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Muscle Creatine Kinase-deficient Mice

I. ALTERATIONS IN MYOFIBRILLAR FUNCTION*

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The regulation of contractile activity in mice bearing a null mutation of the M-isoform of creatine kinase gene, has been investigated in tissue extracts and Triton X-100-treated preparations of ventricular, soleus, and gastrocnemius muscles of control and transgenic mice. Skinned fiber experiments did not evidence any statistical difference in the maximal force or the calcium sensitivity of either muscle type. Rigor tension development at a low MgATP concentration was greatly influenced by phosphocreatine in control but not in transgenic mice as should be expected. In calcium-activated ventricular preparations, although the force developed by each cross-bridge was the same in control and transgenic animals, the rate constant of tension changes appeared to be markedly slowed in transgenic animals. As the ventricular isomyosin pattern was not altered, we suggested that, in transgenic animals, cross-bridge cycling was hindered by a local decrease in the MgATP to MgADP ratio, due to lack of a local MgATP regenerating system. Myokinase activity was not significantly changed while activities of pyruvate kinase or glyceraldehyde-3-phosphate dehydrogenase were found to be increased in transgenic animals. These results show that no fundamental remodelling occurs in myofibrils of transgenic animals but that important adaptations modify the biochemical pathways including glycolytic metabolism.

Creatine kinase (CK) is an important enzyme catalyzing the reversible transfer of a phosphate moiety between ATP and creatine. A major part of muscle creatine kinase exists as dimers composed of two subunits, M and B, giving three isoenzymes, MM, BB, and MB. In addition, there is a fourth isoenzyme in the mitochondria (mitochondrial CK), which differs biochemically and immunologically from the cytosolic forms and can present octameric and dimeric structures (Wyss et al., 1992). Studies with subcellular fractionation or histochemical localization have revealed that CK isoenzymes are present in cytosol or bound to intracellular structures. M-CK has been found in myofibrils and described as a structural protein of the M-band participating in the connections between myosin filaments inside muscle fibers (Wallimann et al., 1977). Additional binding sites have been described on actin filament (Wegmann et al., 1992) or on the entire myosin filament (Otsu et al., 1989). M-CK activity in myofibrils is as high as about 2 IU/mg of protein in skeletal and ventricular muscles and represents 5% of total CK activity in fast-twitch muscle compared to 23% in ventricular muscle (Wallimann et al., 1977; Ventura-Clapier et al., 1987a; for review see Ventura-Clapier et al. (1994)). M-CK has been shown to be functionally coupled to myosin ATPase. That means that myosin ATPase preferentially uses ATP supplied by creatine kinase rather than cytosolic ATP (Bessman et al., 1980; Saks et al., 1984). Myofibrillar CK can rephosphor ylate all of the ADP produced by myosin ATPase (Saks et al., 1976; Wallimann et al., 1984; Arrigo-Dupont et al., 1992) and can provide enough energy for maximal force and normal kinetics even in the absence of MgATP, at the expense of phosphocreatine (PCr) (Ventura-Clapier et al., 1987a; for review see Ventura-Clapier et al. (1994)).

The creatine kinase/phosphocreatine system is considered to fulfill important roles in the energy metabolism of skeletal and cardiac muscles (for reviews see Wallimann et al. (1992), Wyss et al. (1992), and Saks et al. (1994)). In skeletal muscles, activity pattern determines fiber type and metabolic profile. Fast-twitch (white) muscles exhibiting rapid and brief activity patterns are mainly glycolytic and contain high amounts of PCr and CK. The organization of the CK system appears different in these two kinds of muscles; there is an abundance in fast-twitch muscle and compartmentation of the mitochondrial and M-isoenzymes in slow-twitch muscle and ventricle.

