The 5’-AMP-activated Protein Kinase γ3 Isoform Has a Key Role in Carbohydrate and Lipid Metabolism in Glycolytic Skeletal Muscle

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5’-AMP-activated protein kinase (AMPK) is a metabolic stress sensor present in all eukaryotes. A dominant missense mutation (R225Q) in pig Prkag3, encoding the muscle-specific γ3 isoform, causes a marked increase in glycogen content. To determine the functional role of the AMPK γ3 isoform, we generated transgenic mice with skeletal muscle-specific expression of wild type or mutant (225Q) mouse γ3 as well as Prkag3 knockout mice. Glycogen resynthesis after exercise was impaired in AMPK γ3 knockout mice and markedly enhanced in transgenic mutant mice. An AMPK activator failed to increase skeletal muscle glucose uptake in AMPK γ3 knockout mice, whereas contraction effects were preserved. When placed on a high fat diet, transgenic mutant mice but not knock-out mice were protected against excessive triglyceride accumulation and insulin resistance in skeletal muscle. Transfection experiments reveal the R225Q mutation is associated with higher basal AMPK activity and diminished AMP dependence. Our results validate the muscle-specific AMPK γ3 isoform as a therapeutic target for prevention and treatment of insulin resistance.

AMPKγ3 is a heterotrimeric serine/threonine protein kinase composed of a catalytic α subunit and non-catalytic β and γ subunits (1, 2). The mammalian genome contains seven AMPK genes encoding two α, two β, and three γ isoforms. AMPK signaling is elicited by cellular stresses that deplete ATP (and consequently elevate AMP) by either inhibiting ATP production (e.g. hypoxia) or accelerating ATP consumption (e.g. muscle contraction). AMPK is activated allosterically by AMP and through phosphorylation of Thr172 in the α subunit by an upstream AMPK kinase, the tumor-suppressor protein kinase LKB1 (3, 4). AMPK is likely to be important for diverse functions in many cell types, but particular interest has been focused on elucidating the role of AMPK in the regulation of lipid and carbohydrate metabolism in skeletal muscle (5-10). AMPK activity has been correlated with an increase in glucose uptake and fatty acid oxidation and an inhibition of glycogen synthase activity and fatty acid synthesis. Exercise, as well as skeletal muscle contractions in vitro, leads to AMPK activation. Pharmacological activation of AMPK also can be achieved using 5-aminimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR). Once taken up by the cell, AICAR is phosphorylated to 5-aminimidazole-4-carboxamide riboside monophosphate (ZMP) and mimics effects of AMP on AMPK (1, 2). AMPK function is closely related to glycogen storage. AMPK phosphorylates glycogen synthase in vitro (11) and co-immunoprecipitates with glycogen synthase and glycogen phosphorylase from skeletal muscle (12). Mutations of the γ3 or γ2 subunit, respectively, affect glycogen storage in pigs (13, 14) or glycogen storage associated with cardiac abnormalities in humans (15). The recent identification of a glycogen-binding domain in the AMPK β1 subunit provides a molecular relationship between AMPK and glycogen (16, 17). The formation of heterotrimers appears to be rather promiscuous, and the different subunits (α1, α2, β1, β2, γ1, γ2, and γ3) can form a maximum of 12 different AMPK heterotrimers. The functional diversification of the different isoforms is largely unknown.

The dominant Rendement Napole (RN) phenotype identified in Hampshire pigs is associated with a single missense mutation (R225Q) in Prkag3, encoding the muscle-specific AMPK γ3 isoform (13). RN pigs have a ~70% increase in glycogen content in glycolytic skeletal muscle, whereas liver and heart glycogen content is unchanged (18, 19). The mutation has a large impact on meat characteristics and leads to a low pH because of the anaerobic glycogen degradation occurring post-mortem. A second mutation (V224I) identified in pigs at the neighboring amino acid residue is associated with an opposite
Fig. 1. Overview of Tg-Prkag3 transgene and knock-out targeting constructs. Tg-Prkag3 transgene constructs and a knock-out targeting construct including the neomycin resistance (neo') and thymidine kinase (TK) genes are shown in a and b, respectively. The wild type locus and the targeted locus are shown with exons indicated by black boxes and numbers. BamHI and SpeI sites, primers used for long range PCR, and the location of the Cyp27 locus are indicated by arrows. UTR, untranslated region. c, Southern blot of SpeI-digested genomic DNA from wild type (+/+, heterozygous (+/-), and knock-out homozygous (-/-) mice, probed with the flanking Cyp27 probe. d, Western blot analysis of gastrocnemius muscle extracts from Prkag3++/++ and Prkag3---/- mice. The apparent double band for AMPK γ3 was typically observed after separation by SDS-PAGE and may relate to a protease-sensitive site within the protein.

