Expression profiling of the γ-subunit isoforms of AMP-activated protein kinase suggests a major role for γ3 in white skeletal muscle

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AMP-activated protein kinase (AMPK) is a fuel-sensing enzyme that plays a key role in regulation of carbohydrate and fat metabolism in response to cellular stress (10, 21). When activated by energy depletion, AMPK switches off ATP-consuming processes while switching on pathways that generate ATP. AMPK acts via phosphorylation of downstream metabolic enzymes as well as via effects on gene expression. AMPK is expressed in most mammalian tissues, but its actions have most widely been studied in skeletal muscle. The alternation in energy charge during exercise activates AMPK, which is associated with enhanced glucose uptake and fatty acid oxidation. Together, these mechanisms provide the working muscle with additional substrates for ATP-generating processes.

AMPK is a heterotrimeric complex comprising a catalytic α-subunit and two regulatory components (β and γ), all of which are required for full activity. The understanding of the AMPK system is complicated because of the existence of two α- and two β-isofoms (α1, α2, β1, β2) and three γ-isofoms (γ1, γ2, γ3), all encoded by different genes. Thus 12 different heterotrimeric combinations of AMPK may potentially form, and the physiological function of the AMPK holoenzyme depends on the particular isoforms present in the complex (7, 19). A clear tissue distribution profile and the relative expression of different isoforms can shed light on AMPK function in the specific metabolic pathways studied. Previously reported expression differences between subunit isoforms include tissue distribution and cellular location (6, 7, 15, 16, 18, 19). Northern blot analysis in human tissues has provided evidence that the mRNA expression of the γ3-isoform is restricted to skeletal muscle, whereas γ1 and γ2 show broad tissue distributions (7, 15). Furthermore, immunoprecipitation studies of AMPK with subsequent kinase assays have indicated that γ1 is the predominant isoform contributing to kinase activity in skeletal muscle (7, 9). This observation is surprising considering the high γ3 mRNA expression specifically directed to skeletal muscle and the critical role of γ3 in pig skeletal muscle, as indicated by the identification of a mutation causing a 70% increase of muscle glycogen (15). Of note is the finding that this mutation leads to an increase in glycogen content and influences enzyme activities in skeletal muscles with a large proportion of white muscle fibers (2, 14). This implies that, even within divergent skeletal muscle types, the γ-isofoms affects metabolic pathways differently. Additional data on tissue distribution and relative expression of different γ-isofoms can elucidate their specific contribution to AMPK function.

In this study, we have determined the expression patterns of the genes encoding AMPKγ isoforms in various human, mouse, and rat tissues, including skeletal muscles of different fiber type composition. Additionally, we have analyzed human primary muscle cells (HPMC) as a model system for studies of AMPK function. HPMC, originating from satellite cells, can be induced to differentiate in culture to form multinucleated myotubes (1), and they have been used as a model system for studies of insulin action and glucose metabolism. We report

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real-time PCR-based analyses of the distribution and relative amounts of mRNA representing the three AMPK isoforms as well as data on the tissue distribution of each isoform at the protein level. Finally, we show the α- and β-subunit composition in γ3-containing AMPK complexes in mouse skeletal muscle.

**MATERIALS AND METHODS**

**Cell culture, tissues, and RNA isolation.** HPMC originate from satellite cells, a population of stem cells responsible for postnatal growth, repair, and maintenance of skeletal muscle (17). The HPMC used here were cultured from satellite cells isolated from biopsies of rectus abdominis muscle from three healthy subjects. The ethics committee at Karolinska Institutet approved the protocols, and informed consent was obtained from the subjects. Biopsies were obtained during scheduled abdominal surgery. Isolation of satellite cells and initiation of differentiation program was performed as previously described (1). Cultures were harvested on day 0 (corresponding to mononucleated myoblasts) or day 14 (corresponding to multinucleated myotubes) after initiation of differentiation, and preparation of RNA and protein was performed as described previously (1).