Functional consequences and adaptive strategies observed in animal models of long term deficiency in the CK system may give insights into the physiological role of this system in different muscle types. Long term alterations in the creatine kinase/phosphocreatine system have been developed by feeding animals with slowly metabolized analogues of creatine (β-guanidinopropionic acid, β-GPA). These animals exhibit decreased PCr and ATP concentrations in cardiac and skeletal muscles (Shoubridge and Radda, 1984; Kapelko et al., 1988; Zweier et al., 1991). In addition, a clear cardiac hypertrophy and isoenzyme shift from fast V1 to slow V5 myosin have been observed (Mehki et al., 1990). Adaptive strategy in the heart is directed toward an increase in the number of contractile units together with an increased efficiency of each unit to respond to de-
creased metabolic fluxes. In the same animal model, skeletal muscles exhibited an enhanced oxidative metabolism and an isomyosin shift from fast- to slow-type isomyosins (Shoubridge et al., 1985; Moerland et al., 1989).

More recently, a mouse line bearing a null mutation of M-CK has been developed (van Deursen et al., 1993). Genetic M-CK knockout is a unique model of complete isoenzyme-specific CK deficiency in contrast to GPA feeding, a model of substrate deficiency. Another important difference between these two models is that mutant muscles keep mitochondrial and, in principle, brain isoforms of CK which could participate in energy metabolic pathways. It was shown that M-CK deficiency does not lead to compensatory overexpression of other CK isoenzymes. Muscles from these mice, which do not express the muscle form of CK, are able to use PCr but lack the ability to perform burst activity. In order to get insights into the functional characteristics and possible adaptational processes at the level of myofibrils in these transgenic mice, we characterized intrinsic mechanical properties of ventricular, soleus, and gastrocnemius muscles using the skinned fiber technique which allows us to investigate myofibrillar properties without interference with cytosolic substrate and ion changes. The results show that intrinsic mechanical capacities and calcium sensitivity were maintained in transgenic animals, although skinned fibers were not able to utilize PCr. However, contractile kinetics were markedly slowed down despite an unchanged myosin isoenzyme profile. In addition, the energy supply pattern was changed since glycolytic capacity seemed to increase in fast-twitch muscle as well as in ventricular muscle.

MATERIALS AND METHODS

Mouse Model of Muscle Creatine Kinase-deficient Mice

Mice bearing a null mutation of the M-CK gene were obtained as described previously (van Deursen et al., 1993). Heterozygous mutants were interbred to generate mice deficient in M-CK. Offsprings were genotyped 2 weeks after birth.

Six control adult female mice C57BL/6 and 5 adult transgenic mice were anesthetized with an intraperitoneal injection of pentobarbitone according to the recommendations of the Institutional Animal Care Committee (INSERM, Paris, France) and weighed. While under anesthesia, animals were exsanguinated, and various organs were isolated and weighed. Heart, gastrocnemius, and soleus muscles were placed in a modified Krebs solution containing (mM): NaCl, 118; KCl, 4.7; NaHCO3, 25; KH2PO4, 1.2; and MgSO4, 1.2.

Organ samples were frozen for further analysis. Other samples were minced with scissors, placed into cold solution (50 mg wet weight per 1 ml) containing (mM): K2HPO4, 100 (pH 8.7); EGTA, 1; N-acetyl cysteine, 15; and homogenized in a Ultra-Turrax homogenizer. Tissue homogenates were incubated for 60 min at 0 °C for complete extraction of CK and other enzymes, centrifuged at 13,000 × g for 20 min, and the supernatant was used for determination of CK and myokinase and frozen.

Mechanical Experiments

Muscle Preparation—Muscle fiber bundles were dissected from soleus or gastrocnemius from or papillary muscles of the left ventricle of mice in a zero-Ca2+ Krebs solution, pH 7.4. Bundles were incubated for 1 h in a relaxing solution (pCa 9, see solutions below) containing 1% Triton X-100 to solubilize the membranes and were then transferred to the relaxing solution without detergent and kept at 4 °C until use. After the skimming procedure, one bundle was snared at both ends with hair ties. The length and diameter of the muscles were measured by use of a graticule in the dissecting microscope. Muscles were immersed in 2.5-mL chambers arranged around a disk in a temperature-controlled bath positioned on a magnetic stirrer. Each solution was well stirred at high speed. All experiments were performed at 22 °C.

