Skeletal Muscle Reprogramming by Activation of Calcineurin Improves Insulin Action on Metabolic Pathways*

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The protein phosphatase calcineurin is a signaling intermediate that induces the transformation of fast-twitch skeletal muscle fibers to a slow-twitch phenotype. This reprogramming of the skeletal muscle gene expression profile may have therapeutic applications for metabolic disease. Insulin-stimulated glucose uptake in skeletal muscle is both impaired in individuals with type II diabetes mellitus and positively correlated with the percentage of slow- versus fast-twitch muscle fibers. Using transgenic mice expressing activated calcineurin in skeletal muscle, we report that skeletal muscle reprogramming by calcineurin activation leads to improved insulin-stimulated 2-deoxyglucose uptake in extensor digitorum longus (EDL) muscles compared with wild-type mice, concomitant with increased protein expression of the insulin receptor, Akt, glucose transporter 4, and peroxisome proliferator-activated receptor-γ co-activator 1. Transgenic mice exhibited elevated glycogen deposition, enhanced amino acid uptake, and increased fatty acid oxidation in EDL muscle. When fed a high-fat diet, transgenic mice maintained superior rates of insulin-stimulated glucose uptake in EDL muscle and were protected against diet-induced glucose intolerance. These results validate calcineurin as a target for enhancing insulin action in skeletal muscle.

Skeletal muscle fibers can be separated into two major classifications: type I slow-twitch and type II fast-twitch fibers (1). The knowledge that type I and type II muscle fibers exhibit distinctively different contractile and metabolic properties has been accepted for decades (1, 2). However, the intracellular signaling mechanisms controlling skeletal muscle fiber type have not been clearly elucidated. The serine/threonine protein phosphatase calcineurin has been recognized as a major determinant of skeletal muscle fiber type. Early evidence for the involvement of calcineurin in skeletal muscle fiber type selection comes from studies implementing the use of the immunosuppressant drug cyclosporin A, a potent inhibitor of calcineurin activity. Cyclosporin A blockade of calcineurin activity promotes the expression of fast-twitch fibers in rat soleus muscle (3). Conversely, expression of a constitutively active form of calcineurin in cultured muscle cells induces the expression of reporter genes linked to promoters of slow-twitch muscle-specific genes such as myoglobin and slow troponin I (3). Based on these observations, activation of calcineurin was proposed to be involved in the induction of slow muscle genes and the regulation of skeletal muscle fiber type. Consistent with this hypothesis, transgenic expression of a constitutively active form of calcineurin in skeletal muscle increases the percentage of slow-twitch muscle fibers and promotes the expression of myoglobin and slow troponin I (4).

The identification of calcineurin as a regulator of skeletal muscle fiber type may have clinical implications for improving insulin action in individuals with type II diabetes mellitus. A major contributing factor to the progressive development of type II diabetes is reduced insulin-stimulated whole-body glucose disposal, with the greatest defects attributed to skeletal muscle (5). Skeletal muscle insulin resistance in individuals with type II diabetes is associated with impaired insulin signal transduction (6, 7) and defects in glucose transporter 4 (GLUT4)* trafficking (8). Therefore, strategies to improve insulin signal transduction or enhance insulin-stimulated mobilization of GLUT4 vesicles to the cell surface are likely to correct glucose transport defects and lead to improved glucose homeostasis in diabetic individuals. One means of achieving improved glucose transport in skeletal muscle may be through activation of the calcineurin signaling pathway. Activation of calcineurin may promote metabolic adaptations within muscle cells, resulting in improved insulin-stimulated glucose transport. This hypothesis is supported in part by the observation that insulin action on glucose transport is greater in rat skeletal muscles that are highly enriched in slow- versus fast-twitch muscle fibers (9). Fiber type specificity of insulin-stimulated glucose transport appears to be due to increased expression and/or function of proteins involved in the insulin-signaling cascade (10), as well as a greater abundance of GLUT4 (9, 11). Furthermore, insulin-stimulated glucose transport is positively correlated with the percentage of slow-twitch muscle fibers in human skeletal muscle (12). Thus, activation of calcineurin in skeletal muscle represents a novel strategy to improve insulin-stimulated glucose transport and may counterbalance insulin resistance in type II diabetes. We utilized transgenic mice expressing an activated form of calcineurin in skeletal muscle (MCK-CnA* mice) to determine whether skel...

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et al. muscle reprogramming via activation of calcineurin improves insulin-stimulated glucose transport and prevents the development of diet-induced insulin resistance.