Fed C57Bl/6 mice (one male and one female for each tissue, 8–12 wk of age) and Wistar rats (2–4 males per tissue, 8 wk of age) were anesthetized with an intraperitoneal injection of either 2.5% avertin (0.02 ml/g body wt) for mice or pentobarbital sodium (5 mg/100 g body wt) for rats. Tissue samples, including liver, heart, extensor digitorum longus (EDL), gastrocnemius, quadriceps, soleus, and diaphragm muscles, brain, white adipose tissue (WAT), brown adipose tissue (BAT), lung, spleen, kidney, and testis were collected. Thus a variety of rodent hindlimb skeletal muscles were obtained that contained predominantly red, slow-twitch, type I fibers (e.g., the soleus) or predominantly fast-twitch fibers (e.g., the EDL, gastrocnemius, and quadriceps with fiber types Iba, Iib (2, 3)). In the case of the gastrocnemius and quadriceps muscles in rats, the red (predominantly fast-twitch, oxidative-glycolytic type Iba fibers) and white (predominantly fast-twitch, glycolytic type Iib fibers) parts of the muscle could be visually recognized and were analyzed separately. The Ethics Committee on Animal Research in Northern Stockholm approved all experimental protocols. Poly(A+) RNA was isolated from mouse and rat samples using the Quickprep Micro mRNA purification Kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK).

**Relative quantification of mRNA using real-time PCR.** Quantification of AMPK mRNA from adult mouse, rat and human tissues, as well as from HPMC cultures, was performed using real-time PCR with the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Foster City, CA). In each Taqman PCR reaction, 17.5 pmol of each PCR primer, a 6.25 pmol probe and 10 μl TaqMan PCR mix were used. Primer pairs were designed to distinguish between alternative splice variants of the AMPKα1, AMPKα2, and AMPKα3 genes (encoding AMPKα1; GenBank NM_002733) TGGAGCAGAGGT-TCACCGA (F) and TCTCACCTTGACACAGC (R); for PRKAG2 (encoding AMPKβ2; GenBank AJ249976) CCAGATG-CAAGCCTCTTCCA (F) and GTGGATCCCCACGTGAGT (R); for PRKAG3 (encoding AMPKγ3; GenBank NM_017431) CGCTTTTT-GTGGATCTCCATG (F) and GGCCTTTCACATCTAC (R).

**Real-time RT-PCR on human and rat samples.** Reverse transcription on mouse and rat mRNA samples was performed using the First-Strand cDNA Synthesis Kit (Amersham Biosciences) with random hexamer priming in 15-μl reactions, as recommended by the manufacturer. The cDNA was then purified using the QIAquick PCR purification Kit (Qiagen, Hilden, Germany) and used in 25-μl Taq Man PCR reactions with 17.5 pmol of each PCR primer, a 6.25 pmol probe, and 1× TaqMan Universal PCR Master Mix (PE Applied Biosystems, Foster City, CA). In each Taqman amplicon, either the probe or the 3′ end of one of the primers included sequences from adjacent exons. The primers and probes were as follows, 5′-3′: mouse Prkag1 (GenBank AH010706) CAAGTTCCAAGTTGGTG-GTCTTTG (F), CGAACACCTATGCTGAC (R) and TET-CACT-TCGCTACATGTTACAG (P); rat Prkag1 (GenBank U42413) GCTCAAGCTGATGTTTTTG (F), GGCAGACGAAACGGTTA (R), TET-TACTTGCGAGG- TAAAAAGGCTCTTCTTCCA (P); mouse/rat Prkag2 (transcript corresponding to human PRKAG2; GenBank NM_145401 and Ensembl Transcript ID: ENSRNOT00000012111) AAGGCAGCGTTCACTGGCA (F), CTCCATACCTCCTCATACAGG (R) and TET-CACTTCCGGACCTGTTTTGTCA (P); mouse/rat Prkag2-b transcript (corresponding to the human PRKAG2-b (GenBank BQ572978 and AY384865) GCGGGC-CCTGCTGAT (F), AAACGCACCTTGTGTCTCCA (R) used on mouse samples), AAACGGCACTTCTGAGTCCTTT (R) used on rat samples), TET-CCTGCTGAACTCCAGACCCTGAGCTTCTTT (P); mouse/rat Prkag2-b transcript (corresponding to the human PRKAG2-b (GenBank BQ572978 and AY384865) GCGGGC-CCTGCTGAT (F), AAACGCACCTTGTGTCTCCA (R) used on mouse samples), AAACGGCACTTCTGAGTCCTTT (R) used on rat samples), TET-CCTGCTGAACTCCAGACCCTGAGCTTCTTT (P); mouse/rat Prkag3 (GenBank AF525500 and XM_237293) AGAGCCTAGGTAAGCTGTGAC (F), AGATGGC-TGGTGTTGAG (R) and TET-CCTGCTGATCAG (P).