Experimental Apparatus—The tubes were connected to a transducer (model AE 801, SensoNor Microelectronics, Horten, Norway) and a vibrator as described previously (Mayoux et al., 1994). The bandwidth of the transducer and tube was 2 kHz. The permanent magnet and coil came from a standard loudspeaker (Pioneer TS-130A, Pioneer Electric and Research Corp., Forest Park, IL). The coil was glued to a glass tube axis (2 mm in diameter) driven in an axial ball bearing (total moving mass < 1.5 g). A flag with a narrow window was glued on the glass axis between a lamp and a position detector (type Si543, Hamamatsu, Japan), allowing measurements of the displacement length. A feedback with the length signal combined with a power amplifier allowed control of muscle length. The system had a time constant of 1 ms without overshoot. Length and tension changes were monitored on a digital storage oscilloscope (OS4020, Gould, Inc., Cleveland, OH). Tension tracings were digitized at 20 kHz (12-bit analog/digital converter), analyzed on-line using a PC compatible computer, and stored on a videotape.

Solutions—Solutions were calculated using the computer program of Fabiato (1988). All solutions were calculated to contain (mM): EGTA, 10; imidazole, 30; Na+, 30.6; Mg2+, 3.16; and dithiothreitol, 0.3; ionic strength was adjusted to 0.16 M with potassium acetate. pH was adjusted to pH 7.1 with acetic acid. In relaxing artifacts situations, pCa was 9. In activating solution, pCa was 4.5. Relaxing and activating solutions also contained 3.16 mM MgATP and 12 mM PCr. Rigor solutions were obtained by mixing two solutions of pMgATP 2.5 and 6 or pMgATP 4 and 6. EGTA was obtained from Sigma. PCr (Neoton, Schiapparelli Farmaceutica SEARLE, Turin, Italy) was a kind gift of Prof. E. Strumia.

Experimental Protocols

pCa/Tension Relations—pCa/tension relations were determined under isometric conditions by briefly placing each fiber into solutions of increasing calcium concentration until maximal tension was reached. Data were fitted using linearization of the Hill equation for relative tensions above 10% and below 90% of the Hill maximum, T = [(Ca2+)]^n/K + Ca2+)^n) were T is relative tension, n is a constant and K a constant in mM. Hill coefficient, n and pCa for half-maximal activation (pCamil = −log10K/Mn) was calculated for each fiber by means of linear regression analysis. Resting tension was measured at pCa 9. Active tension (expressed as mm/m2) was the total tension at pCa 4.5 minus resting tension.

pMgATP/Rigor Tension Relations—pMgATP/rigor tension relations were established by stepwise decreasing ATP concentrations until maximal rigor tension was obtained. The fiber was then placed in a relaxing solution for 2 to 15 min before a new set of rigor solution was applied. Data were fitted using the Hill equation. The pMgATP for half-maximal rigor tension, pMgATP = (log$_{10}$K/Mn), was calculated for each experimental condition using linear regression analysis.

Stiffness and Kinetic Measurements—To determine fiber bundle stiffness and the rate constant of tension recovery in cardiac preparations, quick length changes (0.3–3% of initial muscle length) were applied in the relaxing and activating solutions. Twelve successive stretches and releases were made, starting with the relaxing solution. Only responses to the released zero was used in the analysis of the relaxant limb. The mean of five to seven stretches of varying amplitudes performed in a given experimental condition. The spike of tension in phase with the length change characterized the elastic phase (Huxley and Simmons, 1971). Stiffness was the extreme tension reached during stretching (mN/mm2) divided by the length change (μm). A first series of length changes was imposed in the relaxing solution to assess passive properties of each fiber. Resting stiffness was calculated by linear regression analysis on the responses to stretches. Then a second series of length changes was initiated in control activating solution. The tension level before the first stretch was taken as the maximal tension and used for normalizations. Active or rigor stiffness were calculated as the difference between total stiffness minus resting stiffness. The rate constant of tension recovery after quick stretches was calculated by a least square regression analysis, according to a simple exponential model, between 50% and 80% of recovery and using stretches of more than 1%.