**EXPERIMENTAL PROCEDURES**

**Transgenic Mice**—A colony of transgenic mice expressing a constitutively active form of calcineurin (13) under control of the muscle creatine kinase promoter/enhancer was established at the Department of Physiology and Pharmacology, Karolinska Institutet, using MCK-CnA* mice that were originally developed at the Department of Molecular Biology, University of Texas Southwestern Medical Center at Dallas (4). Mice were bred on a C57BL/6J background. Wild-type littermates and transgenic mice were used in all experiments. Mice were maintained on a 12-h light-dark cycle and allowed free access to food and water. Food was removed from the cages of 12–14-week-old female mice 4 h prior to study unless indicated otherwise. When examining effects of a high-fat diet, female mice were placed on either a standard chow or a high-fat diet (14) for 20 weeks of age. The Ethics Committee on Animal Research in Northern Stockholm approved all experimental protocols.

**Muscle Incubations**—Incubation media were prepared from pre-gassed (95% O₂, 5% CO₂) stocks of Krebs-Henseleit buffer (KBH) supplemented with 5 mM HEPES and 0.1% bovine serum albumin (radio-[14C]MeAIB). Liberated CO₂ was collected for 60 min, and center wells were transferred to vials containing 0.2 ml of Protosol (PerkinElmer Life Sciences) after 60 min, 0.2 ml of Protosol (PerkinElmer Life Sciences) was injected through the rubber stopper into the center well, and the oxygen needle was removed to close the incubation system. After 60 min, 0.2 ml of Protosol (PerkinElmer Life Sciences) was added for the first 15 min of the incubation period via a needle inserted through the rubber stopper, after which time the oxygen needle was removed to close the system. After 60 min, 0.2 ml of Protosol (PerkinElmer Life Sciences) was transferred to vials containing 1 ml of KBH supplemented with 2 mM pyruvate and 18 mM mannitol. Incubations were performed at 30 °C in a shaking water bath under a constant gas phase (95% O₂, 5% CO₂) unless stated otherwise. For 2-deoxyglucose uptake and a-aminomethylisobutyrate (MeAIB) uptake experiments, muscles were preincubated for 40 min in the absence or presence of insulin. Insulin (120 nM) or cycloheximide (1 μM) was added to the incubation media 15 min prior to the addition of 2-deoxyglucose. Insulin treatment was performed as described (16). Results were determined by Student’s t test. Significance was accepted at p < 0.05.

**RESULTS**

**Calcineurin Protein Expression**—Protein expression of endogenous calcineurin and the truncated constitutively active transgene product was assessed in EDL and soleus muscles of wild-type and MCK-CnA* mice. Endogenous levels of calcineurin protein were reduced in both muscle types in transgenic mice (Fig. 1, Table I); levels were greater in EDL compared with soleus muscle in both wild-type and MCK-CnA* mice. Constitutively active calcineurin was 4.5-fold greater in EDL compared with soleus muscle from MCK-CnA* mice. Constitutively active calcineurin accounted for ~34 and 21% of the total calcineurin pool (endogenous plus transgenic) in MCK-CnA* EDL and soleus muscle, respectively.

**Alterations in Metabolism of MCK-CnA* Mice**—In wild-type mice, insulin increased 2-deoxyglucose uptake 2.9-fold in EDL muscle and 4.2-fold in soleus muscle (Fig. 2A). In EDL muscle from MCK-CnA* mice, basal and insulin-stimulated glucose uptake was increased 36 and 60%, respectively, compared with wild-type EDL muscle (Fig. 2A). Glucose transport rates in soleus muscle were not significantly different between MCK-CnA* and wild-type mice.

System A amino acid uptake was determined using the non-metabolizable amino acid analog MeAIB. Insulin increased amino acid uptake 2-fold in EDL muscle from wild-type mice. In wild-type mice, basal and insulin-stimulated MeAIB uptake was 2- and 1.8-fold greater, respectively, in soleus muscle compared with EDL muscle.
Comparison of the results is presented in Table I. Representative image for protein expression of endogenous (CnA) or constitutively active (CnA*) calcineurin in EDL or soleus muscle from wild-type or MCK-CnA* mice as determined by Western blot analysis. Quantification of the results is presented in Table I.

**Fig. 1.** Protein expression of endogenous and constitutively active calcineurin in MCK-CnA* skeletal muscle. Representative image for protein expression of endogenous (CnA) or constitutively active (CnA*) calcineurin in EDL or soleus muscle from wild-type or MCK-CnA* mice as determined by Western blot analysis. Quantification for protein expression of endogenous (CnA) or constitutively active (CnA*) calcineurin in EDL or soleus muscle from wild-type or MCK-CnA* mice as determined by Western blot analysis.