**Quantitative analysis of protein expression.** Approximately 20 μg of tissue from male mice/rats were ground to powder under liquid nitrogen and homogenized in an ice-cold lysis buffer (50 mM HEPES, 1% Triton X-100, 1 mM DTT, 10% glycerol, 1 mM EDTA, 5 mM NaPP, 50 mM NaF, and proteolytic enzyme inhibitors) by use of a motor-driven pestle in a 1.5-ml microcentrifuge tube. The homogenate was kept on ice for 30 min, and insoluble material was removed by centrifugation (9,000 rpm, 10 min, 4°C). Supernatants were removed, and total protein content was measured by the bicinchoninic acid method (Pierce Chemical, Rockford, IL). The samples were run on 4–12% precast polyacrylamide gels (Invitrogen) with 40 μg protein lysate/lane and transferred to Immobilon-P membranes (Millipore, Bedford, MA) by semidyry transfer (20 V, 45 min). Membranes were blocked with 5% nonfat milk (TBST) in 1× Tris-buffered saline (TBS) (150 mM NaCl, 20 mM Tris, pH 7.6) containing 5% low-fat milk powder for 1 h at room temperature and probed with primary antibodies for 1–18 h in TBS containing 2.5% low-fat milk powder. Antibodies against the
γ1-subunit were raised using the synthetic peptide CALENEHFQETEPSEN (residues 12–25 of γ1), against the γ2-subunit using the CKRRSLVHIPDLSS peptide (residues 29–42 of γ2) or CLTPAGAKQKETE peptide (residues 556–569 of γ2), and against γ3 using the MSVGAEALRQTLCEGL peptide (residues 415–431 of γ3). The antibodies were affinity-purified using the SulfoLink Kit (Pierce Chemical, Rockford, IL) and following the manufacturer’s instructions. After three washing steps with TBS-Tween (0.5% Tween 20 in TBS) for 10 min each, the membrane was incubated for 1 h with horseradish peroxidase-conjugated secondary antibody in TBS containing 2.5% nonfat dry milk. After further extensive washing in TBS-Tween, the blots were developed using enhanced chemiluminescence (Amersham Biosciences) and captured on X-ray film. Densitometry-derived values reflecting band intensities were used for quantification by Image Gauge V3.01 software (Fujifilm, Tokyo, Japan). Quantification was based on Western blot analysis of three different individuals, and the expression level of corresponding protein in EDL muscle was taken as 100%. As size markers in the Western analysis, bacterially expressed AMPK γ-subunit proteins were included in each SDS-PAGE. Briefly, synthetic oligos designed for amplification of the full-length open-reading frames of the different γ-subunits (GenBank nos. AF036535, AJ249976, and AJ249977) were used in RT-PCR with first cDNA strands synthesized with mouse or human skeletal muscle RNA as template. PCR fragments were cloned into the pQE-32 vector (Qiagen) in frame after an NH2-terminal His6 tag. Fusion protein from isopropyl-β-D-thiogalactopyranoside-induced TOP10F bacteria (Invitrogen) was purified by a nitriilotriacetic acid chromatography matrix (Qiagen).

Immunoprecipitation of γ3-containing complexes. For immunoprecipitation studies, 50-mg samples from mouse gastrocnemius or quadriceps muscles were homogenized, and lysate was prepared as described above. AMPKγ3-containing complexes were immunoprecipitated from equal amounts of muscle lysate by incubation with an anti-γ3 antibody prebound to protein A-Sepharose for 2 h on a roller mixer at 4°C. The immune complexes were washed four times with an ice-cold lysis buffer containing 0.5 mM NaCl and resolved using SDS-PAGE, followed by Western blotting with affinity-purified rabbit antibodies as described above. Antibodies against the α1-subunit were raised with the synthetic peptide CRHTLDLNPQKSKH (residues 377–389 of α1); against α2 with the CDDSMAMHPGKLKP peptide (residues 353–366 of α2), against β1 with the CKAPEKEF-LAWQHD peptide (residues 53–66 of β1) and against β2 with the CSVFSLPDLPSKPGDK peptide (residues 44–57 of β2). Protein A conjugated to horseradish peroxidase (Amersham Biosciences) was used for detection.

RESULTS

mRNA expression. The real-time PCR measurements of the relative expression levels of mRNA representing different AMPKγ isoforms in human, mouse, and rat tissues are presented in Fig. 1. The expression of the γ3-isofrom appeared highly specific for skeletal muscle in all species examined. Conversely, expression of γ1 and γ2 showed a broad tissue distribution. ANOVA was performed with the mouse and rat data but did not show any significant effect of sex or covariation between the mRNA levels of the three isoforms (data not shown).

