Gain of function AMP-activated protein kinase γ3 mutation (AMPKγ3R200Q) in pig muscle increases glycogen storage regardless of AMPK activation

Tracy L. Scheffler†, Sungkwon Park‡, Peter J. Roach and David E. Gerrard

1 Department of Animal and Poultry Sciences, Litton-Reaves Hall, Virginia Tech, Blacksburg, Virginia
2 Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana

Abstract

Chronic activation of AMP-activated protein kinase (AMPK) increases glycogen content in skeletal muscle. Previously, we demonstrated that a mutation in the ryanodine receptor (RyR1R615C) blunts AMPK phosphorylation in longissimus muscle of pigs with a gain of function mutation in the AMPKγ3 subunit (AMPKγ3R200Q); this may decrease the glycogen storage capacity of AMPKγ3R200Q+RyR1R615C muscle. Therefore, our aim in this study was to utilize our pig model to understand how AMPKγ3R200Q and AMPK activation contribute to glycogen storage and metabolism in muscle. We selected and bred pigs in order to generate offspring with naturally occurring AMPKγ3R200Q, RyR1R615C, and AMPKγ3R200Q+RyR1R615C mutations, and also retained wild-type littermates (control). We assessed glycogen content and parameters of glycogen metabolism in longissimus muscle. Regardless of RyR1R615C, AMPKγ3R200Q increased the glycogen content by approximately 70%. Activity of glycogen synthase (GS) without the allosteric activator glucose 6-phosphate (G6P) was decreased in AMPKγ3R200Q relative to all other genotypes, whereas both AMPKγ3R200Q and AMPKγ3R200Q+RyR1R615C muscle exhibited increased GS activity with G6P. Increased activity of GS with G6P was not associated with increased abundance of GS or hexokinase 2. However, AMPKγ3R200Q enhanced UDP-glucose pyrophosphorylase 2 (UGP2) expression approximately threefold. Although UGP2 is not generally considered a rate-limiting enzyme for glycogen synthesis, our model suggests that UGP2 plays an important role in increasing flux to glycogen synthase. Moreover, we have shown that the capacity for glycogen storage is more closely related to the AMPKγ3R200Q mutation than activity.

Introduction

AMP-activated protein kinase (AMPK) plays a key role in cellular energy homeostasis in skeletal muscle. AMPK is a heterotrimeric serine/threonine kinase composed of a catalytic α and regulatory β and γ subunits. Decreasing energy charge (ATP:AMP) enhances AMPK activation by allosteric binding of AMP to the γ subunit, and promotes phosphorylation of the activating site, αThr-172, by upstream kinases (Stein et al. 2000; Suter et al. 2006; Gowans et al. 2013). In order to preserve cellular ATP, activated AMPK acutely inhibits anabolic pathways and stimulates catabolic pathways involved in carbohydrate, lipid, and fatty acid metabolism. AMPK also modulates long-term adaptation by coordinating changes in gene and protein expression.

The γ3 subunit is highly expressed in glycolytic skeletal muscle and plays a key role in adaptation and fuel...
metabolism (Mahlapuu et al. 2004). Activating mutations in γ3 contribute to significant elevations in glycogen content in glycolytic muscles of mice (Barnes et al. 2004), pigs (Milan et al. 2000), and humans (Costford et al. 2007). Glycogen synthesis is primarily controlled by glucose transport and glycogen synthase (GS) activity (Azpiazu et al. 2000). AMPK facilitates contraction-induced, insulin-independent glucose uptake by promoting translocation of the muscle-specific glucose transporter (GLUT4) to the cell membrane (Kurth-Kraczek et al. 1999; Jørgensen et al. 2004b). Moreover, chronic activation of AMPK results in adaptive changes in glucose metabolism, including increased GLUT4 protein content (Holmes et al. 1999). However, AMPKγ3 knockout mice exhibit normal glucose tolerance and similar muscle glycogen content as wild type, but show reduced capacity to resynthesize glycogen after exercise (Barnes et al. 2004). Together, this suggests that glucose transport is not the primary step contributing to γ3-mediated glycogen synthesis; instead, glycogen synthase activity or intermediary steps may limit storage of glucose as glycogen.