![Image](image.png)

**TABLE I**

Protein expression and phosphorylation in EDL and soleus muscles from wild-type and MCK-CnA* mice as determined by Western blot analysis

Data are expressed as percentage of EDL muscle from wild-type mice. Values are reported as means ± S.E. Sample size is shown in parentheses (n). NS, not significant.

|                          | Wild type | MCK-CnA* | p value |
|--------------------------|-----------|----------|---------|
| Endogenous calcineurin   |           |          |         |
| EDL                      | 100 ± 14 (4) | 35 ± 3 (4) | p < 0.005 |
| Soleus                   | 25 ± 4 (4)    | 15 ± 2 (6)  | p < 0.005 |
| Transgenic calcineurin   |           |          |         |
| EDL                      | 18 ± 3 (4)    | 4 ± 1 (4)     |         |
| Soleus                   | 4 ± 1 (4)      |             |         |
| Insulin receptor         |           |          |         |
| EDL                      | 100 ± 19 (6)  | 287 ± 45 (6) | p < 0.01 |
| Soleus                   | 333 ± 82 (6)  | 365 ± 76 (6) | NS      |
| IRS1                     |           |          |         |
| EDL                      | 100 ± 20 (6)  | 168 ± 39 (6) | NS      |
| Soleus                   | 263 ± 45 (6)  | 389 ± 72 (6) | NS      |
| Akt                      |           |          |         |
| EDL                      | 100 ± 14 (4)  | 278 ± 18 (4) | p < 0.0005 |
| Soleus                   | 220 ± 29 (4)  | 311 ± 61 (4) | NS      |
| PGC-1                    |           |          |         |
| EDL                      | 100 ± 14 (4)  | 216 ± 8 (4)  | p < 0.0005 |
| Soleus                   | 296 ± 11 (4)  | 235 ± 31 (4) | NS      |
| GLUT4                    |           |          |         |
| EDL                      | 100 ± 5 (4)    | 195 ± 20 (4) | p < 0.005 |
| Soleus                   | 515 ± 44 (4)  | 682 ± 100 (4) | NS     |
| Glycogen synthase        |           |          |         |
| EDL                      | 100 ± 11 (4)  | 160 ± 11 (4) | p < 0.01 |
| Soleus                   | 160 ± 10 (4)  | 171 ± 9 (4)  | NS      |
| Insulin receptor         |           |          |         |
| phosphorylation          |           |          |         |
| EDL insulin              | 100 ± 21 (4)  | 75 ± 5 (4)   | NS      |
| Soleus insulin           | 68 ± 19 (4)   | 90 ± 3 (4)   | NS      |
| GS3α                     |           |          |         |
| phosphorylation          |           |          |         |
| EDL insulin              | 100 ± 11 (4)  | 100 ± 13 (4) | NS      |
| Soleus insulin           | 62 ± 13 (4)   | 71 ± 22 (4)  | NS      |
| GS3β                     |           |          |         |
| phosphorylation          |           |          |         |
| EDL insulin              | 100 ± 15 (4)  | 105 ± 12 (4) | NS      |
| Soleus insulin           | 148 ± 16 (4)  | 140 ± 21 (4) | NS      |

**Fig. 2.** Muscle metabolism is altered in MCK-CnA* mice. A, EDL or soleus muscles from wild-type or MCK-CnA* mice were incubated in the absence (open bars) or presence (closed bars) of insulin, and 2-deoxyglucose uptake was assessed. Data are means ± S.E. for n = 11–12 muscles. *, p < 0.05; **, p < 0.0001 compared with wild-type. B, EDL or soleus muscles from wild-type or MCK-CnA* mice were incubated in the absence (open bars) or presence (closed bars) of insulin, and MeAIB uptake was assessed. Data are means ± S.E. for n = 3–4 muscles. ***, p < 0.0005 compared with wild-type. C, olate oxidation in EDL and soleus muscle from wild-type or MCK-CnA* mice. Data are means ± S.E. for n = 5–6 muscles. ***, p < 0.0001 compared with wild-type.

**Fig. 3.** Insulin action in EDL and soleus muscle from wild-type and MCK-CnA* mice. A, Basal and insulin-stimulated 2-deoxyglucose uptake was assessed. Data are means ± S.E. for 6 muscles. ***, p < 0.0001 compared with wild-type. B, Basal and insulin-stimulated amino acid uptake was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type. C, Basal and insulin-stimulated fatty oxidation was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type.

