGPA. In M-CK-deficient muscles there was respective depletion of PCr, Cr and ATP levels to 31, 41 and 83% of normal. The average cross-sectional area of type 2B fibres in gastrocnemius muscles was very much reduced and was similar to type 1 and type 2A fibres which maintained their normal size. The maximal isometric twitch force developed by gastrocnemius-plantaris-soleus (GPS) muscle complexes of βGPA-treated mutants was reduced by about 30%, but these muscles showed an increased fatigue resistance during 1 and 5 Hz contraction. Mitochondrial enzyme activities in the upper hind limb musculature of null mutants were 20–35% increased by the βGPA diet. Altogether, these results provide evidence that certain functions of the creatine kinase/phosphocreatine (CK/PCr) system are not eliminated solely by the loss of M-CK.

Key words: CK/PCr system; Creatine kinase deficiency; Phosphocreatine; β-Guanidinopropionic acid; Muscle; Skeletal muscle bioenergetics; (M-CK-deficient mice)

1. Introduction

Skeletal muscles contain high levels of phosphocreatine (PCr) and creatine kinase (CK), which are presumed to function in energy buffering and energy transport [1–3]. Thus far, two kinds of animal model have been used to evaluate the physiological importance of the CK/PCr system in energy metabolism of skeletal muscle. One model involves the feeding of synthetic analogues of Cr, such as β-guanidinopropionic acid, which competitively inhibit uptake of creatine (Cr) in skeletal muscle cells [4,5]. Rats put on a diet containing 1% βGPA (w/w) for 6–8 weeks accumulate high concentrations of phosphorylated βGPA (βGPAP) in their skeletal muscles, and display PCr and ATP levels which are respectively 12- and 2-fold reduced compared to normal. A reduction in the flux of high-energy phosphates between PCr and ATP parallels the decline of PCr, as shown by steady-state nuclear magnetic resonance (NMR) magnetization transfer experiments [6,7]. The phosphorylated βGPA cannot effectively substitute for PCr, because it is a poor substrate for CK [8]. βGPA feeding results in a number of adaptive changes in skeletal muscle. Glycogen levels are elevated, while glycolytic enzymes and glycogen consumption during muscle exercise are reduced [7,9]. The aerobic energy-generating capacity of type 2 fibres is enhanced [9]. Mitochondria in perinuclear and subsarcolemmal areas of type 1 fibres frequently contain paracrystalline inclusions and often are

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enlarged [10]. Type 2 fibres only show an increase in size of subsarcolemmal mitochondria.

In the second animal model, which has been generated via gene targeting in mouse embryonic stem (ES) cells, the CK/PCr system is blocked at a nodal point by inactivation of the M-CK gene [11]. Mice deficient in M-CK are viable, exhibit no overt abnormalities and display no specific compensatory expression of other members of the CK gene family in their muscles. Steady-state levels of PCr and ATP are normal, but forward and reverse fluxes through the CK reaction are undetectable by ^{31}P-NMR magnetization transfer techniques. Type 2 fibres exhibit phenotypic adaptations in their anaerobic and aerobic energy metabolism. Their increased glycogen content is accompanied by an enhanced use of glycogen during muscle contraction. Moreover, the intermyofibrillar mitochondrial volume of type 2 fibres is considerably increased. In general, the aerobic energy generating potential of the M-CK-deficient upper hind musculature is about 2-fold higher compared to that of the corresponding muscles of wild-type mice. The maximal isometric twitch force of M-CK-deficient GPS muscle complexes is normal, but these complexes lack burst activity and exhibit an improved endurance. Similar physiological changes have been reported for GPS muscles of βGPA-fed rats [7].

Strikingly, M-CK-deficient muscles were found to hydrolyse PCr during periods of exercise, suggesting that there are routes for PCr breakdown other than those expected in the current models of CK and PCr function [1–3]. Thus, there may still be a role for PCr in skeletal muscles lacking MM-CK activity. To investigate this further, we fed mutant mice a diet containing 1% βGPA. We show that this diet results in additional changes in metabolism, structure and function of M-CK-deficient skeletal muscles.

2. Methods

Animals

Female M-CK-deficient and wild-type mice (having a [C57BL/6 × 129/Sv] hybrid genetic background) were used in all experiments. M-CK-deficient mice were created via targeted inactivation of the M-CK gene in mouse ES cells as described previously [11]. All M-CK deficient and wild-type mice were bred in the Central Animal Facility of the University of Nijmegen. At 3 weeks of age, mutant and wild-type mice in the experimental groups were given free access to water and standard mouse chow containing 1% β-guanidinopropionate (Fluka) for 8–10 weeks. Mutant and wild-type mice in the control groups were fed standard chow without the creatine analogue.

^{31}P-NMR

Fully relaxed ^{31}P-NMR spectra of upper hind limb muscles at rest were collected and analyzed as described previously [11]. Exponential multiplication of the free induction decays (FID) was applied before Fourier transformation, resulting in a line broadening of 18 Hz. Peak areas were measured by Lorentzian curve-fitting procedures after baseline correction. pH values were calculated from the chemical shift of inorganic phosphate (P_i) signal as described previously [12].