The regulation of GS is rather complex; it is negatively regulated by phosphorylation by AMPK (Jensen et al. 2006) as well as several other protein kinases. Although nine phosphorylation sites have been identified, only four sites (2, 2a, 3a, 3b) are considered the most influential for regulating GS activity in muscle (Roach et al. 2012). However, glucose 6-phosphate (G6P) can completely overcome the inhibitory effects of phosphorylation, and thus restore full activity of GS (Roach et al. 2012). In this manner, limiting glucose transport restricts G6P available for glycolysis and glycogen synthesis, whereas higher rates of glucose transport enhance G6P levels and flux toward glycogen synthesis. In fact, Hunter et al. (Hunter et al. 2011) demonstrated that the primary molecular mechanism by which AMPK enhances glycogen storage despite GS phosphorylation is via G6P-induced allosteric activation. Chronic activation of AMPK likely enhances G6P content by increasing hexokinase content (Leick et al. 2011). Regulation of GS phosphorylation is via G6P-induced allosteric activation, and thus limit glycogen storage compared to AMPKγ3R200Q. Yet, the mitochondrial content and oxidative capacity of AMPKγ3R200Q + RyR1R615C is increased and similar to AMPKγ3R200Q muscle, suggesting that the mutation is sufficient to alter fuel storage and utilization (Scheffler et al. 2014). Thus, our objective was to use our AMPK and RyR1 pig model to understand how AMPKγ3R200Q and AMPK activation contribute to glycogen storage and metabolism in muscle.

Materials and Methods

Animals

Animals were bred and reared at the Purdue University Swine Center and the Virginia Tech Swine Center, and all procedures were carried out in accordance with the guidelines of each university’s Institutional Animal Care and Use Committee. Pigs heterozygous at the RyR1 and AMPKγ3 loci were bred to generate all possible genotype combinations. Female and castrated male pigs were reared under standard conditions and fed ad libitum. At approximately 120 kg, animals were transported to the university’s meat science center and harvested. Immediately after exsanguination, muscle samples (~5–10 g) were collected from the lumbar region of the longissimus muscle. Samples were immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

Genotype determination

Genotypes were determined using polymerase chain reaction (PCR) restriction fragment length polymorphism technique. DNA was isolated from blood or tissue and used for PCR amplification. PCR products were digested with appropriate restriction enzyme overnight and fragments were separated on an agarose gel stained with ethidium bromide for visualization. For determination of AMPKγ3 genotype, the primers were (5′−3′) AATGTGCAGA–CAAGGATCTC (forward) and CCCAGAAGCTCTGCTT (reverse). AMPKγ3 products were digested with restriction enzyme BsrBI. Pigs were evaluated for RyR1 genotype (Fujii et al. 1991) following the procedures outlined by O’Brien et al. (O’Brien et al. 1993). Those that were homozygous “normal” (wild type) at both RyR1 and AMPK loci were considered control while those pigs designated RyR1R615C were homozygous mutant. RyR1 heterozygotes were excluded because they tend to exhibit an intermediate phenotype. In contrast, AMPKγ3 mutation is dominant, so
both homozygous mutant and heterozygotes were utilized (designated AMPK/3R200Q). Finally, pigs denoted as AMPK/3 + RyR1 mutants were either heterozygous or homozygous mutant at AMPK/3 locus, and homozygous mutant at the RyR1 locus.

Glycogen synthase and phosphorylase activity

Enzyme activities were determined in muscle homogenates. Muscles were powdered in liquid nitrogen and homogenized on ice in buffer containing protease and phosphatase inhibitors (50 mmol/L Tris pH 7.8, 10 mmol/L EDTA, 10 mmol/L EGTA, 100 mmol/L NaF, 35 mg/mL tosyllysine chloromethyl ketone hydrochloride, 2 mmol/L benzamidine, 10 mg/mL leupeptin, and 0.5 mmol/L β-mercaptoethanol). Glycogen synthase activity was quantified by the incorporation of [U-14C] glucose from UDP[U-14C]glucose into glycogen. The assay was conducted in the absence or presence of 10 mmol/L G6P (−G6P or +G6P), and activity ratio (−G6P/+G6P) was also determined. Glycogen phosphorylase activity was measured in the direction of glycogen synthesis from [U-14C]glucose 1 phosphate (G1P) in the absence or presence of 3 mmol/L 5’AMP. Glycogen phosphorylase ratio activity was determined by dividing activity without AMP by activity with AMP (−AMP/+AMP).

Glycogen

Glycogen content was determined as previously described (Scheffler et al. 2013). Briefly, frozen muscle was powdered in liquid nitrogen and subjected to acid hydrolysis at 95°C. Another portion of frozen muscle was homogenized in perchloric acid, centrifuged, and the resulting supernatant was used for glucose, glucose-6-phosphate, and lactate analysis. Muscle glucose (free glucose and glucose-6-phosphate resulting from glycogen hydrolysis), glucose-6-phosphate, and lactate concentrations were determined using enzyme analytical methods (Bergmeyer 1974). These metabolite concentrations were used to calculate total glycogen content (in glucose equivalents) using the formula: glycogen = glucose + G6P + (lactate/2).