Comparison of the results is presented in Table I. Representative image for protein expression of endogenous (CnA) or constitutively active (CnA*) calcineurin in EDL or soleus muscle from wild-type or MCK-CnA* mice as determined by Western blot analysis. Quantification of the results is presented in Table I.

**Fig. 4.** Insulin action in EDL and soleus muscle from wild-type and MCK-CnA* mice. A, Basal and insulin-stimulated 2-deoxyglucose uptake was assessed. Data are means ± S.E. for 6 muscles. ***, p < 0.0001 compared with wild-type. B, Basal and insulin-stimulated amino acid uptake was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type. C, Basal and insulin-stimulated fatty oxidation was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type.

**Fig. 5.** Protein expression of molecules involved in the regulation of insulin action in EDL and soleus muscle from wild-type and MCK-CnA* mice. A, Basal and insulin-stimulated 2-deoxyglucose uptake was assessed. Data are means ± S.E. for 6 muscles. ***, p < 0.0001 compared with wild-type. B, Basal and insulin-stimulated amino acid uptake was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type. C, Basal and insulin-stimulated fatty oxidation was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type.

**Fig. 6.** Insulin action in EDL and soleus muscle from wild-type and MCK-CnA* mice. A, Basal and insulin-stimulated 2-deoxyglucose uptake was assessed. Data are means ± S.E. for 6 muscles. ***, p < 0.0001 compared with wild-type. B, Basal and insulin-stimulated amino acid uptake was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type. C, Basal and insulin-stimulated fatty oxidation was assessed. Data are means ± S.E. for 5–6 muscles. ***, p < 0.0001 compared with wild-type.
different in skeletal muscle from MCK-CnA* compared with wild-type mice. Similar to the pattern observed for insulin receptor phosphorylation, insulin led to a robust stimulation of IRS1-associated PI 3-kinase activity, with comparable effects observed in both muscle types of each genotype (Fig. 4B). Insulin-stimulated Akt phosphorylation in skeletal muscle of wild-type mice was not different between EDL and soleus muscles compared with wild-type mice (Fig. 4C). However, insulin-stimulated Akt phosphorylation in EDL muscle from MCK-CnA* mice was increased 2-fold compared with wild-type mice (Fig. 4C).

**Blood Chemistry**—Blood glucose, blood lactate, non-esterified free fatty acid levels, and plasma insulin were determined in wild-type and MCK-CnA* mice (Table II). No significant differences were found in any of these parameters.

**Elevated Skeletal Muscle Glycogen Content in MCK-CnA* Mice**—Muscle glycogen content was increased 125 and 64% in EDL and soleus muscle from MCK-CnA* mice, respectively, compared with wild-type mice (Fig. 5A). Activation of the calcineurin transgene induced a 60% increase in glycogen synthase protein expression in EDL muscle from MCK-CnA* compared with wild-type mice (Fig. 5B, Table I). Expression of glycogen synthase was not increased in soleus muscle from MCK-CnA* mice. Insulin-stimulated phosphorylation of GSK3α/β was markedly increased in skeletal muscle from wild-type and MCK-CnA* mice, with similar responses observed between genotypes (Fig. 5C, Table I).

**MCK-CnA* Mice Are Protected against Deleterious Effects of a High-fat Diet**—2-Deoxyglucose uptake was assessed in skeletal muscle from wild-type and MCK-CnA* mice maintained on either a chow or high-fat diet from 4 to 20 weeks of age. Insulin-stimulated glucose uptake was 72% greater in EDL muscle from chow-fed MCK-CnA* compared with wild-type mice (Fig. 6A). High-fat feeding for 16 weeks resulted in a 21% reduction in insulin-stimulated glucose uptake in EDL muscle from both wild-type and MCK-CnA* mice (Fig. 6A). However, the rate of glucose uptake in high-fat-fed MCK-CnA* mice was 70% higher than that observed in high-fat-fed wild-type mice. Fat feeding did not alter the expression of endogenous or transgenic calcineurin in quadriceps muscle (data not shown).

We determined the effect of a high-fat diet on glucose tolerance in wild-type and MCK-CnA* mice. Wild-type mice developed glucose intolerance when maintained on a high-fat diet (Fig. 6B). Blood glucose levels in high-fat-fed wild-type mice were significantly elevated compared with chow-fed wild-type mice at all measured time points between 30 and 120 min. In contrast, MCK-CnA* mice were protected against the development of diet-induced glucose intolerance.