Chemical analyses of metabolite concentrations

Mice were anaesthetized (2,2,2-tribromoethanol; 0.2 mg/g body weight, injected intraperitoneally) and GPS muscle groups were exposed and frozen in clamp tongs precooled in liquid nitrogen. The frozen muscles were pulverized in a mortar cooled with dry ice, weighed portions of pulverized muscles were diluted 10-fold (w/v) with cold 4% perchloric acid and subsequently homogenized. Glycogen, lactate, ATP, ADP, AMP, PCr and Cr were assayed by standard enzymatic analyses as described in Bergmeyer [13]. βGPA and βGPAP levels were determined by the method of Bonas and co-workers [14] as described by Fitch and Chevli [15].

Histochemistry and electron microscopy

Mice were anaesthetized as described above. For histochemistry, GPS muscle complexes were excised and quickly frozen to −150°C in liquid nitrogen-chilled isopentane. Subsequently, cross-sections (8 μm) were cut and stained for myosin ATPase (pH 4.3, 4.6 and 10.8), succinate dehydrogenase (SDH) and glycogen according to standard histochemical procedures [16]. Stained sections were examined using a Leitz Orthoplan light microscope. Cross-sectional areas of type 1, type 2A and type 2B fibres in the gastrocnemius muscle were measured according to standard point sampling methods (n = 20 fibres per fibre type per muscle).

For electron microscopy the individual muscles of the GPS complex were dissected during immersion fixation with 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4; 330 mosM). The specimens were oriented longitudinally for prolonged overnight fixation at 4°C and rinsing (3 h overnight) in 0.1 M phosphate buffer at 4°C. Postfixation was carried out in the same buffer supplemented with 1% osmium tetroxide at 4°C for 1–2 h. The tissues were then rinsed twice in the same buffer and temperature for at least 1 h. Dehydration was performed in an ascending series of aqueous ethanol. Tissues were transferred via a mixture of propylene oxide and Epon to pure Epon 812 as embedding medium. Ultrathin grev sections were cut, contrasted with aqueous 3% uranyl acetate, rinsed and counterstained with lead citrate, air-dried and examined in a Philips electron microscope EM 300 or 301.

Enzyme assays

Fresh upper hind limb muscles were excised and homogenized in a Teflon-glass homogenizer in 1:10 dilution (w/v) of buffer containing 50 units/ml heparin, 250 mM sucrose, 2 mM EDTA and 10 mM Tris-HCl at pH 7.4 (4°C). Homogenates were centrifuged at 600 × *g* and cytochrome *c* oxidase (COX) and citrate synthase (CS) activities were assayed in the supernatants according to the methods of Cooperstein and Lazarow [17] and Srere [18], respectively.

Muscle force measurements

M-CK deficient and wild-type mice fed a diet with βGPA for 8–10 weeks were anaesthetized as described above. The hind limb was fixed between a pair of brass posts at the point of the condyli of the femur. The distal tendons of GPS muscles were connected to a force transducer (Statham Transducing cell UC2; [19]). Isometric contractions were elicited by supramaximal stimulation (square pulses of 15 V and 2 ms duration) of the muscle via the sciatic nerve using a bipolar platinum electrode. We determined successively: the maximal twitch force of three single contractions; the twitch force of the first 24 contractions at 1 and 5 Hz stimulation; the twitch forces during 180 s at 1 and 5 Hz stimulation (after 1 Hz stimulation muscles were allowed to recover for 10 min).

3. Results

Energy metabolites in muscles at rest

The steady-state levels of phosphorus metabolites in M-CK-deficient and wild-type muscles of βGPA-treated mice were determined by in vivo ^{31}P-NMR spectroscopy. Representative spectra ($n = 4$ in each group) of upper hind limb muscles from βGPA-treated mice deficient in M-CK (−/− GPA), βGPA-fed wild-type mice (+/+ GPA) and wild-type mice (+/+) are shown in Fig. 1. Spectra of muscles from mice deficient in M-CK (−/−) are identical to that shown for +/+ muscles [11]. Typically, the βGPAP resonance (located 0.45 ppm upfield from the PCr resonance in control spectra) was the major phosphagen in +/+ GPA muscle, while the PCr resonance was decreased such that it is not seen anymore as a discrete peak in Fig 1C. Strikingly, βGPA-treated muscles of −/− mice showed no accumulation of βGPAP. Despite this absence of βGPAP, the PCr resonance was significantly below normal. Intracellular pH values, as determined from the chemical shifts of the inorganic phosphate (P_i) peaks, were normal in both +/+ GPA and −/− GPA mice.

The concentrations of energy metabolites in muscles of +/+ GPA and −/− GPA mice as determined by biochemical methods are summarized in Table 1 (see

Fig. 1. Representative ^{31}P-NMR spectra of upper hind limb muscles from (A) wild-type mice, (B) βGPA-fed wild-type mice and (C) βGPA-fed mice deficient in M-CK. Each spectrum is the average of 128 free induction decays generated with 70° magnetization pulse angles at 8 s intervals. Resonance assignments are indicated on the figure.