Western bloting

Procedures for sample processing were determined based on preliminary testing. For detection of hexokinase 2 (HK2), UGP2, and GS, muscle samples were homogenized and sonicated in HEPES buffer (25 mmol/L HEPES, 1 mmol/L EDTA, 1 mmol/L benzamidine, 1 mmol/L 4-(2-aminoethyl)-benzene sulfonyl fluoride, 1 μmol/L apro tinin, 1 μmol/L leupeptin, 1 μmol/L pepstatin, pH 7.5) and retained as a total homogenate. Protein concentration was determined using BCA protein assay kit (Pierce, Rock ford, IL) and samples were diluted to yield equal protein concentration and mixed with Laemmli buffer. Protein samples were separated using SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked in StartingBlock Blocking Buffer (Thermo Scientific). Blots were probed with primary antibody (UGP2 and HK2, Sigma, St. Louis, MO) and GS (Bioss; Woburn, MA) prepared in blocking buffer with 0.05% tween and washed in Tris-buffered saline with 0.05% tween. Then, blots were incubated with the appropriate IRDye® 680 or 800 conjugated anti-IgG antibody (LI-COR® Biosciences, Lincoln, NE). Bands were visualized using Odyssey® Infrared Imaging System (LI-COR® Biosciences) and quantified using the manufacturer’s software.

Statistical analysis

Data were analyzed using SAS-JMP (Statistical Analysis Software; Cary, NC). The model was a two-way ANOVA with main effects of AMPK (wild type or mutated) and RyR1 (wild type or mutated) genotypes and their interaction (AMPK*RyR1). Data are represented as LSM ± SE. If the interaction was significant, differences between genotype combinations were determined using Tukey’s adjustment for multiple comparisons. P < 0.05 was considered significant.

Results

Previously, we reported that pigs with both AMPK and RyR1 mutations have blunted AMPK phosphorylation and decreased GLUT4 protein expression compared to pigs possessing only AMPK mutation (Park et al. 2009). Yet, despite these differences, AMPK + RyR1 mutant muscle exhibits some traits associated with AMPK activation, such as increased oxidative capacity (Scheffler et al. 2014). Therefore, our goal was to understand the contribution of AMPK/3R200Q and AMPK activation to glycogen storage and metabolism in pig longissimus muscle.

Muscle glycogen (sum of equivalents), glycogen, G6P, and lactate

AMPK genotype influenced (P < 0.0001) glycogen content in longissimus muscle (Fig. 1A); In contrast, RyR1R615C decreased glycogen (P = 0.0003), consistent with increased lactate content (Fig. 1B, P < 0.001), possibly linked to increased phosphorylase activation (see below). In addition, both AMPK and RyR mutations influenced G6P levels (P < 0.0001 and P = 0.005, respectively). To better reflect total initial glycogen levels in
muscle, we calculated the sum of major glycolytic metabolites (glucose equivalents, Fig. 1D). AMPK\(_{c3R00Q}\) muscle had approximately 70% greater glucose equivalents, regardless of RyR genotype. RyR1 R615C genotype tended to decrease glycogen (\(P = 0.08\)), but the change was relatively small (\(\sim 5\%\)).

**Glycogen synthase activity and content**

Glycogen synthase is considered to be the rate-limiting enzyme for glycogen synthesis. Phosphorylation contributes to down-regulation of GS activity, but inactivation can be completely overcome by increasing the allosteric activator, G6P. Thus, GS is typically assayed in the absence and presence of G6P, and the resulting ratio (\(\frac{\text{GS activity in absence of G6P}}{\text{GS activity in presence of G6P}}\)) is used as an indication of the phosphorylation state of GS. An AMPK\(_{c3R200Q}\) genotype interaction (\(P = 0.04\)) influenced GS activation in the absence of G6P, as demonstrated by the lower GS activity (\(P < 0.05\)) in AMPK\(_{c3R200Q}\) muscle relative to all other genotypes (Fig. 2A). However, G6P increased (\(P < 0.0001\)) GS activity about twofold in muscle with AMPK\(_{c3R200Q}\) regardless of the RyR1 mutation. For GS activity ratios, AMPK\(_{c3R200Q}\) muscle exhibited the lowest activity, whereas AMPK\(_{c3R200Q} + \text{RyR1}^{R615C}\) was intermediate, and control and RyR1\(^{R615C}\) were the highest. The low activity ratio of AMPK\(_{c3R200Q}\) muscle reflects both inactivation of GS by phosphorylation in the absence of G6P and increased activity in the presence of G6P, whereas the intermediate value of AMPK\(_{c3R200Q} + \text{RyR1}^{R615C}\) muscle is due exclusively to increased activity with G6P.