Enzyme Analysis

Enzyme activities were determined spectrophotometrically at 340 nm (Gilford Spectrophotometer, Corning, NY), 50 °C, by using coupled enzyme systems. Results are given in IU/g wet weight. Myokinase activity was assayed by monitoring the coupled enzyme activity of hexokinase and glucose-6-phosphate dehydrogenase producing NADPH. Activity was assayed in a medium containing (in mM): HEPES, 20; MgCl2, 6; dithiothreitol, 0.5; ADP, 1.2; glucose, 20; NADP, 0.6; and 2 IU/mL glucose-6-phosphate dehydrogenase and hexokinase. Glyceroldehyde-3-phosphate dehydrogenase was determined at pH 7.6, in a medium containing (in mM): triethanolamine, 82.8; 3-phosphoglycerate, 6; ATP, 1.1; SDHA, 0.9; MgSO4, 1.7; NADH, 0.2; and 15 IU/mL 3-phosphoglycerate kinase. Pyruvate kinase activity was measured at
Table I

Anatomical data of control and M-CK-deficient mice

<table>
<thead>
<tr>
<th></th>
<th>Body</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>HW/BW</th>
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<tr>
<td>Control (5)</td>
<td>27.0 ± 1.3</td>
<td>156 ± 14</td>
<td>1223 ± 94</td>
<td>376 ± 28</td>
<td>171 ± 14</td>
<td>5.73 ± 0.34</td>
</tr>
<tr>
<td>Transgenic (5)</td>
<td>25.3 ± 1.0</td>
<td>135 ± 6</td>
<td>1145 ± 88</td>
<td>347 ± 19</td>
<td>178 ± 9</td>
<td>5.34 ± 0.11</td>
</tr>
</tbody>
</table>

* HW/BW, heart weight/body weight ratio.
* Numbers in parentheses, number of animals.
* NS, not significant.

Myosin Isoforms

Myosin Preparation—Frozen muscles were thawed on ice, cut into small pieces, and washed with 5 volumes of 20 mM NaCl, 5 mM sodium phosphate, and 1 mM EGTA (pH 6.5). Myosin was then extracted with 100 volumes of 200 mM sodium pyrophosphate, 5 mM EGTA, and 1 mM dithiothreitol (pH 8.5); after 30 min of gentle shaking, the mixture was centrifuged at 10,000 x g. The supernatant containing myosin was diluted with 1 volume of glycerol and stored at -20 °C (d'Albis et al. 1979).

Electrophoresis took place in a cold cupboard, at 10 °C for 28 h, in a cold buffer consisting of 20 mM sodium pyrophosphate (pH 6.5), 10% glycerol, 0.01% mercaptoethanol, and 2 mM MgCl₂. Cylindrical (6 × 0.5 cm) gels contained 4% polyacrylamide (3.88% acrylamide and 0.12% N,N'-methylenebisacrylamide). Between 1 and 5 μg of myosin was loaded on each gel. Electrophoresis was carried out at a constant voltage of 90 V, for 22 h, between 0 and 2 °C (d'Albis and Gratzer, 1973; Hoh, 1975).

Effect of Phosphocreatine on Relaxation of Rigor Tension—The next series of experiments was undertaken to study the influence of PCr on the relaxation of rigor tension in control and transgenic muscles. Rigor tension is the tension induced in muscle fibers of control (Fig. 1) where omission of PCr in activating solution led to an increase in calcium sensitivity from 5.68 ± 0.03 to 5.98 ± 0.03 (n = 4, p < 0.001).

Effect of Phosphocreatine on Relaxation of Rigor Tension—In ventricular muscle that the development of rigor tension is greatly influenced by CK bound in myofibrils. The concentration of MgATP necessary to obtain half-maximal rigor is greatly reduced when PCr is provided as a substrate. In order to characterize more precisely this effect in control and transgenic mice, complete pMgATP/rigor tension relations have been established in presence or absence of PCr in sets of solutions of decreasing MgATP concentrations. In Fig. 2, pMgATP/rigor tension relations have been drawn using Hill equation and pMgATP/rigor force values given in Table II for ventricle, soleus, or gastrocnemius. When PCr was added, a clear shift of the relation was observed in control muscles. While pMgATP/tension relations without PCr were identical in control and transgenic animals, no shift could be evidenced in the presence of PCr for the three muscle types of transgenic animals. pMgATP values in the presence of PCr, as well as CK efficacy, which is defined as the difference between pMgATP values in the presence and absence of PCr, were both highly significantly different between control and transgenic mice (Table II). This result is in complete agreement with the absence of M-CK in these animals and clearly shows that myofibrils from transgenic mice have no enzyme able to utilize PCr.
Myofibrillar Function in M-Creatine Kinase-deficient Mice

