An increase in the myocardial PCr/ATP ratio in GLUT4 null mice[1]

ROBERT G. WEISS,[2] JOHN C. CHATHAM,* DIMITRIOS GEORGAKOPOLOUS, MAUREEN J. CHARRON,‡ THEO WALLIMANN,# LAURENCE KAY,# BERND WALZEL,# YIBIN WANG,§ DAVID A. KASS, GARY GERSTENBLITH, AND V. P. CHACKO†

Cardiology Division, Department of Medicine and †NMR Research Division, Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; *Department of Medicine, University of Alabama, Birmingham, Alabama, USA; ‡Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York, USA; #Institute for Cell Biology, ETH Hoenggerberg, Zürich, Switzerland; and §Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland, USA

SPECIFIC AIMS

ATP and creatine phosphate (PCr) are the prime myocardial high-energy phosphates. Although the cardiac PCr/ATP ratio is decreased in several pathological conditions, such as ischemia and heart failure, the cardiac PCr/ATP ratio is not increased in any species. Our aim was to study in vivo energetics in transgenic mice lacking expression of the GLUT4 protein (G4N), which is the predominantly insulin-sensitive transporter found in adipose tissue and skeletal and heart muscle. Because G4N have a primary genetic abnormality of the glucose transport protein and have been shown to have severe left ventricular hypertrophy, we anticipated low cardiac PCr/ATP in G4N. Instead, we report an increase in the cardiac PCr/ATP in G4N and investigate the contributing mechanisms.

PRINCIPAL FINDINGS

1. Adult GLUT4 nulls have severe left ventricular hypertrophy and depressed systolic function

Noninvasive studies in intact 7- to 12-month-old G4N and wild-type (WT) littermate mice were performed using multi-slice, cardiac-gated magnetic resonance imaging (MRI) to quantify left ventricular mass and function (**Fig. 1**). G4N had severe left ventricular hypertrophy (6.8±0.7 vs. 3.5±0.3 LV mass/body mass (mg/g), mean ± SE, $P < 0.0008$). Although young G4N were previously shown to have normal systolic function, we observed a significant decrease in left ventricular ejection fraction in older G4N vs. age-matched WT littermates (42±7% vs. 66±4%, respectively, $P=0.009$). The lower EF in G4N was not due to lower filling volumes as LV end diastolic and end systolic volumes trended higher in G4N than in WT animals.

Invasive studies in ventilated, open-chest animals also demonstrated a decrease in systolic function, as evidenced by a 30% lower + dP/dt_{max} in G4N than in WT animals (+dP/dt_{max} 7,245±1,014 vs. 10,622±723, mmHg/s, $P=0.025$). Despite the hypertrophy, diastolic function was not significantly altered as demonstrated by resting end-diastolic pressure, time constant of relaxation, and the time to early peak diastolic filling. Myocardial expression of atrial natriuretic factor and brain natriuretic peptide were significantly increased in adult G4N animals, providing additional evidence of contractile dysfunction in adult G4N.

2. Adult GLUT4 nulls have a high cardiac PCr/ATP ratio

Noninvasive in vivo studies of cardiac energetics in intact mice were performed with image-guided, spatially localized ^{31}P-MR spectroscopy and demonstrated a significant 60% increase in the cardiac PCr/ATP ratio in G4N compared with WT animals (3.3±0.2 vs. 2.0±0.2, respectively, $P<0.001$) (Fig. 1). Because a significant increase in the cardiac PCr/ATP has not been reported in any other species, we conducted additional studies to confirm this finding. A separate series of in vivo ^{31}P-MRS studies were also performed in anesthetized, ventilated mice with the sternum retracted, and these, too, demonstrated a ~60% increase in the cardiac PCr/ATP ratio in G4N ($P<0.0001$). Other ^{31}P-MRS studies were conducted in isolated, perfused hearts supplied identical substrates, and these too revealed a ~60% increase in the cardiac PCr/ATP in G4N compared with WT animals (3.1±0.5 vs. 1.9±0.1, respectively, $P=0.044$). Mean intracellular pH as measured by ^{31}P NMR did not differ between the two groups under in vivo or in vitro conditions. Thus, a higher cardiac PCr/ATP was observed in G4N in three independent settings: in vitro isolated perfused hearts,