Because total activity (+G6P) is often related to protein content (Manchester et al. 1996), we evaluated GS protein content by western blot. Unexpectedly, GS content was about 20% higher (\(P < 0.05\)) in wild-type pigs relative to all other genotypes (Fig. 3).

**Enzymes in glycogen synthesis pathway**

Although GS is considered the rate-limiting enzyme, glycogen synthesis rate is dependent on glucose supply. The first step is the uptake of glucose into the cell via glucose transporters. Previously, we determined that GLUT4 protein content in muscle with AMPK\(_{c3R200Q}\) was enhanced relative to control, whereas GLUT4 in AMPK\(_{c3R200Q} + \text{RyR1}^{R615C}\) muscle was similar to control (Park et al. 2009). Enzymes downstream of glucose entry are HK2, phosphoglucomutase, and UGP2. Hexokinase 2 protein content was not affected by genotype (Fig. 4). However, AMPK\(_{c3R200Q}\) increased (\(P < 0.0001\)) the content of UGP2 approximately threefold (Fig. 5).

**Glycogen phosphorylase**

Since glycogen content is the net result of glycogen synthesis and degradation, we evaluated the activity of glycogen
phosphorylase (GP), the rate-limiting enzyme for glycogen breakdown. In the absence of AMP, the AMPK mutation contributed to increased GP activity ($P = 0.002$; Fig. 6), suggesting greater phosphorylation (activation) of GP in muscle with AMPK$^{c3R200Q}$. AMPK and RyR1 genotype also exerted significant ($P < 0.05$ and $P = 0.03$, respectively), but rather small effects on GP activity ($+AMP$). Although AMPK$^{c3R200Q}$ was associated with increased activity, RyR1$^{R615C}$ decreased GP ($+AMP$) activity. Therefore, the GP activity ratio ($/-AMP$) was lowest in control and highest in AMPK$^{c3R200Q} + $RyR1$^{R615C}$ muscle.

**Discussion**
AMPK signaling plays important roles in acute and adaptive regulation of muscle metabolism. Under acute energy
stress, AMPK is well-documented to protect cellular energy levels by down-regulating anabolic pathways, such as glycogen synthesis. Yet, AMPK’s role in limiting glycogen synthase activity is not consistent with the dramatic increase in muscle glycogen in pigs and mice with an AMPK-activating mutation. Increases in muscle glycogen despite inactivation of GS are largely mediated by allosteric activation of GS by G6P (Bouskila et al. 2010; Hunter et al. 2011). Enhanced glucose transport contributes to elevated G6P, which overcomes the inhibitory effect of phosphorylation on glycogen synthase, and thus promotes glycogen storage (Hunter et al. 2011). However, we previously demonstrated that pigs with AM PKc3R200Q exhibit enhanced AMPK activity and a concordant increase in GLUT4 content, whereas these effects are blunted in muscle with AM PKc3R200Q + RyR1R615C (Park et al. 2009). In turn, we expected these differences in AMPK activity and GLUT4 content would be associated with altered glycogen storage capacity and metabolism.

Curiously, AM PKc3R200Q + RyR1R615C muscle contains similar glycogen as AM PKc3R200Q muscle, which is approximately 70% greater than wild-type and RyR1R615C littermates. In muscle, control of glycogen synthesis is distributed primarily between glucose transport and glycogen synthase (Azpiazu et al. 2000). GLUT4 content and translocation contribute significantly to glucose uptake into the cell, and initially, these steps were considered rate-limiting for glycogen synthesis (Ren et al. 1993). Consistent with this concept, transgenic overexpression of GLUT4 in muscle increases glucose transport activity and enhances glycogen storage, without affecting activation of glycogen synthase (Hansen et al. 1995). However, GLUT4 knockout mice also possess greater muscle glycogen in the fasted state despite markedly reduced glucose transport (Kim et al. 2005), suggesting that glucose transport is not always rate-limiting for glycogen synthesis. Along these lines, RyR1R615C blunted AM PKc3R200Q-induced increases