Mechanical characteristics of skeletal and cardiac skinned fibers from control and M-CK-deficient mice

Values are mean ± S.E.

<table>
<thead>
<tr>
<th>Muscles</th>
<th>Resting tension</th>
<th>Active tension</th>
<th>pCa50</th>
<th>nH</th>
<th>pATP50</th>
<th>pATP50 + PCr</th>
<th>CKeff ± 6.99</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min/mm²</td>
<td>min/mm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricle</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.35 ± 0.68 (8)</td>
<td>37.9 ± 4.4 (8)</td>
<td>5.723 ± 0.04 (10)</td>
<td>2.83 ± 0.16 (10)</td>
<td>3.44 ± 0.07 (8)</td>
<td>4.99 ± 0.06 (8)</td>
<td>1.55 ± 0.09 (8)</td>
</tr>
<tr>
<td>Transgenic</td>
<td>3.27 ± 0.60 (7)</td>
<td>47.2 ± 5.3 (7)</td>
<td>5.761 ± 0.027 (7)</td>
<td>2.75 ± 0.22 (7)</td>
<td>3.47 ± 0.08 (8)</td>
<td>3.58 ± 0.03 (8)</td>
<td>0.11 ± 0.06 (8)</td>
</tr>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>5.82 ± 0.63 (6)</td>
<td>103 ± 21 (6)</td>
<td>5.825 ± 0.074 (8)</td>
<td>3.12 ± 0.28 (8)</td>
<td>3.65 ± 0.07 (8)</td>
<td>4.90 ± 0.08 (8)</td>
<td>1.25 ± 0.07 (8)</td>
</tr>
<tr>
<td>Transgenic</td>
<td>6.99 ± 1.8 (1)</td>
<td>123 ± 28 (6)</td>
<td>5.746 ± 0.022 (6)</td>
<td>4.00 ± 0.68 (6)</td>
<td>3.87 ± 0.07 (8)</td>
<td>3.92 ± 0.10 (8)</td>
<td>0.05 ± 0.06 (8)</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.3 ± 6.1 (4)</td>
<td>275 ± 47 (6)</td>
<td>5.849 ± 0.062 (8)</td>
<td>3.20 ± 0.29 (8)</td>
<td>3.43 ± 0.08 (6)</td>
<td>4.98 ± 0.10 (8)</td>
<td>1.66 ± 0.06 (8)</td>
</tr>
<tr>
<td>Transgenic</td>
<td>7.8 ± 2.0 (7)</td>
<td>155 ± 28 (7)</td>
<td>5.937 ± 0.043 (7)</td>
<td>5.10 ± 0.59 (7)</td>
<td>3.55 ± 0.05 (8)</td>
<td>3.57 ± 0.10 (8)</td>
<td>0.02 ± 0.09 (8)</td>
</tr>
</tbody>
</table>

- CKeff efficacy of creatine kinase expressed as a pmMgATP50 value at 12 mM PCr minus the value at 0 mM PCr.
- Numbers in parentheses, number of fibers.
- NS, not significant.
- p < 0.001 relative to respective value for fibers of control mice.
- p < 0.05 relative to respective value for fibers of control mice.

Before the length change. The rate constant of tension recovery reflects the cross-bridge cycling rate, while the extent of recovery characterizes the cross-bridge state. The rate constant of tension recovery following stretches was greatly decreased in transgenic mice compared to control while the extent of recovery was not different (Fig. 3). This result suggests a decrease in the cross-bridge cycling rate in transgenic ventricular fibers.