Corresponding bacterially expressed His-tagged proteins (BCT) were included as a positive control.

effect, low glycogen and high pH, compared with the RN allele (14). We have found that γ3 is the predominant AMPK γ isoform in glycolytic (white, fast-twitch type II) muscle, whereas it is expressed at very low levels in oxidative (red, slow-twitch type I) muscle and is undetectable in brain, liver, or white adipose tissue (20). Furthermore, γ3 primarily forms heterotrimeric complexes with α2 and β2 isoforms in glycolytic skeletal muscle. Here we report the characterization of the metabolic consequences of genetic modification of AMPK γ3 expression in skeletal muscle.

MATERIALS AND METHODS

Animal Care—Mice were maintained in a temperature- and light-controlled environment and were cared for in accordance with regulations for the protection of laboratory animals. The regional animal ethical committee approved all experimental procedures. Animals had free access to water and standard rodent chow. In some experiments, female mice were placed on either a standard chow or a high fat diet (21) from 4 to 16 weeks of age.

Generation of Transgenic Mice—The complete coding sequence of mouse Prkag3 was amplified by reverse transcriptase-PCR using skeletal muscle mRNA (Clontech). The forward (5’-CACCATGGAGC-CGGAGCGAGACA) and reverse (5’-GTCCTAGGCGCTTAGGGCC-ATC) primers included the translation start and stop codons (in bold), respectively. The forward primer also included a Kozak element (CAC, underlined above) (22) in front of the start codon to facilitate initiation of translation. The reverse transcriptase-PCR product (~1.5 kb) was ligated into the pCRII TA TOPO cloning vector (Invitrogen). A clone encoding the wild type transcript (Fig. 1c) was used for the transgene constructs. The R225Q mutation (13) was introduced by in vitro mutagenesis (QuickChange site-directed mutagenesis kit, Stratagene) changing codon 225 from arginine to glutamine. EcoRI fragments containing the wild type or the mutant (225Q) form were ligated into the pMLC vector (23) along with flanking sequences for the myosin light chain 1 (MLC1) promoter and enhancer and the SV40 3’-untranslated region (Fig. 1a). The constructs were cut from the plasmid and microinjected into mouse oocytes (CBA/CgJ and C57Bl/6J).

The founder mice were tested for transgenesis by PCR analysis using a forward primer in Prkag3 exon 12 (5’-GCTGCCCAGCAGAAACCTCAG-AAC) and a reverse primer in SV40 3’-untranslated region. This ampliﬁcates the SV40 3’-untranslated region intron (66 bp) and thereby allows for conﬁrmation of the transgene both in genomic DNA (ampliﬁcates size = 453 bp) and in cDNA (ampliﬁcates size = 387 bp). Genomic DNA prepared from mouse tails was used for PCR.

Generation of Knock-out Mice—Prkag3 knock-out mice were generated through traditional gene-targeting techniques. Briefly, exons 1–4 and exons 11–13 of Prkag3 were cloned into the pKO923 selection plasmid (Stratagene) with a neomycin resistance (neo') gene inserted. The predicted result was a Prkag3 transcript with exons 1–4 joined with exons 11–13, including a frameshift after residue 211 and a premature stop codon at residue 235, skipping most of the 489 amino acids encoded by the wild type transcript (Fig. 1b). The construct was linearized using NotI and used for electroporation of embryonic stem cells. Knock-out recombinant embryonic stem cells were injected into blastocysts. Screening for knock-out recombinant embryonic stem cells was performed using Southern analysis, with SpeI digestions and a 1034-bp cDNA probe, representing the mitochondrial vitamin D (3) 25-hydroxylase (Cyp27) gene (Fig. 1b). The probe was amplified by reverse transcriptase-PCR from mouse skeletal muscle mRNA. Long range PCR (MasterAmp high fidelity long PCR kit, Epicenter Technologies) was used to screen for knock-out recombinant embryonic stem cells and identify of heterozygous (Prkag3+/-) and homozygous (Prkag3---/-) carriers of the knock-out recombinant allele, respectively. Founder mice were back-crossed to C57Bl/6J mice for three generations. In all experiments, knock-out homozygote mice were compared with homozygous wild type littermates.