**DISCUSSION**

We hypothesized that activation of calcineurin in transgenic mice could alter the gene expression program of fast-twitch skeletal muscle, thereby resulting in a slow-twitch muscle phenotype with enhanced insulin-stimulated glucose transport. We studied EDL and soleus muscles because they exhibit distinctively different contractile and metabolic properties. EDL muscles from wild-type mice are predominantly composed of fast-twitch fibers and contain as little as 2% type I (slow-twitch) fibers, whereas soleus muscles contain ~50% type I fibers, with the balance accounted for by type IIa/b (fast-twitch) muscle fibers in each case (18). MCK-CnA* mice have been...
Calcineurin, Muscle Reprogramming, and Metabolism

Table II
Blood metabolite and insulin levels in wild-type and MCK-CnA* mice

Blood glucose, blood lactate, plasma insulin, and plasma free fatty acid (FFA) levels were analyzed in wild-type and MCK-CnA* mice in the ad libitum fed state and the 4-h and 16-h fasted states. Results are reported as means ± S.E. for n = 7 wild-type and n = 8 MCK-CnA* mice. No statistical differences were observed.

| Condition          | Wild type | MCK-CnA* |
|--------------------|-----------|----------|
| Blood glucose (mmol/liter) |           |          |
| Fed                | 6.5 ± 0.2 | 6.5 ± 0.3 |
| 4-h fasted         | 7.2 ± 0.3 | 8.0 ± 0.4 |
| 16-h fasted        | 3.8 ± 0.4 | 4.1 ± 0.4 |
| Blood lactate (mmol/liter) |           |          |
| Fed                | 1.9 ± 0.3 | 1.7 ± 0.2 |
| 4-h fasted         | 1.9 ± 0.2 | 1.8 ± 0.2 |
| 16-h fasted        | 1.7 ± 0.2 | 2.2 ± 0.3 |
| Plasma insulin (ng/ml) |        |          |
| Fed                | 1.18 ± 0.18 | 1.37 ± 0.46 |
| 4-h fasted         | 1.89 ± 0.30 | 2.37 ± 0.59 |
| 16-h fasted        | 1.09 ± 0.16 | 1.44 ± 0.26 |
| Plasma FFA (mmol/liter) |      |          |
| Fed                | 0.52 ± 0.04 | 0.57 ± 0.06 |
| 4-h fasted         | 0.41 ± 0.03 | 0.49 ± 0.06 |
| 16-h fasted        | 1.12 ± 0.13 | 1.05 ± 0.11 |

Fig. 5. Muscle glycogen in MCK-CnA* muscle. A, intramuscular glycogen levels in EDL or soleus muscle from wild-type or MCK-CnA* mice. Data are means ± S.E. for n = 6–7 mice. *, p < 0.05, **, p < 0.001 compared with wild-type mice. B, glycogen synthase (GS) protein expression in wild-type and MCK-CnA* EDL and soleus muscle as determined by Western blot analysis. Quantification of results is presented in Table I. C, GSK3α/β phosphorylation. EDL or soleus muscles from wild-type or MCK-CnA* mice were incubated in the absence or presence of insulin, and GSK3α/β phosphorylation was determined by Western blot analysis. No significant differences were observed. Quantification of results is presented in Table I.

Fig. 6. Glucose uptake and glucose tolerance in high-fat-fed mice. A, basal (open bars) and insulin-stimulated (closed bars) glucose uptake was determined in EDL muscle from wild-type or MCK-CnA* mice following 16 weeks on either a standard chow or a high-fat diet. Data are means ± S.E. for n = 5–8 mice. *, p < 0.05, **, p < 0.005 compared with wild-type mice under identical dietary conditions; †, p < 0.05 compared with chow-fed mice of identical genotype. B, glucose tolerance was assessed in wild-type or MCK-CnA* mice maintained on either a standard chow (open circles, wild-type; closed circles, MCK-CnA* mice) or a high-fat (open squares, wild-type; closed squares, MCK-CnA* mice) diet for 16 weeks. Data are means ± S.E. for n = 5–9 mice. *, p < 0.05, **, p < 0.005 compared with chow-fed mice of identical genotype.

utilized to demonstrate that activation of calcineurin promotes fast-to-slow-twitch muscle fiber type transformation (4). Here we provide direct evidence that muscle fiber type transformation is associated with a shift in the metabolic parameters to a more insulin-responsive phenotype. Insulin-stimulated glucose uptake in EDL muscle from MCK-CnA* mice was increased 60% compared with wild-type mice, concomitant with increased expression of proteins involved in the regulation of glucose transport. In addition, amino acid transport and fatty acid oxidation were increased in fast-twitch muscle from MCK-CnA* mice to levels characteristic of slow-twitch soleus muscle. Thus, multiple fiber type-specific physiological processes are under the control of calcineurin. Importantly, activated calcineurin protected against the development of diet-induced glucose intolerance. Our results provide evidence to suggest that calcineurin is a plausible target for improving insulin action in skeletal muscle.