[1] To read the full text of this article, go to http://www.fasebj.org/cgi/doi/10.1096/fj.01–0462fje; to cite this article, use *FASEB J.* (February 25, 2002) 10.1096/fj.01–0462fje

[2] Correspondence: Carnegie 584, The Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21287-6568, USA. E-mail: rgweiss@rad.jhu.edu

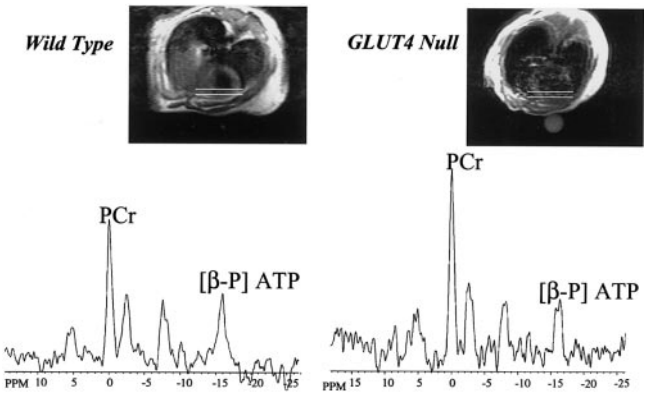


Figure 1. Image-guided, spatially localized cardiac ^{31}P NMR Spectroscopy of intact mice. Representative axial ^{1}H-MR images (above) and ^{31}P-MR spectra (below) acquired in the same examination without repositioning the animals are presented from a WT (left) and G4N (right) mouse. ^{31}P-NMR spectra from the cardiac regions (slice thickness ~1 mm) annotated on the ^{1}H spin echo, axial MR images indicate the relative myocardial content of phosphocreatine (PCr) and ATP under these noninvasive, lightly sedated conditions. Note that the heart is larger and the myocardial PCr/ATP ratio is higher in the G4N than in the WT animal.

in vivo open-chest animals, and in vivo intact animals under physiological conditions.

3. The increased PCr/ATP in GLUT4 nulls is cardiac-specific

To determine whether the unique increase in PCr/ATP in G4N mice was specific to the heart or also occurred in skeletal muscle, we conducted more in vivo experiments. The PCr/ATP ratio in thigh muscle trended lower in G4N than in WT (2.9±0.2 vs. 3.5±0.2, respectively, P=0.066), whereas in chest muscle the PCr/ATP ratio did not significantly differ between the strains (3.1±0.1 vs. 3.1±0.3, =NS). Thus, the increased PCr/ATP ratio in G4N relative to WT is specific to cardiac muscle.

4. The increased cardiac PCr/ATP is related to higher total creatine with unchanged [ADP] and-$\Delta G_{\sim ATP}$

To understand the mechanism contributing to the uniquely higher in vivo cardiac PCr/ATP in G4N, we studied in vitro energetics and creatine transport uptake protein (CreaT) expression. To determine whether the PCr/ATP ratio is increased by a higher [PCr] or a lower [ATP], high-energy phosphate and total creatine levels were measured by conventional biochemical techniques in the isolated hearts frozen at the end of the perfusion period. [ATP] did not differ between G4N and WT (3.7±0.6 vs. 3.7±0.4 μmol/g wet wt, respectively, P=NS) (**Table 1**). However, total creatine (PCr+Cr) concentrations were significantly higher in G4N (17.4±2.1 μmol/g wet wt) than in WT animal hearts (10.9±1.3 μmol/g wet wt, P=0.017). From the in vitro biochemical and ^{31}P NMR metabolite ratios, we calculate [ADP] of 40 ± 6 μmol/L in WT littermates and similar values, 38 ± 16 μmol/L, in G4N (P=NS). The free energy of ATP hydrolysis, -$\Delta G_{\sim ATP}$, is calculated at 60.1 ± 1.0 and 60.0 ± 0.4 kJ/mol, for G4N and WT animals, respectively, (P=NS). Thus, the increase in myocardial PCr/ATP ratios that occurs with GLUT4 ablation is due to an increased total creatine pool and is not associated with differences in [ATP], calculated [ADP] or -$\Delta G_{\sim ATP}$.