Relative Quantification of mRNA—Quantification of mRNA representing different isoforms of AMPK γ subunits from adult mouse tissues was performed using reverse transcription and real time PCR. Relative quantities of mRNA were calculated for duplicate tissue samples from 1–2 mice and normalized for Actb (β-actin).

Cell Culture and Transfections—Briefly, cDNA encoding the γ3 subunit of AMPK was inserted into cloning vector pDONR201 included in the Gateway cloning system (Invitrogen) per the manufacturer’s instructions. Site-directed mutagenesis was used to create γ3 V224I and γ3 R225Q cDNA constructs, which were cloned into Gateway cloning system pDEST26 (Invitrogen) for subsequent expression in mammalian cell culture.

Cultured COS7 cells were transiently transfected with cDNA encod-
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Table I: Relative mRNA expression levels from endogenous Prkag1 and Prkag3 and transgenic Prkag3 in mice as measured by real time PCR

| Tissue          | Mouse line      | Prkag1 endogenous | Prkag3 endogenous | Prkag3 transgenic |
|-----------------|-----------------|-------------------|-------------------|-------------------|
| Liver           | WT              | 2.4 ± 0.9         | 0.0               | 0.0               |
|                 | AMPK γ3wt       | 1.7               | 0.0               | 0.0               |
|                 | AMPK γ3225Q     | 2.7 ± 1.3         | 0.0               | 0.0               |
| Heart           | WT              | 22.9 ± 9.3        | 1.2 ± 0.4         | 0.0               |
|                 | AMPK γ3wt       | 12.5              | 0.1               | 0.0               |
|                 | AMPK γ3225Q     | 12.9 ± 10.9       | 0.3 ± 0.1         | 0.0               |
| Extensor digitorum longus<sup>a</sup> | WT | 19.9 ± 14.9       | 24.2 ± 3.0        | 0.0               |
|                 | AMPK γ3wt       | 23.3 ± 3.5        | 18.3 ± 4.2        | 1646 ± 492       |
|                 | AMPK γ3225Q     | 18.7              | 14.1              | 73.2              |
| Gastrocnemius<sup>b</sup> | WT | 30.9 ± 0.8        | 100 ± 25.3        | 0.0               |
|                 | AMPK γ3wt       | 26.3 ± 8.5        | 62.1 ± 13.2       | 659 ± 222         |
|                 | AMPK γ3225Q     | 19.4 ± 8.0        | 11.1 ± 2.4        | 99.5 ± 28.3       |
|                 | AMPK γ3<sup>−/−</sup> | 9.5              | 3.8               |                   |
| Quadiceps<sup>b</sup> | WT | 45.7 ± 3.1        | 41.3 ± 3.2        | 167.2             |
|                 | AMPK γ3wt       | 27.5              | 26.8              |                   |
|                 | AMPK γ3225Q     | 35.0              | 12.9              |                   |
| Soleus<sup>c</sup> | WT | 33.4 ± 8.7        | 3.0 ± 0.8         | 182.8             |
|                 | AMPK γ3wt       | 30.8 ± 0.6        | 2.6 ± 0.8         | 11.1 ± 6.9        |
|                 | AMPK γ3225Q     | 28.7 ± 5.2        | 2.7 ± 0.5         | 6.5 ± 4.5         |
| Diaphragm<sup>c</sup> | WT | 7.0 ± 4.0         | 1.3 ± 0.6         | 5.0               |
|                 | AMPK γ3wt       | 12.5              |                   |                   |
|                 | AMPK γ3225Q     | 10.3 ± 8.0        | 1.3 ± 0.8         | 0.9 ± 0.9         |
| Brain           | WT              | 1.5 ± 0.0         | 0.0               |                   |
|                 | AMPK γ3wt       | 2.2 ± 0.3         | 0.0               |                   |
|                 | AMPK γ3<sup>−/−</sup> | 1.7              | 0.0               |                   |
| White adipose tissue | WT | 1.3 ± 0.4         | 0.0               |                   |
|                 | AMPK γ3wt       | 0.9               | 0.0               |                   |
|                 | AMPK γ3225Q     | 1.2               | 0.0               |                   |