The mRNA expression of AMPKγ isoforms in the HPMC established from rectus abdominus muscle showed severalfold higher mRNA expression of γ2 than of γ1 and γ3 (Figs. 1A and 2). Moreover, differentiation of HPMC from myoblasts into myonucleated myotubes was accompanied by an approximately fivefold increase in mRNA levels of γ3, with only minor changes in γ1 and γ2 (Fig. 2).

In mice, an 8- to 33-fold higher γ3 mRNA expression was observed in skeletal muscles containing mostly fast-twitch (type II) fibers (EDL, gastrocnemius and quadriceps) compared with the soleus muscle, which is composed mostly of slow (type I) fibers (Fig. 1B). Furthermore, when red (type IIa) and white (type IIb) portions of rat gastrocnemius and quadriceps muscles were analyzed separately, γ3 mRNA expression was found to be three- to ninefold higher in the white vs. red portions of the muscle (Fig. 1C). In these white muscle por-
tions, the γ3/γ1 and γ3/γ2 mRNA expression ratios were in the range 9-13 and 27-44, respectively. The proportion of fast-twitch glycolytic (type IIb) fibers in the mouse and rat muscle samples, as indicated by real-time PCR quantification of *Atp1b2* mRNA, showed a highly significant correlation with the expression of γ3 mRNA (Pearson coefficient = 0.84, *P* < 0.001; Fig. 1, B and C). The γ1 and γ2 expression levels did not vary between red and white portions of rat skeletal muscle. However, in mice and rats, we observed slightly lower γ2 expression in the soleus muscle, with a greater proportion of type I fibers compared with skeletal muscle composed predominantly of type II fibers.

Expression of γ2 mRNA showed a broad tissue distribution in all of the species tested. In mice and rats, we detected the highest levels of γ2 mRNA in testis and BAT, whereas we found the highest human γ2 mRNA expression in heart (Fig. 1). In mice and rats, real-time PCR analysis was also used to quantify the relative proportions of the two different γ2 transcripts previously observed in humans [PRKAG2-a and PRKAG2-b (7, 13)], which showed that Prkag2-a is the highly predominant transcript in most tissues (Table 1). In fact, the highest Prkag2-b proportions of the total Prkag2 were found in skeletal muscle ( ≈48% in mice) and brain ( ≈16%).

**Protein expression.** To complement the data on the relative abundance of γ mRNA with data at the protein level, we performed Western blot analysis on mouse and rat tissues. This showed a positive correlation between mRNA and protein expression in different skeletal muscles and confirmed that γ3 is expressed primarily in white skeletal muscle (Fig. 3, A and B). For example, the levels of γ3 mRNA and γ3 protein were both highest in white parts of gastrocnemius and quadriceps muscles in rats (Figs. 1C and 3B). By using an anti-γ2 antibody, which detects both the longer (γ2a) and the shorter (γ2b) variants of the protein, we also confirmed highly predominant expression of γ2 protein encoded by the Prkag2-a transcript in relation to the Prkag2-b (Fig. 3C).

Some discrepancies between our mRNA and protein expression data could be noted. For example, although very low γ1 mRNA expression was observed in mouse brain and WAT, the amount of the γ1 protein in these tissues reached similar levels to what was observed for heart and skeletal muscle. Likewise, the relative abundance of γ2 protein in mouse heart appears to be lower than one would predict on the basis of our mRNA expression analysis (Figs. 1B and 3A). This may reflect a true biological difference; however, we cannot exclude the possibility that these differences were caused by minor variations between samples used for mRNA and protein isolation.

To define the subunit composition of γ3-containing complexes in the skeletal muscle, we immunoprecipitated extracts from mouse gastrocnemius and quadriceps muscle with γ3-specific antibody and performed immunoblot analysis to determine α- and β-subunit isoforms (Fig. 3D). In both of these muscles types, the γ3-subunit was detected only in association with the AMPK α2- and β2-isofoms.

**DISCUSSION**

In this study, we demonstrate that expression of the AMPKγ3 isoform shows a high degree of specificity for white skeletal muscle and clearly appears to be the most highly expressed γ isoform in this muscle type. The expression profile described fits well with the dramatic effect on glycogen content in pig skeletal muscle caused by a mutation in the PRKAG3 gene encoding γ3 (15). The increased glycogen levels found are particularly pronounced in white skeletal muscles, which is in good correlation with our data (14). The results are also consistent with previously published Northern blot results in humans, showing that γ3 is specifically expressed in skeletal muscle, whereas γ1 and γ2 are more widely distributed (7, 15).