Ref. [11] for levels of energy metabolites in +/+ and −/− muscles). βGPA levels in −/− GPA muscle extracts were almost 4-fold higher compared to those in extracts of +/+ GPA muscles. After acid hydrolysis of the tissue extracts, βGPA levels in −/− GPA extracts were not increased, indicating that βGPA is indeed not phosphorylated in muscles lacking M-CK expression. Hydrolysis of +/+ GPA muscle extracts revealed a ratio of βGPA to βGPAP of about 1:3. Total βGPA (βGPA + βGPAP) levels were identical in muscles of −/− GPA and +/+ GPA mice. In −/− GPA muscles, Cr, PCr, total Cr (PCr + Cr) and ATP concentrations were reduced to respectively 31, 41, 39 and 83%, while in +/+ GPA muscles they declined to respectively 32, 20, 25 and 80% (see Table

Table 1
Chemical analysis of energy metabolites in GPS muscles of wild-type and M-CK-deficient mice FED with βGPA

Chemical	+/+ GPA	−/− GPA
ATP	6.34 ± 0.68	5.80 ± 0.38
ADP	0.73 ± 0.04	1.44 ± 0.15
AMP	0.06 ± 0.01	0.20 ± 0.04
ATP+ADP+AMP	7.13 ± 0.64	7.41 ± 0.64
Cr	3.0 ± 0.9	2.1 ± 0.5
PCr	3.3 ± 1.6	9.0 ± 1.2
Cr+PCr	6.3 ± 2.3	11.1 ± 1.0
βGPA	4.9 ± 1.9	18.7 ± 2.9
βGPAP	13.9 ± 2.2	0.4 ± 0.7
βGPA+βGPAP	18.7 ± 2.4	18.3 ± 2.4
Glycogen	74.7 ± 16.2	57.6 ± 12.3
Lactate	2.7 ± 0.7	3.1 ± 1.0

Values are means ± SD in μmol/g wet weight (glycogen is expressed as μmol glucose/g wet weight); $n = 5$.

1 and ref. 11). ADP and AMP levels were not altered by the βGPA diet in both M-CK knock-out and wild-type mice.

As reported earlier [11], glycogen levels in GPS complexes of −/− mice have been shown to be 1.6-fold higher than in the corresponding muscle complexes of +/+ mice (42.3 ± 7.2 versus 26.1 ± 5.8 μmol glucose/g wet weight). Glycogen levels in GPS complexes were increased by the βGPA diet in both +/+ and −/− mice (Table 1), but the changes were more dramatic in wild-type than in M-CK-deficient mice (a 2.9-fold versus a 1.4-fold increase). Steady-state levels of lactate, which have been shown to be identical in muscles of −/− and +/+ mice (6.8 ± 1.4 and 7.0 ± 2.5 μmol/g wet weight, respectively, see also Ref. [11]), were significantly reduced as a result of the βGPA diet. In −/− GPA muscles the decrease in lactate was 2-fold compared to −/− muscles and in +/+ GPA muscles the reduction was 2.6-fold compared to +/+ muscles.

Histochemical and morphological characterizations

Analogue feeding caused a slight reduction in body weight in both mutant and wild-type mice. Body weights of −/−, −/− GPA, +/+ and +/+ GPA mice ($n = 7$ in each group) were 19.8 ± 0.8, 16.5 ± 2.3, 20.4 ± 1.1 and 17.3 ± 1.5, respectively. Such reduction in body mass of mice on a βGPA diet has also been reported by Moerland and co-workers [20].

Cross-sections of GPS muscle complexes were stained for the different types of myosin ATPase and SDH, which allows discrimination between type 1, type 2A and type 2B fibres. In M-CK-deficient as well as wild-type mice, the number and the distribution of the three fibre types were similar with and without βGPA ingestion in all three muscles of the GPS complex. The influence of βGPA feeding on fibre size was evaluated by analyzing the cross-sectional areas of the three fibre types in the medial portion of the gastrocnemius muscle (Table 2). The mean area of type 2B fibres in −/− GPA mice was 42% smaller than in −/− mice, whereas the mean areas of both type 1 and type 2A fibres were not significantly changed by the diet. In +/+ GPA mice, the mean area of type 2B fibres was 32% reduced compared to +/+ mice and, as in mutants, no alterations of significance were noticed in the areas of type 1 and type 2A fibres.

Previously, we have reported that mutant type 2 fibres stained for SDH activity present a more coarsely granulated aspect than the corresponding fibres of wild-type animals, which is indicative for an increased intermyofibrillar mitochondrial volume [11]. As shown in Fig. 2, type 2 fibres of βGPA-fed mutants retained the coarsely granulated aspect, whereas it was not observed in the corresponding fibres of wild-type mice treated with βGPA. These data were confirmed by transmission electron microscopy (Fig. 3A and C). As in null mutants [11], mitochondria in type 2 fibres of −/− GPA mice were frequently packed in rows between the myofibrils and a high proportion of the intermyofibrillar and subsarcolemmal mitochondria were enlarged (Fig. 3). On the other hand, mitochondria had a normal distribution in type 2 fibres of +/+ GPA mice and only subsarcolemmal mitochondria were frequently larger than normal. Unlike in βGPA-fed rats [10], no mitochondria with paracrystalline inclusions were noticed in type 1 fibres of soleus muscles of +/+ GPA and −/− GPA mice (Fig. 3).