<sup>a</sup> WT, wild type.
<sup>b</sup> Muscle containing primarily glycolytic muscle fibers.
<sup>c</sup> Muscle containing primarily oxidative muscle fibers.

Tissue were lysed, insoluble material was removed, and lysates were incubated with a triglycerides/glycerol blanked kit (Roche Applied Science) to determine cholesterol levels. Glycogen and triglycerides were calculated as pmol of phosphate incorporated into the ADR1 peptide/min in the presence of equal amounts of the heterotrimer.

Western Blot Analysis—Quantitative analysis of the expression of different AMPK subunits was performed as described previously (20). Skeletal muscle protein lysate was separated by SDS-PAGE, transferred to Immobilon-P membranes (Millipore), and probed with primary AMPK isoform- or phospho-specific antibodies and secondary horseradish peroxidase-conjugated antibodies.

Glycogen and Triglyceride Analyses—Mice were studied under fed or fasted conditions or after swim exercise, as described previously (25). Fasted mice swam for four 30-min intervals separated by 5-min rest periods. After the last swim interval, mice were studied immediately or dried and returned to cages for 2.5 h (recovery). At the onset of the recovery period, mice received an intraperitoneal glucose injection (0.5 mg/g body mass) and were subsequently given free access to chow and water. Gastrocnemius muscles were removed from anesthetized mice (Avertin; 2,2,2-tribromoethanol 99% and tertiary amyl alcohol, 15 μl/g of body mass), cleaned of fat and blood, and quickly frozen in liquid nitrogen. Glycogen content was determined fluorometrically on HCl extracts as described previously (26). Triglyceride content was determined with a triglyceride/glycerol blanked kit (Roche Applied Science) using Seronorm™ lipid (SERO) as a standard.

Glucose Tolerance Test—Glucose (2 g/kg of body mass) was administered to fasted mice by intraperitoneal injection. Blood samples were obtained via the tail vein prior to and 15, 30, 60, and 120 min following glucose injection for measurement of glucose concentration (One Touch Basic glucose meter; Lifescan).

Skeletal Muscle Incubation Procedure—Incubation medium was prepared from a stock solution of Krebs-Henseleit bicarbonate buffer (KHB) supplemented with 5 mmol/liter HEPES and 0.1% bovine serum albumin (RIA grade) and continuously gassed with 95% O₂, 5% CO₂. Mice were anesthetized, and extensor digitorum longus (EDL) muscles were isolated and preincubated at 30 °C for 30 min in KHB containing 5 mmol/liter glucose. Thereafter, muscles were incubated in 1 ml of identical medium containing 0.3 mmol/liter [1-14C]oleate (0.4 Ci/ml) for 60 min. The medium was acidified by 0.5 ml of 15% HCl, and its radioactivity was assayed by liquid scintillation analyzer. AMPK activities were calculated as pmol of phosphate incorporated into the ADR1 peptide/min in the presence of equal amounts of the heterotrimer.

Statistical Analyses—Differences between two groups were determined by an analysis of variance with multiple comparisons. Differences between more than two groups were determined by one-way analysis of variation followed by Fisher’s least significant difference post hoc analysis. Significance was accepted at p < 0.05.