We examined whether changes in insulin signal transduction contribute to the increased insulin responsiveness to glucose transport in EDL muscle from MCK-CnA* mice. Although insulin receptor protein expression was increased in EDL muscle from MCK-CnA* mice, insulin-stimulated receptor auto-phosphorylation was unchanged. Furthermore, insulin signal transduction at the level of PI 3-kinase activity, a key component of the insulin-signaling cascade (19), was not altered in skeletal muscle from MCK-CnA* mice. Although components of the signaling cascade between PI 3-kinase and GLUT4 translocation have not been fully elucidated, the serine/threonine
protein kinase Akt is a common step in several PI 3-kinase-dependent signaling pathways that regulate metabolism and cell growth. Nevertheless, the direct role of Akt in the regulation of glucose transport is a matter of debate (20–22). Overexpression of activated calcineurin was associated with a 2-fold increase in Akt protein expression and insulin-stimulated phosphorylation in EDL muscle. This finding is consistent with studies in cardiomyocytes, whereby calcineurin signaling has been proposed to activate the hypertrophic program, which may then indirectly lead to Akt activation (23). Although the functional significance of the elevation in Akt protein expression and insulin-stimulated phosphorylation in EDL muscle from MCK-CnA mice as related to glucose transport is not clear, collectively our data provide evidence that adaptive mechanisms independent of changes in early components of the insulin signal transduction pathway are involved in improving glucose transport in response to calcineurin activation.

PGC-1 has been implicated as a regulator of muscle fiber type in skeletal muscle of transgenic mice (24). PGC-1-mediated regulation of skeletal muscle fiber type has been proposed to occur via a mechanism downstream of calcium-sensitive signaling intermediates such as calcineurin and/or the calcium/calmodulin-dependent protein kinase (CaMK) (24). We observed a fiber type-specific difference in protein expression of PGC-1. Protein expression of PGC-1 was increased in slow-twitch soleus muscle from wild-type mice and up-regulated in EDL muscle from MCK-CnA mice. Thus, calcineurin is directly implicated in the muscle fiber type-specific regulation of PGC-1. Our results and those of others (24, 25) support the hypothesis that a calcineurin/PGC-1 pathway regulates, at least in part, muscle fiber type-specific control of insulin-stimulated glucose transport. Adenoviral expression of PGC-1 in L6 myotubes is sufficient to induce a marked increase in GLUT4 protein expression (25), thus leading to elevated basal and insulin-stimulated glucose transport. Consistent with this, we show that activation of calcineurin in fast-twitch EDL muscle yields an adaptive increase in GLUT4 expression, which may solely explain improvements in insulin-stimulated glucose transport. However, protein expression levels of GLUT4 in the soleus muscle from wild-type mice were considerably higher than those observed in EDL muscle from MCK-CnA mice, indicating that additional mechanisms cooperate with calcineurin to regulate fiber type-specific expression of GLUT4. A likely candidate would be one or more of the known CaMK isoforms. Transgenic activation of CaMKIV in skeletal muscle is sufficient to promote muscle fiber type transformation and up-regulation of slow muscle genes (26). Calcineurin and CaMKIV synergistically induce MEF2 (myocyte enhancer factor 2) transcriptional activity in C2C12 muscle cells (27). Interestingly, deletion of the MEF2 binding domain of the GLUT4 promoter abolishes expression of a reporter gene in skeletal muscles of transgenic mice (28). Therefore, increased activation of calcineurin and CaMK may be required to achieve the levels of GLUT4 that are observed in slow-twitch skeletal muscle.

Once transported inside the muscle cell, glucose is primarily stored as glycogen. Both the rate of glucose transport (29) and the level of glycogen synthase expression (30) strongly influence skeletal muscle glycogen storage. Because both glucose transport and glycogen synthase protein expression are increased in EDL muscle from MCK-CnA mice, the increase in glycogen content is not surprising. Nevertheless, activation of calcineurin in EDL muscle did not lead to a slow-twitch phenotype in terms of muscle glycogen storage because intramuscular glycogen was found to be lower in soleus compared with EDL muscle from wild-type mice. Activation of calcineurin in skeletal muscle leads to parallel adaptations induced by contractile activity (31), presenting the possibility that constitutively active calcineurin recapitulates the consequences of exercise training to enhance glycogen storage in a manner that is not evident even in type I muscle of sedentary animals.