To determine whether the aerobic energy generating capacity of skeletal muscles had increased because of the βGPA diet, as has been reported for βGPA-fed rats [9], we measured the activities of two mitochondrial marker enzymes, COX and CS, in upper hind limb muscles of +/+ GPA and −/− GPA mice (Table 3) and compared these activities to those of +/+ and −/− muscles (previously reported in ref. 11). This comparison showed that both enzyme activities were elevated by the βGPA diet in wild-type as well as in M-CK-deficient mice. Remarkably, the extent of the increments was similar in wild-type and M-CK-deficient mice.

Muscle force measurements

The maximal isometric twitch force of GPS muscles of +/+ GPA mice (0.95 ± 0.06 g/g body weight, $n = 6$) was similar to that of +/+ and −/− GPS muscles (0.97 ± 1.0 and 0.98 ± 0.18 g/g body weight, respectively, $n = 7$ in each group). In contrast, maximal force of −/− GPA muscles (0.68 ± 0.08 g/g body weight, $n = 6$) was approx. 30% lower than those of the other three groups of mice. We also analyzed muscle force of +/+ GPA and −/− GPA mice during a series of 24 initial contractions at two different work loads and compared these results to those of +/+

Table 2
Influence of βGPA on fibre size in wild-type and M-CK-deficient muscle

Mouse	Type 1	Type 2A	Type 2B
+/+ (5)	1100±190	1180±390	2530±600
+/+ GPA (6)	960±200	870±220	1740±370
−/− (7)	880±120	1200± 80	2360±460
−/− GPA (7)	870±220	890±280	1270±250

Fibre areas were measured in the gastrocnemius muscle: type 1 fibres in the deep region and type 2A and 2B in the medial region. Values are means±SD indicated in μm^2; the number of animals is given in parentheses.

and −/− mice reported earlier in ref. 11. During 1 Hz contraction (Fig. 4A), the initial twitch force of +/+ muscles gradually declined by approx. 8%. Muscles of +/+ mice fed with βGPA exhibited a 19% reduction within the first 7 to 9 contractions, while the final loss of force was about 12%. Muscles of −/− mice show a 20% reduction during the first 4 to 5 contractions and subsequently remain nearly constant until the final twitch [11]. Mutant muscles treated with βGPA also declined in force during the initial 4 to 5 contractions, but the relative reduction was significantly less than that observed in muscles of −/− mice. During 5 Hz contraction (Fig. 4B), +/+ muscles maintained their initial force throughout the experiment, whereas the force of +/+ GPA muscles declined gradually to a constant level of approx. 68% of the initial force within the first 19 to 20 contractions. Muscles of −/− mice typically lose about 45% of their initial force within the first 8 contractions. Mus-