RESULTS AND DISCUSSION

Three novel mouse models were used to genetically dissect the functional role of the AMPK γ3 isoform in skeletal muscle. AMPK γ3-transgenic (Tg-Prkag3<sup>3wt</sup> and Tg-Prkag3<sup>225Q</sup>) and Prkag3<sup>−/−</sup> mice had normal growth rates (data not shown). The expression of Prkag3 wild type (Tg-Prkag3<sup>3wt</sup>) or an R225Q mutant form (Tg-Prkag3<sup>225Q</sup>) transgene was restricted to skeletal muscles containing a high proportion of glycolytic fibers.
consistent with the expression profile of the endogenous Prkag3 transcript (Table I). We found a marked overexpression of the wild type transgene in EDL, gastrocnemius, and quadriceps muscle (−16.5-, 6.6-, and 1.7-fold, respectively), but no or only a moderate overexpression of the mutant transgene (−0.7-, 1.0-, and 1.8-fold, respectively). Positional effects or number of integrated copies most likely explain the difference in expression level between the two transgenic models. Levels of endogenous Prkag3 transcript in Tg-Prkag3 mice tended to be decreased.

The amount of expressed γ3 protein, as well as a, β, or the other γ subunits, was unchanged in Tg-Prkag3 mice (Fig. 2). Thus, AMPK expression in Tg-Prkag3 mice resembles the expression pattern in wild type mice, both in regard to tissue distribution and protein expression. The mutant form (225Q) presumably replaced endogenous γ3, based on the relative mRNA expression. However, endogenous and exogenous forms, as assessed by Western blot, were indistinguishable because they differed by a single amino acid substitution. An absolute quantification of the relative expression of the mutant and wild type protein is not crucial for the interpretation of our results because the R225Q mutation is fully dominant. Moreover, there is no significant difference in glycogen content between pigs expressing 50 or 100% of the mutant form. Overexpression of the wild type transgene led to an increase in the amount of γ3 protein and a concomitant increase in α1, α2, and β2 subunits (Fig. 2). Thus, the total amount of AMPK heterotrimers in glycolytic muscles was increased in Tg-Prkag3 mice.

Southern and Western blot analysis confirmed the successful disruption of Prkag3 and concomitant complete absence of γ3 expression in skeletal muscle in Prkag3 knock-out (Prkag3−/−) mice (Fig. 1, c and d). The homozygous knock-out animals were fully viable, and a standard pathological examination revealed no obvious phenotypic consequences of the γ3 disruption. Real-time PCR analysis of mRNA from skeletal muscle unexpectedly revealed a low abundance of Prkag3 transcripts, as the PCR primers were designed against a part of the 3'-region that was not deleted by the gene-targeting event (Fig. 1b and Table I). The low abundance of this aberrant transcript likely reflects degradation by the nonsense-mediated mRNA decay pathway (29). Western blot analysis did not reveal any compensatory increase in γ3 expression in γ1 or γ2 isoform expression in skeletal muscle (Fig. 2), indicating that these isoforms do not compete with γ3 for the same pool of α-β chains or do not form AMPK heterotrimers in the same cell or cellular compartment.

Glycogen content in the glycolytic portion of the gastrocnemius muscle was 2-fold higher in Tg-Prkag3 mice compared with wild type mice under both fed and fasted conditions, whereas glycogen content was unaltered in Tg-Prkag3 mice (Fig. 3). The results provide definitive evidence that R225Q is the causative mutation for the RN phenotype in pigs (13) because the phenotype is replicated in mice by introducing this single missense mutation. Furthermore, this mutation alters the biochemical regulation of AMPK, as the increase in AMPK expression in the Tg-Prkag3 mice failed to cause a glycogen phenotype.

Glycogen content after swimming exercise was similar between wild type and all genetically modified mice (Fig. 3a). Thus, the R225Q mutation does not impair glycogen utilization during exercise. Because postexercise glycogen levels were appropriately depleted in Prkag3−/− mice, our data also suggests that AMPK γ3 is not required for glycogen degradation. Glycogen content 2.5 h after exercise was significantly higher in Tg-Prkag3 mice compared with wild type mice (Fig. 3a). A
similar tendency (N. S) for increased glycerogen content after exercise was also noted in Tg-Prkag3<sup>wt</sup> mice. In contrast, glycerogen content was significantly lower in Prkag3<sup>−/−</sup> versus wild type mice 2.5 h after exercise, demonstrating that γ3 is important for glycerogen resynthesis.