To validate calcineurin as a therapeutic target for the treatment of skeletal muscle insulin resistance, we determined whether calcineurin-induced changes in glucose metabolism could protect against insulin resistance commonly associated with high-fat feeding in rodents models (14, 32). High-fat feeding reduced insulin-stimulated glucose uptake in EDL muscle from both wild-type and MCK-CnA mice. However, glucose uptake in high-fat-fed MCK-CnA mice was 70% higher than that observed in high-fat-fed wild-type mice and 35% higher than for Chow-fed wild-type mice, indicating that activated calcineurin preserves insulin action. Skeletal muscle insulin resistance in the high-fat-fed wild-type mice was associated with impaired glucose tolerance. In contrast, high-fat-fed MCK-CnA mice were protected against the development of glucose intolerance. These data demonstrate that enhanced insulin-stimulated glucose uptake driven by targeted activation of calcineurin in skeletal muscle can protect against the disturbances in whole-body glucose disposal that are commonly associated with skeletal muscle insulin resistance.

In conclusion, activation of the calcineurin pathway drives adaptive responses to enhance insulin action in skeletal muscle. Activated calcineurin leads to the induction of multiple genes involved in the regulation of glucose transport in skeletal muscle. These changes are associated with increased glucose uptake and glycogen deposition in skeletal muscle. Activation of calcineurin also results in increased amino acid uptake and fatty acid oxidation. These metabolic adaptations are coincident with an up-regulation in protein expression of PGC-1. Importantly, MCK-CnA mice fed a high-fat diet maintain superior rates of insulin-stimulated glucose uptake in EDL muscle and are protected against the development of diet-induced glucose intolerance. Future strategies aimed at activating the calcineurin pathway in skeletal muscle may prove fruitful in the treatment of skeletal muscle insulin resistance in type II diabetes mellitus.

REFERENCES

1. Saltin, B., and Gollnick, P. D. (1983) in Handbook of Physiology (Peachey, L. D. D., ed.) pp. 555–632. American Physiological Society, Bethesda, MD
2. Hara, D. S., Chu, M. M., Feng, J. L., Kaisar, K. K., Lowry, C. V., and Lowry, O. H. (1982) Am. J. Physiol. 242, C218–C226
3. Chin, E. R., Olson, E. N., Richardson, J. A., Yang, Q., Humphries, C., Shelton, J. M., Wu, H., Zhu, W., Basse-Buoy, R., and Williams, R. S. (1998) Genes Dev. 12, 2499–2509
4. Naya, F. J., Mercer, B., Shelton, J., Richardson, J. A., Williams, R. S., and Olson, E. N. (2000) J. Biol. Chem. 275, 4545–4548
5. DeFronzo, R. A., Gunnarsson, R., Bjorkman, O., Olsson, M., and Wahren, J. (1985) J. Clin. Invest. 76, 149–155
6. Kim, Y. B., Nikoulina, S. E., Ciaraml, T. P., Henry, R. R., and Kahn, B. B. (1980) J. Clin. Investig. 104, 735–741
7. Krook, A., Bjorhem, M., Galuska, D., Jiang, X. J., Fahlin, R., Myers, M. G., Jr., Wallberg-Henriksson, H., and Zierath, J. R. (2000) Diabetes 49, 284–292
8. Ryder, J. W., Yang, J., Galuska, D., Rincon, J., Bjorhem, M., Krook, A., Lunding, S., Pedersen, O., Wallberg-Henriksson, H., Zierath, J. R., and Holman, G. D. (2000) Diabetes 49, 647–654
9. Herzka, E. A., Bourre, R. E., Rodnick, K. J., Koranyi, L., Permutt, M. A., and Holloszy, J. O. (1990) J. Am. J. Physiol. 259, E583–E589
10. Song, X. M., Ryder, J. W., Kawano, Y., Chibalin, A. V., Krook, A., and Zierath, J. R. (1999) Am. J. Physiol. 277, R1690–R1696
11. Daugaard, J. R., Nielsen, J. N., Kristiansen, S., Andersen, J. L., Hargreaves, M., and Richter, E. A. (2000) Diabetes 49, 1092–1098
12. Hickey, M. S., Cig relocate, J. O., Azevedo, J. L., Hearn, E. S., Pories, W. J., Israel, R. G., and Dohm, G. L. (1995) Am. J. Physiol. 268, E453–E457
13. O’Keefe, J. S., Tanaka, J., Kincad, R. L., Toec, M. J., and O’Neill, E. A. (1992) Nature 357, 692–694
14. Zierath, J. R., Housekevec, K. L., Goud, L., and Kahn, B. B. (1997) Diabetes 46, 215–223
15. Ryder, J. W., Kawano, Y., Chibalin, A. V., Rincon, J., Tsao, T. S., Stenbit, A. E., Combatsiaris, T., Yang, J., Holman, G. D., Oharron, M. J., and Zierath, J. R. (1999) Biochem. J. 342, 321–328
16. Krook, A., Whitehead, J. P., Dobson, S. P., Griffiths, M. R., Owens, M., Baker,
Calcineurin, Muscle Reprogramming, and Metabolism