Indeed, inhibition of myofibrillar CK slows down tension kinetics in skinned rat cardiac muscle, probably due to local accumulation of protons and MgADP (Ventura-Clapier et al., 1987b). This was also observed in mouse heart where withdrawal of PCr decreased the rate constant from 103 ± 17 s⁻¹ to 38 ± 4 s⁻¹ (n = 4, p < 0.05).

Isomyosin Patterns—A decrease in cross-bridge cycling rate may arise from an altered pattern in myosin isoenzyme distribution. In order to see if transgenic animals exhibit a change in myosin isoforms, the myosin phenotype was determined in the different muscles.

Two types of gel electrophoresis were used to analyze the content in myosin isoforms of the cardiac and skeletal muscles, respectively. The cardiac ventricular myosins are best separated by gel electrophoresis under nondissociating conditions (Lompré et al., 1981), as shown here in a control rat heart, which displayed the three isoforms VI, V2, and V3 (Fig. 4a).

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Both control and transgenic mouse ventricles displayed only the VI isoform.

To analyze the myosin isoform content in the skeletal muscles, gel electrophoresis in the presence of SDS allowed the separation of the slow type isoform 1 and the three fast type isoforms 2 (Fig. 4b). The only transgenic mouse soleus muscle contained the 2A and the 1 isoforms in the same proportions, 35% and 65%, respectively, as the control muscle. The gastro-

![Graphs showing relative pMgATP/rigor tension relations with or without PCr, obtained in ventricular (V), soleus (S), and gastrocnemius (G) skinned fibers from control (continuous lines) and mutant mice (dashed lines). pMgATP versus rigor tension relations were calculated and plotted according to the Hill equation T = (K/K + [MgATP]), where T is relative tension, K is a constant, and n the Hill coefficient. Each curve was drawn using the means of the n values and pMgATP for half-maximal tension (pMgATP50) calculated for each fiber and averaged in Table II. Arrows indicate CK efficacy values in control animals. Addition of PCr (solid lines) was able to shift the pMgATP/tension relation of all muscles of control animals but not of mutant animals.](image-url)
No significant changes were seen for soleus muscle. 

Glycolytic Enzymes and Myokinase Determinations—Since no change in isomyosin pattern could be detected in muscles of transgenic mice, the question arose as to how MgATP and MgADP concentrations could be controlled in transgenic animals. Indeed, it has been shown that myofibrillar CK, by keeping high ATP/ADP ratio and low proton concentrations close to myosin ATPase, ensures optimal efficiency of myosin ATPase in skeletal (Bessman et al., 1980) as well as cardiac muscle (Ventura-Clapier et al., 1987a, 1987b). Other MgATP regenerating enzymes may exist in cytosol or may be loosely bound to myofibrils. To check for possible overexpression of such enzymes, glycolytic enzymes as well as myokinase activities were measured in the different muscles. Results are shown in Table III. Myokinase activity was not significantly increased in transgenic animals while some glycolytic enzymes like pyruvate kinase and glyceraldehyde-3-phosphate dehydrogenase increased in ventricles and glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase increased in gastrocnemius. No significant changes were seen for soleus muscle.

The rate constant of tension recovery following stretches is an estimate of the kinetics of cross-bridge cycling and reflects the rate-limiting step in the cycle; it was shown to vary with myosin isofrom composition as well as following alterations in concentrations of substrates or products of myosin ATPase (Ventura-Clapier et al., 1987a; Mekht and Ventura-Clapier, 1988; Mayoux et al., 1994). We have observed a 3-fold decrease in cross-bridge cycling rate in cardiac myofibrils of transgenic animals compared to control without any shift in myosin isoforms. A similar change was observed in control mice when PCr was omitted in the solution. Thus, this decreased rate of force changes can be attributed to changes in ATP/ADP ratio in the vicinity of myosin ATPase with a consequent product inhibition of ATPase activity. Accumulation of MgADP as a result of a lack of myofibrillar CK will induce an increase in force production and a decrease in rate of cross-bridge cycling, leading to a lower energy consumption and better economy of force production. As a consequence, the rates of force production and relaxation of the muscle twitch would be decreased. However, for cardiac muscle having cyclic activity, this would tend to increase the end-diastolic pressure and to decrease the ventricular filling, except if the intrinsic heart rate is decreased. Unfortunately, no information are as yet available concerning heart rate, developed pressure, or the force-length relationship.
of cardiac muscle in transgenic animals. Although the cross-bridge cycling rate of skeletal muscle could not be determined in this study, it is highly probable that tension kinetics would be slowed also. Further studies are needed to clarify the contraction kinetics of the intact muscles in these animals.