Because creatine is not synthesized in myocytes and was found to be significantly higher in G4N than in WT adult animals, we quantified the creatine transporter (CreaT) protein. Our aim was to determine whether it is depressed in G4N as in other experimental models of cardiac dysfunction and in humans with heart failure or elevated and possibly responsible for the increased cardiac creatine and PCr/ATP. The results from six WT and seven G4N demonstrated that the myocardial CreaT expression in G4N does not differ from that observed in WT animals. CreaT expression was significantly depressed in skeletal muscle of G4N compared with that in WT animals.

DISCUSSION

Ablation of GLUT4 expression appears to result in the first mammal with an increased myocardial PCr/ATP ratio, a well-established marker of cardiac bioenergetic status. The increased PCr/ATP ratio in G4N is specific to cardiac muscle and the magnitude of the increase is considerable (~60%). The higher cardiac PCr/ATP findings in G4N were confirmed in three distinct experiments from isolated, perfused hearts, to open-chest, surgically exposed intact hearts, to the entirely intact in vivo setting at physiological heart rates. The consistency of the findings from these different studies unequivocally demonstrates that the higher cardiac PCr/ATP in G4N is not an artifact of the preparation, protocol, or measurement technique.

Differences in substrate utilization could contribute to an altered cardiac PCr/ATP ratio but probably do

TABLE 1. *In vitro NMR and biochemical determinations*[a]

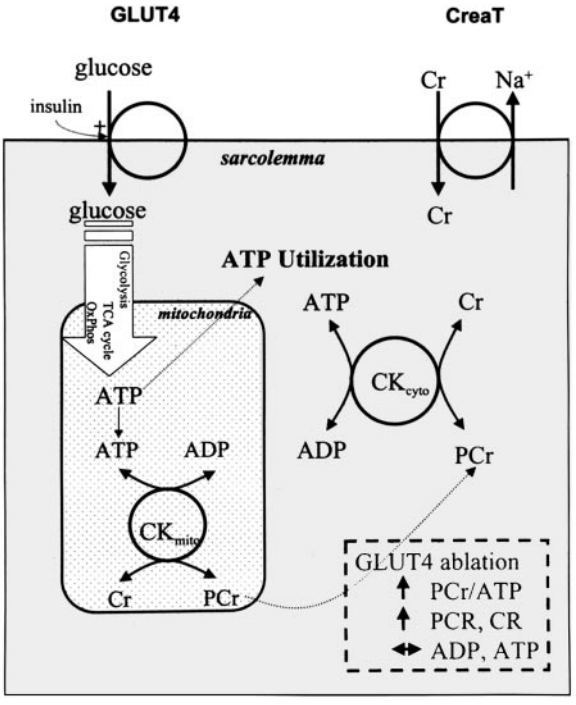
	Wild-type	GLUT4 null	*P* value
[ATP] nmol/mg prot.	32.9 ± 2.8	29.8 ± 4.9	NS
[PCr] nmol/mg prot.	63.0 ± 4.0	89.3 ± 12.4	0.09
[Cr] nmol/mg prot.	32.6 ± 6.8	50.6 ± 9.0	0.16
[PCr+Cr] nmol/mg prot.	95.6 ± 10.7	139.9 ± 8.2	0.017
PCr/ATP	1.9 ± 0.1	3.1 ± 0.5	0.044
PCr/Cr	2.1 ± 0.2	2.2 ± 0.8	NS
[ADP] (umol/L)	40 ± 6	38 ± 16	NS
-$\Delta G_{\sim ATP}$ (kJ/mol)	60.0 ± 0.4	60.1 ± 1.0	NS