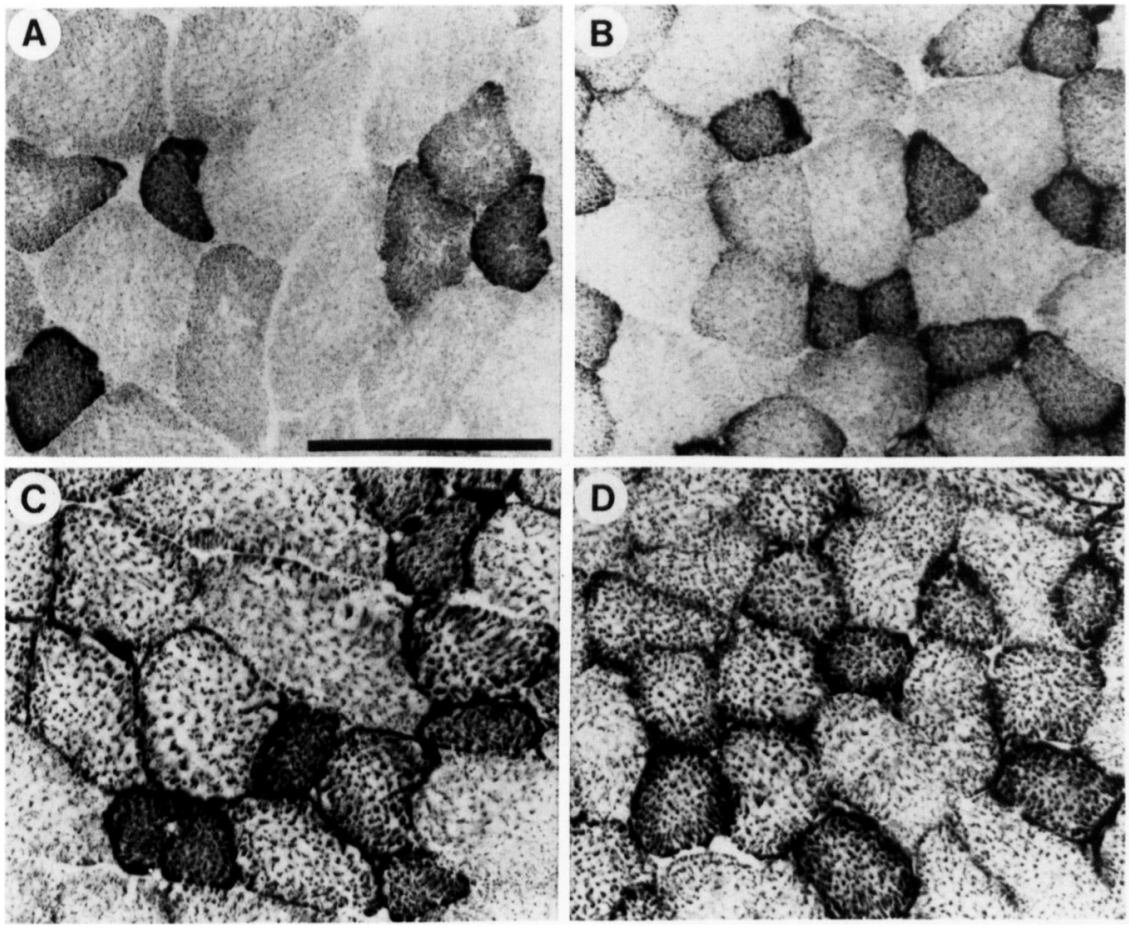

Fig. 2. Cross-sections of gastrocnemius muscles stained for SDH activity from (A) wild-type mice, (B) βGPA-fed wild types, (C) M-CK-deficient mice and (D) βGPA-fed mice deficient in M-CK. Type 2A fibres are stained dark, while type 2B fibres are stained pale. Typically, type 2 fibres of M-CK-deficient mice exhibit a coarsely granulated aspect due to an increase in their intermyofibrillar volume. Note that the size of type 2B fibres in the βGPA-fed mice is relatively small compared to that of their controls. The bar in (A) indicates 100 μm.

cles of −/− GPA mice exhibit a similar pattern of force development during the initial 24 contractions but the reduction of force (after 8 contractions) was just about half of that observed in muscles of −/− mice. We also measured the isometric twitch forces developed during 180 s of 1 and 5 Hz stimulation (Fig.