Fasted insulin and glucose levels (data not shown) and glucose tolerance (Fig. 3b) were normal in transgenic and Prkag3<sup>−/−</sup> mice. Thus, despite a very distinct phenotype for skeletal muscle glycerogen content, blood glucose homeostasis was normal in Tg-Prkag3<sup>225Q</sup> mice, consistent with the phenotype noted in pigs carrying the R225Q mutation.<sup>3</sup>

AMPK phosphorylation under basal conditions or after activation with AICAR or contraction was similar between genotypes (Fig. 3c). Phosphorylation of the AMPK downstream target ACC was elevated under basal conditions and after AICAR stimulation in Tg-Prkag3<sup>−/−</sup> mice (Fig. 3d). This was unexpected because our transfection experiments (see below) revealed that the R225Q mutant γ3 isoform is AMP-independent and thus would be predicted to be AICAR-insensitive. However, the elevated ACC phosphorylation may be an indirect effect of the R225Q mutant γ3 isoform caused by an altered metabolic state of the cell. In fact, the γ3 isoform may not mediate ACC phosphorylation, consistent with unaltered ACC phosphorylation in Prkag3<sup>−/−</sup> mice (Fig. 3d).

Glucose transport in isolated EDL muscle from fasted mice was determined in response to insulin, in response to AICAR, or after electrically stimulated contractions (Fig. 3e). Basal glucose transport was similar between genotypes. Insulin-stimulated glucose transport was normal in Tg-Prkag3<sup>wt</sup> and Prkag3<sup>−/−</sup> mice but was significantly reduced in Tg-Prkag3<sup>225Q</sup> mice. The reduction in insulin-stimulated glucose transport was not observed in fed mice (data not shown). AICAR-induced glucose transport was normal in Tg-Prkag3<sup>wt</sup>, but significantly reduced (−50%) in Tg-Prkag3<sup>225Q</sup>, compared with wild type fasted (Fig. 3e) and fed (data not shown) mice. Thus, the mutation in γ3 may occur at a site that is directly involved with the interaction with both AMP and AICAR, rendering a mutant form that is partially resistant to AICAR. This interpretation is supported by evidence that this region of the γ subunit directly binds AMP and that the presence of this mutation at the corresponding site in the γ1 (R70Q) or γ2 (R302Q) subunit impairs AMP and ATP binding (30, 31). However, we cannot exclude a partial inhibition because of excessive glycerogen content (32, 33). Interestingly, the AICAR effect on glucose uptake in EDL muscle was completely abolished in Prkag3<sup>−/−</sup> mice (Fig. 3e). Thus, AMPK complexes containing the γ3 subunit are required for AICAR-induced glucose transport in skeletal muscle, and other γ isoforms fail to compensate for the loss of γ3 function. This result is consistent with the reduced glycerogen resynthesis in vivo after exercise in Prkag3<sup>−/−</sup> mice (Fig. 3a).

In contrast to the results for AICAR, in vitro contraction of isolated EDL muscle led to a similar increase in glucose uptake in all genotypes in fasted (Fig. 3e) or fed mice (data not shown). Similarly, AICAR- but not contraction-induced glucose uptake was abolished in AMPK α2, but not in α1 knock-out mice (34). Because the γ3 subunit primarily forms heterotrimers with α2 (20), disruption of either α2 or γ3 should confer a similar

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<sup>3</sup> B. Essen-Gustavsson, M. Jensen-Waern, R. Jonasson, and L. Andersson, unpublished observation.
glucose transport defect in skeletal muscle. AICAR-induced glucose uptake was also abolished in kinase-dead AMPK α2-transgenic mice (35). In contrast to the α2 AMPK knock-out and the Prkag3−/− mice, contraction-mediated glucose transport was significantly blunted (30%) in kinase-dead AMPK α2-transgenic mice, possibly because of contraction-induced hypoxia, as these mice have an impaired hypoxia response. Collectively, these results challenge the hypothesis that contraction increases glucose transport through an AMPK-mediated mechanism. In contrast, activation of AMPK is directly linked to AICAR-stimulated glucose transport. Although AICAR and contraction both increase AMPK activity, the AMPK response to in vitro contraction may be inconsequential for activation of glucose transport. Although γ3-containing AMPK complexes are required for AICAR-mediated fatty acid oxidation in chow-fed mice. AICAR-mediated fatty acid oxidation was similar between genotypes (Fig. 3f), consistent with the observed normal level of ACC phosphorylation in the Prkag3−/− mice (Fig. 3d).