In Triton X-100-treated fibers, loosely bound enzymes are usually detached from the myofibrillar structures. In intact cells, many enzymes including glycolytic enzymes, AMP deaminases, and myokinase are bound to myofibrillar proteins, mainly to the thin filament and may participate in MgADP/MgATP regulation in myofibrils (Maughan and Godt, 1989). Indeed, we observed in total tissue extracts of both cardiac and skeletal muscles of transgenic mice, an increase in glycolytic enzyme activities, with no increase in total myokinase activity. It is thus possible that a fraction of these enzymes is bound to myofibrils in vivo and ensures local rephosphorylation of MgADP.

When PCR was omitted, it was clear that calcium sensitivity of control cardiac fibers was increased. Such a result was already obtained in rat heart (Ventura-Clapier et al., 1987a) and is due to cross-bridge slowing and cooperative interaction between attached cross-bridges. Surprisingly, such an increased calcium sensitivity was not observed in soleus, gastrocnemius, or ventricular muscles of transgenic mice, and a similar force/calcium relationship was observed in control and transgenic muscles, suggesting that another mechanism compensated for the increased calcium sensitivity following changes in the local ATP/ADP ratio. Calcium sensitivity is determined by the binding of calcium to troponin C as well as by interactions between the other constituents of the thin filament. Calcium sensitivity of cardiac or skeletal muscle is developmentally regulated, and the role of troponin T isoforms is often put forward to explain changes in calcium sensitivity in spite of unchanged troponin C expression (Solaro et al., 1988; Nasser et al., 1991; Pan and Potter, 1992). One may suggest that a phenotypic change in the proteins constitutive of the thin filament will participate in maintaining constant calcium sensitivity in these transgenic muscles. Alternatively, at least in cardiac muscle, cAMP-mediated phosphorylation of the inhibitory unit of troponin (troponin I) decreases the sensitivity of myofilbrils for calcium by diminishing the Ca2+-affinity of troponin C (Ray and England, 1976). Phosphorylation of troponin I has been shown to be very stable (Garvey et al., 1988). It may thus be possible that myofilbrillar transgenic mice exhibit an enhanced phosphorylation level of contractile proteins which would decrease calcium sensitivity and compensate for the change induced by the altered ATP/ADP ratio inside myofilbrils. Unfortunately, experimental data in support of such a hypothesis are lacking.

The skeletal muscle function of mice deficient in muscle CK has been investigated previously (van Deursen et al., 1993). Mice lacking M-CK have lost the ability to sustain maximal force output during short periods of high work, while apparently being adapted for endurance exercise. However, cardiac function of mutant mice has not been investigated at present. To elucidate the role of the CK system in energy metabolism, other strategies designed to reduce the activity of the CK system were used, such as feeding animals with creatine analogs. This affects the creatine kinase/phosphocreatine system at the substrate site. Alternatively, acute iodoacetamide poisoning of CK has also been used (Fossa and Hoefeler, 1987; Kupriyanov et al., 1991). In these models, where the function of isolated heart was impaired, a decreased developed pressure and rate pressure product were described (Mekhfi et al., 1990; Zweier et al., 1991). Furthermore, impairment of diastolic function and a deeper rise in stiffness at increased afterloads in association with increased energy breakdown were observed (Kapelko et al., 1988; Kupriyanov et al., 1991). Even more interesting was the observation, in these models, of phenotypic conversion of fast-twitch to slow-twitch fibers in skeletal muscle together with isomyosin transitions (Moerland et al., 1989) and cardiac enlargement and increased economy of contraction by a shift from the fast isoform of myosin to the slow isoform in heart (Mekhfi et al., 1990). It could be concluded that CK/PCr system alterations induce contractile abnormalities and that alterations in metabolic state per se, may lead to changes in the expression of contractile proteins.