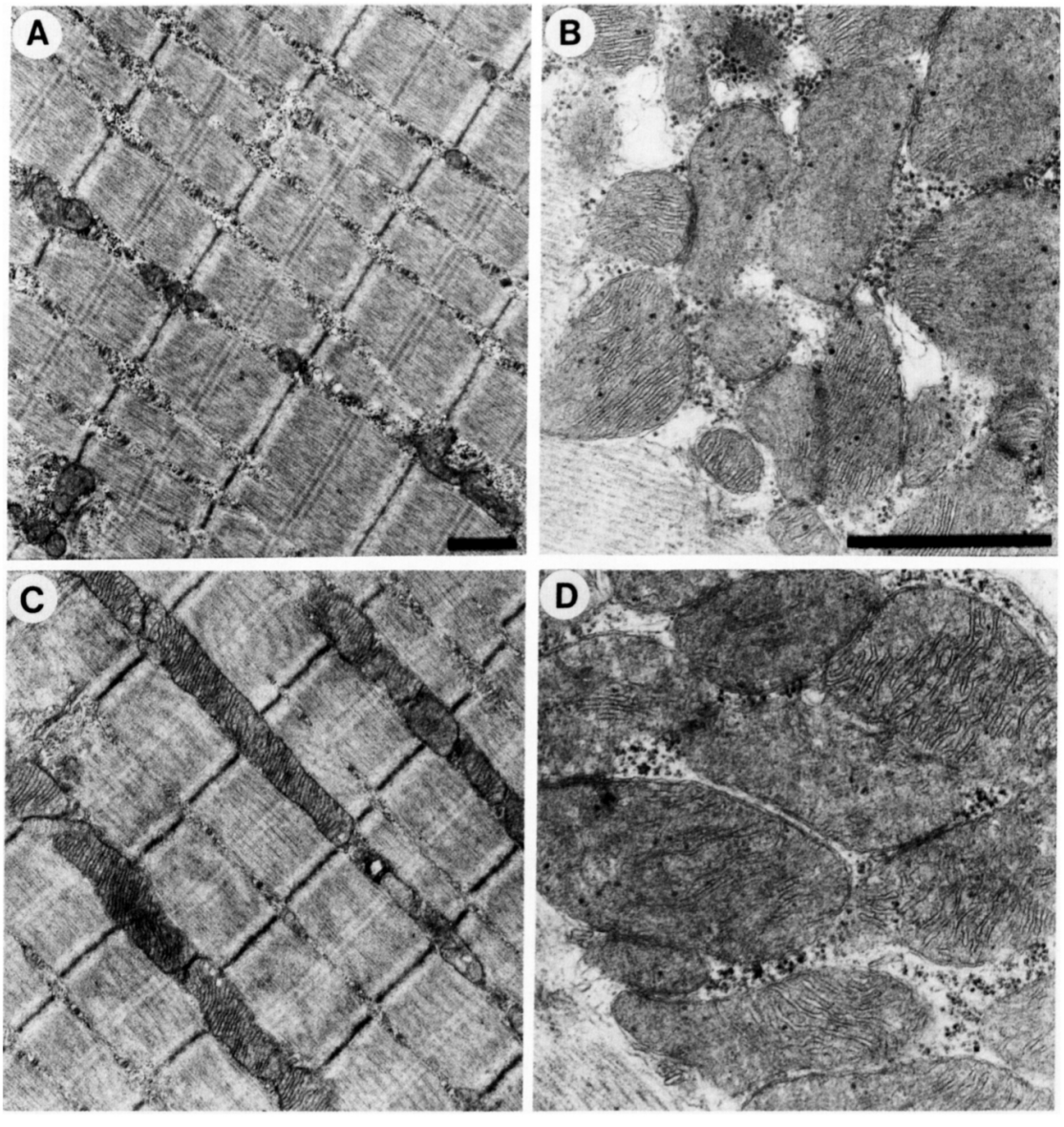

Fig. 3. Ultrastructural analyses of Cr-depleted skeletal muscles. (A and C) Representative electron micrographs of longitudinal sections through myofibres in superficial parts of gastrocnemius muscles of +/+ GPA (A) and −/− GPA mice (C). As in null mutants, the intermyofibrillar mitochondria in type 2 fibres of βGPA-fed mutants are frequently packed in rows and many mitochondria have an increase in size. (B and D) Mitochondria in soleus muscles of +/+ GPA (B) and −/− GPA mice (D). Note that mitochondria are enlarged and lack paracrystalline structures. The bars in (A) and (B) represent 1 μm.

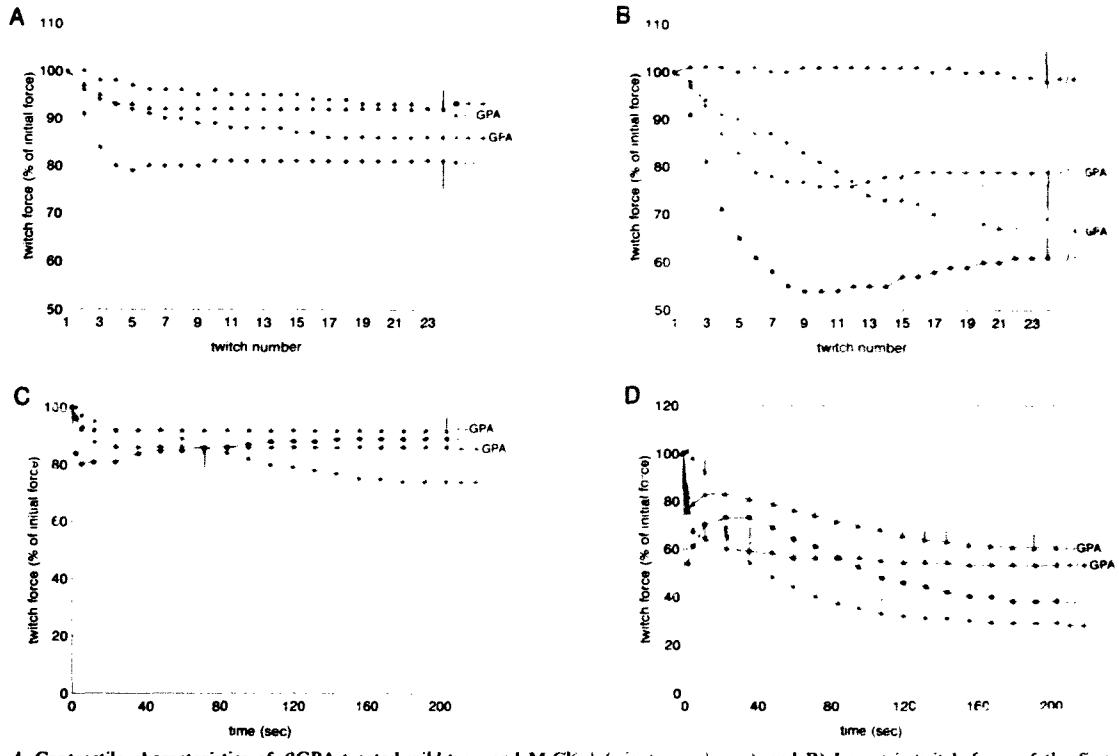

Fig. 4. Contractile characteristics of βGPA-treated wild-type and M-CK-deficient mu[illegible]A and B) Isometric-twitch force of the first 24 contractions of GPS muscle complexes during 1 (A) and 5 Hz (B) stimulation. Shown are means (+ or − S.D.) of six muscles. Squares, muscle force of wild-type mice; closed circles, muscle force of M-CK-deficient mice; asterisks, muscle force of βGPA-fed wild types; triangles, muscle force of βGPA-fed mice deficient in M-CK. (C and D) The isometric twitch force of GPS muscle complexes during 1 (C) and 5 Hz (D) stimulation for 180 s. Shown are means (+ or − S.D.) of five muscles. Symbols are as indicated above. The isometric twitch force is given as the percentage of the initial for[illegible]. Note that the initial twitch force of −/− GPA mice is approx. 30% smaller compared to those of +/+, +/+ GPA and −/− mice.