[a] [ATP], [PCr], [Cr], and [PCr+Cr] indicate the concentrations of ATP, PCr, unphosphorylated creatine, and total creatine, respectively, in the frozen heart tissue. PCr/ATP was measured by ^{31}P-NMR spectroscopy in isolated hearts and $-\Delta G_{\sim ATP}$ is the free energy of ATP hydrolysis.

not explain the higher cardiac PCr/ATP in G4N. Total myocardial creatine (CR) is significantly higher in G4N than in WT, accounting for the higher cardiac PCr/ATP ratios, whereas the [PCr]/[CR], [ATP], [ADP], and $-\Delta G_{\sim ATP}$ all do not differ between the two strains. Thus, the mechanism by which GLUT4 ablation increases cardiac PCr/ATP is not via a change in substrate availability mediated by [ADP], but by an increase in total creatine and phosphocreatine.

Creatine is not synthesized in myocytes but is transported from the blood into the cell via a membrane transport protein (CreaT). A reduction in CreaT has been described in experimental models and human pathological conditions characterized by depressed contractile function. Because adult G4N have depressed contractile function, one could also expect a reduction in CreaT here as well. However, expression of the myocardial CreaT is preserved in G4N animals and likely contributes to the prevention of cardiac [CR] depletion in this model of severe LVH with contractile dysfunction. In contrast, skeletal muscle CreaT is not preserved and skeletal PCr/ATP of G4N trends lower than that of WT animals, as reported here and before. Although expression of the two major CreaT proteins is not elevated in G4N, adaptive regulation by hormones, signaling cascades, and/or the transmembrane Na^+ gradient could elevate CreaT transport capacity over a prolonged period such that the total intracellular creatine content in heart is increased in G4N. The current evidence further demonstrates that expression of the cardiac creatine transporter is not reduced in all settings of contractile dysfunction and is consistent with the hypothesis that preservation of the myocardial CreaT in adult G4N may contribute to the truly unique cardiac energetic status of these animals with ventricular dysfunction and marked hypertrophy.

GLUT4 and CreaT are distinct membrane transport proteins whose expression are sometimes related (see **Fig. 2**). The expression of GLUT4 in muscle is increased when creatine and creatine phosphate levels fall such as during ischemia, hypoxia, exercise, and with creatine analog exposure. This increase in GLUT4 expression is likely mediated by an AMP-activated protein kinase pathway. Less is understood about glucose regulation of creatine metabolism. In humans, muscle creatine uptake during oral creatine supplementation is augmented by glucose. Skeletal muscle creatine content is known to be enhanced by several factors that affect glucose metabolism, such as insulin and insulin growth factor, which are unchanged in adult G4N. These data demonstrate that genetic manipulation of GLUT4 provides a strategy for maintaining cardiac CreaT expression and increasing myocardial PCr and CR content.



In summary, diffuse ablation of the GLUT4 gene responsible for insulin-sensitive glucose uptake results in depressed contractile function and altered cardiac high-energy phosphates in adult animals. The myocardial PCr/ATP is unexpectedly higher in G4N than in WT animals and is independent of external workload and substrate supply. An increase in the cardiac PCr/ATP ratio has not been observed in other animals or been shown to increase with interventions such as creatine loading. The increased PCr/ATP in G4N is cardiac specific and due to an increase in total cardiac creatine with unchanged energetic cost, i.e., similar [ADP] and $-\Delta G_{\sim ATP.}$ Thus, significant in vivo reductions in systolic function can occur with an increased PCr/ATP ratio, and depressed myocardial contractility does not require reduced baseline creatine kinase reserve. In contrast to other animal models with contractile dysfunction and human heart failure, a depression in the myocardial creatine transporter (CreaT) is not observed in G4N, and this may contribute to the higher PCr/ATP and [CR] in G4N. Additional studies are required to evaluate the possibility that modification of the myocellular creatine pool occurs in other conditions associated with altered glucose uptake protein and whether this alteration may represent a unique genetic approach to influence energy metabolism in hypertrophy and/or heart failure. FJ