17. Lowery, O. H., and Passonneau, J. V. (1972) A Flexible System of Enzymatic Analysis, pp. 189–193, Academic Press, New York.

18. Tsao, T. S., Li, J., Chang, R. S., Stenbit, A. E., Galaska, D., Anderson, J. E., Zerath, J. R., McCarter, R. J., and Charron, M. J. (2001) FASEB J. 15, 958–969.

19. Okada, T., Kawano, Y., Sakakibara, T., Hazeki, O., and Ui, M. (1994) J. Biol. Chem. 269, 3568–3573.

20. Kitamura, T., Ogawa, W., Sakagoe, H., Ino, Y., Kuroda, S., Takata, M., Matsumoto, M., Maeda, T., Konishi, H., Kikkawa, U., and Kasuga, M. (1998) Mol. Cell. Biol. 18, 3708–3717.

21. Kohn, A. D., Summers, S. A., Birnbaum, M. J., and Roth, R. A. (1996) J. Biol. Chem. 271, 31372–31378.

22. Wang, Q., Sonwar, R., Bilan, P. J., Liu, Z., Jin, J., Woodgett, J. R., and Klip, A. (1999) Mol. Cell. Biol. 19, 4008–4018.

23. De Windt, L. J., Lim, H. W., Taigen, T., Wengeler, D., Condorelli, G., Dorn, G. W., Hittais, R. N., and Molkentin, J. D. (2000) Circ. Res. 86, 253–263.

24. Lin, J., Wu, H., Turr, P. T., Zhang, C. Y., Wu, Z., Ross, O., Michael, L. F., Puigserver, P., Isotani, E., Olson, E. N., Lowell, B. B., Bassel-Duby, R., and Spiegelman, B. M. (2002) Nature 418, 797–801.

25. Michael, L. F., Wu, Z., Cheatham, R. B., Puigserver, P., Adelmann, G., Lehman, J. J., Kelly, D. P., and Spiegelman, B. M. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3820–3825.

26. Wu, H., Kanatous, S. B., Thurmond, F. A., Gallardo, T., Isotani, E., Bassel-Duby, R., and Williams, R. S. (2002) Science 296, 349–352.

27. Wu, H., Naya, F. J., McKinsey, T. A., Mercer, B., Shelton, J. M., Chin, E. R., Simard, A. R., Michel, R. N., Bassel-Duby, R., Olson, E. N., and Williams, R. S. (2000) EMBO J. 19, 1963–1973.

28. Thai, M. V., Guruswamy, S., Cao, K. T., Pessin, J. E., and Olson, A. L. (1998) J. Biol. Chem. 273, 14285–14292.

29. Ben, J. M., Marshall, B. A., Gulve, E. A., Gao, J., Johnson, D. W., Holluszy, J. O., and Mueckler, M. (1993) J. Biol. Chem. 268, 16113–16115.

30. Manchester, J., Skurat, A. V., Roach, P., Haushicha, S. D., and Lawrence, J. C., Jr. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10707–10711.

31. Wu, H., Rothermel, B., Kanatous, S., Rosenberg, P., Naya, F. J., Shelton, J. M., Hutcheson, K. A., DiMaio, J. M., Olson, E. N., Bassel-Duby, R., and Williams, R. S. (2001) EMBO J. 20, 6414–6423.

32. Klaman, L. D., Ross, O. Peroni, O. D., Kim, J. K., Martino, J. L., Zabolotny, J. M., Moghal, N., Lubkin, M., Kim, Y. B., Sharpe, A. H., Stricker-Krongrad, A., Shulman, G. I., Neel, B. G., and Kahn, B. B. (2000) Mol. Cell. Biol. 20, 5479–5489.