Table 3
Activities of mitochondrial enzymes in skeletal muscle of controls and null mutants fed with βGPA

Mouse	CS	COX
+/+ GPA	230 ± 27	863 ± 190
−/− GPA	349 ± 41	1379 ± 208

Upper hind limb mu.cles were dissected and subsequently homogenized. Enzyme activities were measured in 600 × g supernatant of the homogenate. Values are means ± S.D. indicated in milliunits per milligram of protein; $n = 7$.

4C and D). These experiments show that the isometric endurance of both wild-type and M-CK-deficient GPS muscles was improved after the βGPA-treatment.

4. Discussion

In theory, disruption of M-CK is expected to inactivate the CK/PCr system in skeletal muscles because of a blockade in the transfer of chemical energy from PCr to ATP catalysed by CK. However, we have shown previously that PCr levels decline in mutant muscles at work, suggesting that the M-CK-mediated hydrolysis is not the exclusive route for PCr utilization in active muscles [11]. It would therefore appear that the CK/PCr system is not completely crippled by M-CK inactivation only. As a first step to investigate this further, we have depleted PCr and Cr from mice deficient in M-CK by feeding them a βGPA diet. Phenotypic alterations in their skeletal muscles were studied and compared with those of βGPA-fed wild types.

The decrease in PCr and Cr levels and the accumulation of βGPA and βGPAP that we observed in skeletal muscles of wild-type mice treated with βGPA were as expected from earlier studies on rats [5,6,7,21]. Skeletal muscles of mice deficient in M-CK did not accumulate βGPAP. This is not due to a defect in the uptake of the creatine analogue, as βGPA levels in

dietary M-CK-deficient muscles were found to be similar to the βGPA plus βGPAP levels in muscles of wild-type mice. Because mitochondrial CK (Mi-CK) activity is not impaired in M-CK-deficient muscles [11], our results imply that normally MM-CK is the only CK isoform involved in phosphorylation of βGPA in skeletal muscle. It is possible that βGPA is an inactive substrate for Mi-CK, but it is also conceivable that βGPA cannot reach Mi-CK since it may not be able to enter the mitochondrial intermembrane space where the enzyme is located. The extent of residual PCr after 8 weeks of βGPA feeding is significantly higher in mutants (41%) than in wild types (20%), but the depletion of Cr is similar. Remarkably, the decrease of ATP levels in mutant and wild-type mice fed a βGPA diet is less than that in muscles of βGPA-fed rats (20% versus 50%) [6,7,21]. We surmise that this difference is due to species or age specific variation in the response to the creatine analogue.

The changes in energy metabolites described above, are accompanied by a variety of adaptations in skeletal muscle tissue. In Cr-depleted muscles of both wild-type and M-CK-deficient mice, the aerobic capacity seems to increase as judged by enhanced activities of two mitochondrial marker enzymes, CS and COX. The absolute increase of the enzyme activities was similar in both types of mice, which is remarkable because important differences in basal aerobic potential and intermyofibrillar mitochondrial volume do exist between the type 2 fibres of M-CK-deficient and wild-type mice [11]. The observation that the mitochondria do not phosphorylate βGPA suggests that the increment of mitochondrial enzyme activities will probably an indirect effect of the creatine analogue accumulation. As in βGPA-fed rats, the increase in aerobic capacity in both types of βGPA-fed mice might involve a decrease in type 2B fibre size in the absence of mitochondrial proliferation or an elaboration of the mitochondrial reticulum, or both [9]. In addition, the enlargement of many subsarcolemmal mitochondria, as seen in all fibre types of wild-type mice and in type 1 fibres of M-CK-deficient mice, might also play a role. This explanation may not hold true for type 1 fibres as previous work on βGPA-fed rats has shown that an increase in size of subsarcolemmal mitochondria in the soleus muscle [10] was not accompanied by an increase in mitochondrial enzyme activities [9].

Another phenotypic parallel between muscles of Cr-depleted M-CK-deficient and wild-type mice is the selective hypotrophy of type 2B fibres. This size reduction is most pronounced in mutants, where type 2B fibres can become nearly as small as type 1 and type 2A fibres. Although the actual cause of this differential change in fibre size is not yet known, two possible explanations can be put forward. Both PCr and Cr levels have been shown to be different between the various types of fibres [22]. βGPA-induced changes in PCr and Cr levels might also be fibre type specific. This might result in discriminatory effects on muscle size via an as yet unknown control mechanism. Alternatively, the explanation may be more trivial and simply reflect a type 2B specific disuse atrophy [23].

Chronic ingestion of βGPA results in an almost 3-fold increase in glycogen in GPS muscles of wild-type mice, which is consistent with a build up of glycogen in muscles of βGPA-treated rats [9]. The glycogen accumulation may result from an impaired activation of glycogenolysis at the onset of muscle contraction, as has been suggested to be the case in βGPA-fed rats [7]. Because Cr-depletion as well as M-CK inactivation are known to elevate the level of glycogen [7,9,11], it is difficult to speculate on the specific cause(s) of the elevated glycogen levels in muscles of βGPA-fed null mutants. It is conceivable that the use of glycogen during muscle exercise is reduced, as in Cr-depleted rats [7]. Alternatively, it may be an adaptation of the system to larger fluctuations in glycogen consumption as is the case in M-CK-deficient muscles [11].