AMPK has been identified as a molecular target for pharmacological intervention to treat insulin resistance and type II diabetes mellitus. However, genetic validation of this target is lacking. We challenged wild type, Tg-Prkag3R225Q, and Prkag3−/− mice with a high fat diet for 12 weeks and evaluated metabolic responses. Muscle glycogen content was unaffected by the high fat diet (data not shown). However, triglyceride content was increased (Fig. 4b), and insulin action on glucose transport was impaired in wild type and Prkag3−/− mice (Fig. 4c) in skeletal muscle. This phenotype closely resembles the original phenotype described for mutant pigs (13). The high frequency of the R225Q mutation (PRKAG3R225Q) in Hampshire pigs was likely caused by the strong selection for lean meat content in commercial pig populations, as pigs carrying this mutation are leaner (more muscle, less fat) than wild type pigs. Mutant pigs also have a higher oxidative capacity, as measured by an increase in activity of citrate synthase and β-hydroxyacyl-coenzyme A dehydrogenase (36, 37). The relationship between the R225Q mutation and increased oxidative capacity in both mutant pigs and transgenic mice prompted us to investigate whether this was associated with an
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altered muscle fiber type composition. Real time PCR analysis of Atp1b1 and Atp2b2 (20), markers of glycolytic and oxidative skeletal muscle fibers (38), revealed a similar ratio of Atp1b1 and Atp2b2 expression in gastrocnemius and EDL muscle from Tg-Prkag3R225Q and Prkag3−/− mice (data not shown). Furthermore, myoglobin protein expression in Tg-Prkag3R225Q and Prkag3−/− did not differ from wild type mice (data not shown). Thus, R225Q alters skeletal muscle oxidative capacity without altering fiber type composition.

AMPK activity and phosphorylation of Thr172 were determined in CO2 cells transfected with AMPK trimers containing α2, β2, and either wild type γ3 or mutant γ3 (R225Q or V224I) (20, H9252). AMPK activity and phosphorylation on Thr172 in the absence of AMP were elevated in cells transfected with α2-β2-γ3 V224I and unchanged in cells transfected with α2-β2-γ3 V224Q. Both results resulted in diminished AMP dependence on AMPK (Fig. 5). The ranking of basal AMPK activity in the three genotypes is consistent with the in vivo effects of the corresponding pig mutations, as the R225Q and V224I mutants are responding pig mutations, as the R225Q and V224I mutants are AMPK 3 isoform must be interpreted in light of its very specific tissue distribution, in contrast to the broad tissue distribution of other AMPK subunits (20). Ablation of AMPK γ3 leads to impaired AICAR-induced glucose uptake and to reduced glycogen resynthesis after exercise. The R225Q mutation may not directly alter the accumulation of glycogen in skeletal muscle, as glucose uptake and tolerance are unaltered in AMPK γ3R225Q mice. Rather the R225Q mutation may indirectly alter glycogen storage by altering skeletal muscle oxidation. Evidence for a role of the R225Q mutation in increasing fatty acid oxidation is revealed when AMPK γ3R225Q mice are challenged with a fat-rich diet. This is paradoxical because expression of the endogenous γ3 isoform is restricted to glycolytic muscles. However, a major role of the AMPK γ3 isoform may be to ensure that glycogen content in glycolytic skeletal muscle is restored, maintaining a high glycolytic potential through shifting the metabolic fate of fuel toward fat oxidation and glycogen storage.

We provide a biological validation of the muscle-specific γ3 isoform as a putative drug target for the prevention of triglyceride accumulation and the development of insulin resistance in skeletal muscle. Targeting the AMPK γ3 isoform offers an entry point for tissue-specific regulation of glucose and lipid metabolism in skeletal muscle. A compound mimicking the effect of the R225Q mutation may be efficacious in the treatment of type II diabetes mellitus.

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