**Table 1. Relative expression of the Prkag2-a and Prkag2-b transcripts* in mouse and rat tissues as measured by real-time PCR**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Prkag2-a/Prkag2-b mRNA Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Rat</td>
</tr>
<tr>
<td>Liver</td>
<td>657.9</td>
</tr>
<tr>
<td>Heart</td>
<td>25.2</td>
</tr>
<tr>
<td>EDL</td>
<td>1.2</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>1.1</td>
</tr>
<tr>
<td>Red gastrocnemius</td>
<td>NA</td>
</tr>
<tr>
<td>White gastrocnemius</td>
<td>NA</td>
</tr>
<tr>
<td>Quadriceps†</td>
<td>1.2</td>
</tr>
<tr>
<td>Red quadriceps</td>
<td>NA</td>
</tr>
<tr>
<td>White quadriceps</td>
<td>NA</td>
</tr>
<tr>
<td>Soleus</td>
<td>7.4</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>9.8</td>
</tr>
<tr>
<td>Brain</td>
<td>5.4</td>
</tr>
<tr>
<td>WAT</td>
<td>82.8</td>
</tr>
<tr>
<td>BAT</td>
<td>538.7</td>
</tr>
<tr>
<td>Lung</td>
<td>19.0</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.7</td>
</tr>
<tr>
<td>Kidney</td>
<td>85.7</td>
</tr>
<tr>
<td>Testis</td>
<td>822.7</td>
</tr>
</tbody>
</table>

*The Prkag2-a and Prkag2-b transcripts tested here correspond to the human PRKAG2-a and PRKAG2-b transcripts previously described (13). †The samples of mouse gastrocnemius and quadriceps were taken without discrimination between white and red fractions. NA, not applicable. WAT and BAT, white and brown adipose tissue, respectively.
Fig. 3. Protein expression profiles of AMPKγ subunit isoforms as revealed by Western blot analyses of mouse (A) and rat (B) tissues. In rats, the white (W) and red (R) parts of gastrocnemius and quadriceps muscle were separated. Samples were resolved by SDS-PAGE and immunoblotted with antibodies against AMPKγ1 (epitope ALENEHFQETPESN, residues 12–25 of γ1), γ2 [epitope KRRSLRVIHPDLS, residues 29–42 of γ2 (A) or LTPAGAKQETETE, residues 556–569 of γ2 (B)], or γ3 (epitope MSVGEALRQRTLCLEG, residues 415–431 of γ3). Quantification is based on results from 3 different animals and shown as means ± SE. Inset: representative blot from 1 animal. The apparent double band for AMPKγ3 in A is frequently seen after separation on a SDS-PAGE gel and may relate to the protease-sensitive site within the protein. C: relative abundance of 2 protein variants of AMPKγ2 (γ2a and γ2b) in mouse quadriceps, heart, and liver assessed by Western blot analysis. In this case, the anti-γ2 antibody used detects both the longer (γ2a) and the shorter (γ2b) variants of the protein (epitope LTPAGAKQETETE, residues 556–569 of γ2). D: subunit composition of γ3-containing AMPK complexes in skeletal muscle. AMPK complexes were immunoprecipitated from mouse gastrocnemius and quadriceps muscles using anti-γ3 antibody bound to protein A-Sepharose. The presence of different α- and β-subunits in γ3-containing complexes was estimated by Western blot analyses. Corresponding bacterially expressed His-tagged proteins (BCT) were included in each SDS-PAGE run to serve as size markers. In D, equal amounts of bacterially expressed α1/α2 and β1/β2 proteins were loaded.