M-CK inactivation [11] and βGPA feeding alone have no effect on the absolute force of GPS muscle complexes. However, the two ways of interfering with the CK/PCr system in combination result in a significant reduction of muscle force. Interestingly, the reduction in body weight in mutant and wild-type mice fed a βGPA diet was similar but cross-sectional areas of type 2B fibres were more reduced in dietary mutants than in dietary wild types. Because the maximal isometric muscle force is expressed on a body weight basis, the reduction in muscle force in βGPA-treated mutant mice might reflect a decrease in their muscle weight to body weight ratio.

M-CK inactivation and βGPA feeding both cause a dramatic loss of force at the onset of muscle contraction. The degree of reduction is comparable in both animal models, but the decrease is much faster in M-CK deficient mice than in Cr-depleted wild-type mice. This suggests that the thermodynamic efficiency of ATP hydrolysis is most rapidly affected in case of M-CK deficiency. Strikingly enough, βGPA feeding caused an improvement in endurance, which is consistent with an increase in aerobic potential in both wild-type and M-CK-deficient muscles. Transitions to myosin isoforms with intrinsically lower ATPase activities, as reported for mice fed a βGPA diet [20], or an increase of muscle ADP levels may be involved in the maintenance of high force during prolonged exercise [24]. We conclude that the effects of βGPA feeding and M-CK inactivation are additive as further changes in the metabolism, morphology and physiological functioning of muscle can be invoked by diet-induced reduction of PCr and Cr levels in mutant mice. This shows that the coupling of the CK/PCr system to the

intricate pathways of molecular and morphological muscle biology is not mediated solely by M-CK and are therefore rather complex. Our findings underscore the crucial importance of the CK/PCr system for muscle cells designed for burst performance. On the other hand, the CK/PCr system seems to have a minor impact on the function of fibres designed for endurance activity.

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References

[1] Bessman, S.P. and Geiger, P.J. (1982) Science 211, 448–452.
[2] Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K. and Eppenberger, H.M. (1992) Biochem. J. 281, 21–40.
[3] Wyss, M., Smeitink, J., Wevers, R.A. and Wallimann, T. (1992) Biochim. Biophys. Acta 1102, 119–166.
[4] Sheilds, R.P. and Whitehair, C.K. (1973) Can. J. Biochem. 51, 1046–1049.
[5] Fitch, C.D., Jellinek, M. and Mueller, E.J. (1974) J. Biol. Chem. 249, 1060–1063.
[6] Shoubridge, E.A. and Radda, G.K. (1984) Biochim. Biophys. Acta 805, 79–88.
[7] Meyer, R.A., Brown, T.R., Krilowicz, B.L. and Kushmerick, M.J. (1986) Am. J. Physiol. 250, C264–C274.
[8] Chevli, R. and Fitch, C.D. (1979) Biochem. Med. 21, 162–167.
[9] Shoubridge, E.A., Challiss, R.A.J., Hayes, D.J. and Radda, G.K. (1985) Biochem. J. 232, 125–131.
[10] Gori, Z., De Tata, V., Pollera, M. and Poggesi, [illegible] (1988) Br. J. Exp. Path. 69, 639–650.
[11] van Deursen, J., Heerschap, A., Oerlemans, F., Ruitenbeek, W., Jap, P., ter Laak, H. and Wieringa, B. (1993) Cell 74, 621–631.
[12] Heerschap, A., Bergman, A.H., van Vaals, J.J., Wirtz, P., Loermans, H.M.T. and Veerkamp, J.H. (1988) NMR Biomed. 1, 27–31.
[13] Bergmeyer, H.U. (1974) Methods of Enzymatic Analysis. Academic Press, New York.
[14] Bonas, J.E., Cohen, B.D. and Natelson, S. (1963) Microchem. J. 7, 63–77.
[15] Fitch, C.D. and Chevli, R. (1975) Anal. Biochem. 68, 196–201.
[16] Lillie, R.D. and Fulmer, H.M. (1976) Histopathologic technic and practical histochemistry. McGraw-Hill, New York.
[17] Cooperstein, S.J. and Lazarow, A. (1951) J. Biol. Chem. 189, 665–670.
[18] Srere, P.A. (1969) Methods Enzymol. 13, 3–11.
[19] Degens, H., Turek, Z. and Binkhorst, R.A. (1993) Mech. Ag. Dev. 66, 299–311.
[20] Moerland, T.S., Wolf, N.G. and Kushmerick, M.J. (1989) Am. J. Physiol. 257, C810–C816.
[21] Fitch, C.D., Jellinek, M., Fitts, R.H., Balwin, K.M. and Holloszy, J.O. (1975) Am. J. Physiol. 228, C1123–C1125.
[22] Kushmerick, M.J., [illegible], T.S., and Wiseman, R.W. (1992) Proc. Natl. Acad. Sci. USA 89, 7521–7525.
[23] Dubowitz, V. (1985) Muscle Biopsy: A Practical Approach. Lavenham Press, Lavenham.
[24] Dantzig, J.A., Hibberd, M.G., Trentham, D.R. and Goldman, Y.E. (1991) J. Physiol. 432, 639–680.