No obvious change in size and distribution of the three fiber type populations was observed in M-CK-deficient mice (van Deursen et al., 1993). However, M-CK-deficient type 2A and 2B fibers exhibited a clear metabolic phenotype change by elaborating an intermyofibrillar mitochondrial network, with a high number of relatively large mitochondria, the potential for aerobic energy generation being increased approximately twice (Veksler et al, 1995) explaining improved endurance performance during low intensity exercise (van Deursen et al., 1993). In addition, we showed in the companion paper that mitochondria in ventricular and soleus muscles from transgenic mice have an increased sensitivity to ADP (Veksler et al., 1995). Increase in mitochondrial content in “glycolytic” muscles and increased sensitivity to ADP in “oxidative” muscles appear to represent adaptations toward increased energy turnover via the adenylate pathway. Absence of marked isomyosin shift, either in skeletal or cardiac muscle in M-CK knockout mice is in contrast with what was observed in rat cardiac or mice skeletal muscles following β-GPA feeding (Shoubridge et al., 1985; Moerland et al., 1989; Mekhfi et al., 1990). In these situations, a better economy of contractile force development was achieved by a switch from fast to slower myosin isoforms. The reason for such a difference is not straightforward. Despite the absence of an isomyosin shift in mouse heart following β-GPA feeding, these hearts can potentially switch totally from fast to slow myosin

### Table III

<table>
<thead>
<tr>
<th></th>
<th>Myokinase</th>
<th>Fructose-6-phosphokinase</th>
<th>Pyruvate kinase</th>
<th>Glyceroldehyde-P dehydrogenase</th>
<th>Lactate dehydrogenase</th>
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<tbody>
<tr>
<td><strong>Ventricle</strong></td>
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<tr>
<td>Control</td>
<td>96 ± 11</td>
<td>2.05 ± 0.31</td>
<td>37.4 ± 6.2</td>
<td>2.31 ± 0.48</td>
<td>52 ± 14</td>
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<td>Transgenic</td>
<td>120 ± 12</td>
<td>2.71 ± 0.77</td>
<td>74 ± 12&quot;</td>
<td>8.49 ± 0.42&quot;</td>
<td>69 ± 11</td>
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<td><strong>Soleus</strong></td>
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<tr>
<td>Control</td>
<td>214 ± 21</td>
<td>2.14 ± 0.37</td>
<td>69 ± 30</td>
<td>111 ± 28</td>
<td>66.8 ± 4.3</td>
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<tr>
<td>Transgenic</td>
<td>237 ± 43</td>
<td>2.08 ± 0.48</td>
<td>14.8 ± 4.1</td>
<td>69 ± 11</td>
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<tr>
<td><strong>Gastrocnemius</strong></td>
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<tr>
<td>Control</td>
<td>235 ± 24</td>
<td>7.9 ± 2.9</td>
<td>251 ± 51</td>
<td>8.4 ± 1.0</td>
<td>239 ± 10</td>
</tr>
<tr>
<td>Transgenic</td>
<td>334 ± 47</td>
<td>21.3 ± 6.7</td>
<td>372 ± 28</td>
<td>16.2 ± 1.7&quot;</td>
<td>342 ± 24&quot;</td>
</tr>
</tbody>
</table>

*p < 0.05 relative to respective value in control mice.

*p < 0.001 relative to respective value in control mice.

*p < 0.01 relative to respective value in control mice.
as has been shown under the influence of hypothyroidic treatment (Ng et al., 1991). The main difference between the two models is that ATP as well as PCr contents are preserved in the case of the M-CK mutation in comparison with β-GPA feeding where both compounds appear to be decreased. A consequence of this would be that the expression of proteins of the contractile apparatus is more under the control of the concentrations of metabolites. On the other hand, it should be borne in mind that these strategies may involve "exotic" pathways and that thorough examination of biochemical and physiological characteristics of these animals would be of potentially high significance in the understanding of the role of a given pathway.

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REFERENCES