The data presented here show the mRNA expression ratios between the three γ isoforms in several tissues, with a high degree of consistency between the three mammalian species investigated. Moreover, our observations of protein expression in mice and rats largely confirm the tissue distribution of each γ isoform observed at the mRNA level. Yet, our data on γ3 expression in skeletal muscle are obviously in contrast to previously published results of Western blot analysis of AMPK subunit isoforms in rats, which showed undetectable levels of γ3 in white quadriceps, but small amounts of γ3 in red quadriceps (9). We also find a surprising contrast between the results presented here and previously reported data showing a very low proportion of γ3-related AMPK activity in rat skeletal muscle (7), 9), whereas the activity was considerably higher in brain and testis (7). The latter observations, however, were not direct measurements of γ3 expression but were based on the AMPK activity, measured as the level of phosphorylation of the SAMS-peptide, after immunoprecipitation with isoform-specific antibodies. Hence, we cannot exclude the possibility that this principal difference in methodology may explain some of the discrepancies. For example, the presence of inactive γ3-containing complexes or free γ3 (not bound in AMPK complex) may explain why high γ3 expression can be observed despite low γ3-associated activity. Such explanations, however, do not seem to clarify why we observe practically no γ3 expression in brain and testis in contrast to the previous data showing relatively high γ3-related activity in these tissues (7). In fact, we found γ1/γ3 and γ2/γ3 mRNA expression ratios higher than 150 in brain samples from rats, mice, and humans. The situation was similar in testis, where the human and rat/mouse γ3 mRNA fractions of the total γ-mRNA were <2% and 0.02%, respectively. The reason(s) for these conflicting data may have to do with the different anti-γ3 antibodies used, their specificity, and location in the peptide sequence. For example, one possibility is the existence of more or less tissue-specific splice variants affecting an epitope for antibody binding. The anti-γ3 antibody used in this study binds a carboxy-terminal epitope whereas the antibodies used in the previous studies (7, 9) bind NH2-terminal epitopes. Although the reason(s) for the conflicting data discussed above
is still unclear, the protein expression data in mice and rats presented here are fully consistent with our mRNA data. Also, the specificity of our anti-γ3 antibody has been confirmed using a recently developed γ3 knockout mouse line that showed positive staining for γ3 expression in wild-type mice and no staining for γ3 expression in knockout mice when tested with this antibody in Western blot analysis (data not shown).

The striking difference in γ3 expression between white and red skeletal muscle fiber types probably accounts for most of the variation in the γ3/γ1 expression ratio between skeletal muscle samples studied. For example, a first glance at our data may give the impression that the γ3/γ1 expression ratio in skeletal muscle is much higher in human than in mouse skeletal muscle. Although we cannot exclude the possibility of a true expression difference between these species, this observation is rather likely to reflect differences in fiber composition between the samples compared.

In this study, the mRNA expression in skeletal muscle appeared to be generally higher for γ1 than for γ2. Interestingly, in human heart, the γ2 expression level was close to fivefold higher than the γ1 expression level. Several mutations in the PRKAG2 gene (encoding AMPKγ2) causing cardiac hypertrophy have been reported (4, 8). In rats and mice, however, we found that the γ2/γ1 mRNA expression ratios were considerably lower (1.3 and 0.6, respectively). If this reflects a general difference in γ1 and γ2 protein expression between rodents and humans, it may raise doubts for the use of rodents as model animals for functional analysis of γ2 in heart.

Our investigation of AMPKγ mRNA expression in HPMC, a possible model for functional studies of AMPK in adult skeletal muscle, showed that γ2 remains the most abundant γ-isofrom in these cells throughout the differentiation program. Thus γ2 expression reaches its full level very early during muscle cell differentiation, whereas γ1 and γ3 are upregulated at a later stage. This indicates a dramatic difference between HPMC and adult skeletal muscle regarding the γ1/γ2/γ3 mRNA expression ratio and may raise caution for using HPMC as a model for studies of regulation and action of AMPK in adult muscle.

We found that the γ3-subunit of AMPK, in mouse gastrocnemius and quadriceps, was associated solely with the α2- and β2-isofroms. This finding may reflect the relative abundance of α- and β-subunit isofroms in white skeletal muscle. Previous studies have shown that α2- and β2-subunits have considerably higher levels of expression in white compared with red skeletal muscle (21) and that interaction can occur between γ3- and all α- and β-isofroms (7). In this study, however, we did not detect any α1 or β1 protein, as assessed by immunoblot analysis after precipitation with our anti-γ3 antibody, despite the strong corresponding signals obtained with the α2 and β2 immunoblots. Thus the relatively small amount of α1 and β1 expected to be present in these muscles does not seem to associate with γ3. Therefore, our data indicate that γ3 preferentially forms a complex with α2 and β2.

Both exercise and the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) are known to display a fiber type-specific effect on AMPK activity, with the most marked increase in skeletal muscle composed predominantly of type IIb fibers (12). Glucose uptake is also stimulated by AICAR in a fiber type-specific manner, with the largest effects noted in white skeletal muscle (5). It is therefore intriguing that our data reveal α2β2γ3 as the major AMPK heterotrimer in skeletal muscle composed predominantly of white fibers. This indicates that targeting γ3-containing AMPK complexes might give an opportunity to promote glucose uptake specifically in skeletal muscle and thereby reverse some metabolic abnormalities in type 2 diabetes patients.

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