![Figure 5. UDP-glucose pyrophosphorylase 2 protein content of AMPK and RyR genotypes. Data are LSM ± SE (n = 6–7 pigs per genotype).](https://example.com/figure5)

![Figure 6. Glycogen phosphorylase activity in AMPK and RyR genotypes. (A) Glycogen phosphorylase activity in the absence of AMP. (B) Glycogen synthase activity in the presence of AMP. (C) Activity of glycogen phosphorylase expressed as ratio (−/+ AMP). Data are LSM ± SE (n = 5 pigs per genotype).](https://example.com/figure6)
in GLUT4 content (Park et al. 2009), but did not diminish
glycogen storage. Although GLUT4 content significantly
contributes to glucose transport, other mechanisms may
permit glycogen storage in AMPK$\gamma^2$R200Q + RyR1R615C
muscle.

Glycogen synthase is considered the key regulatory
enzyme in glycogen synthesis. Due to the complex regula-
tion of GS, including multiple phosphorylation sites, GS
activity is typically assayed in the absence and presence of
G6P. GS activity (−G6P) was reduced only in
AMPK$\gamma^2$R200Q muscle, which is consistent with increased
AMPK phosphorylation and activity in AMPK$\gamma^3$R200Q but
not AMPK$\gamma^3$R200Q + RyR1R615C muscle (Park et al. 2009),
and with activated AMPK functioning as a glycogen syn-
thase kinase (Jørgensen et al. 2004a). However, GS activity
(+G6P) was nearly double in AMPK mutated muscle
(AMPK$\gamma^2$R200Q and AMPK$\gamma^3$R200Q + RyR1R615C) com-
pared to wild type and RyR1R615C. Activity with G6P, also
referred to as total GS activity, is thought to parallel GS
content. Increasing GS content is sufficient to augment
muscle glycogen synthesis and glycogen level without
altering glucose transport (Manchester et al. 1996). How-
ever, AMPK$\gamma^2$R200Q did not increase GS protein content,
as evidenced by western blot quantification. Similarly, Yu
et al. (Yu et al. 2006) observed that muscles from
AMPK$\gamma^3$R225Q mice exhibit increased GS activity com-
pared to wild type, but do not have greater GS protein
content. The disconnect between GS activity in the pres-
ence of G6P and GS protein may reflect an as yet unap-
preciated, stable mechanism to modify the enzyme
activity. It is also possible that other mechanisms may
make GS more sensitive to G6P in AMPK$\gamma^2$R200Q and
AMPK$\gamma^3$R200Q + RyR1R615C muscle.

Allosteric regulation of GS, and hence increased total GS
activity, is the primary mechanism by which AMPK (Hun-
ter et al. 2011) and insulin (Bouskila et al. 2010) promote
glycogen accumulation. The increased flux through glucose
transporters and HK2 contributes to elevated G6P and
allosteric activation of GS. The AMPK and RyR mutations
increased G6P levels in muscle. Elevated G6P in RyR1R615C
muscle is likely due to increased glycolytic flux, which is
also consistent with the elevated lactate levels observed in
muscle; pigs with this mutation are susceptible to stress
and rapid metabolism (MacLennan and Phillips 1993). On
the other hand, increased G6P in AMPK pigs may be asso-
ciated with greater glucose transport. Others have shown
that chronic activation of AMPK increases GLUT4 and
increased HK2 activity (Holmes et al. 1999; Granlund et al.
2011). In AMPK$\gamma^2$R200Q + RyR1R615C muscle, neither
GLUT4 (Park et al. 2009) nor HK2 content (this study)
was different from control. We did not determine HK2
activity, although it is possible that AMPK$\gamma^3$R200Q enhances
G6P levels by increasing HK2 activity but not content.

High GS activity ratio (−/+ G6P) indicates that dephos-
phorylated (active) GS predominates. In mice genetically
engineered to have altered expression of GS or its phos-
phatase, there is a positive relationship between GS activity
ratio and glycogen content (Roach 2002). However, in our
model, high activity ratio is a poor predictor of glycogen
content because both control and RyR1R615C muscle exhi-
bit the highest activity ratios, but contain less glycogen
than AMPK$\gamma^3$R200Q and AMPK$\gamma^3$R200Q + RyR1R615C
muscle. The inverse relationship between GS activity ratio and
glycogen content is consistent with other reports incorpo-
rating genetic (Yu et al. 2006), exercise (Manabe et al.
2013), and fasting/refeeding (Jensen et al. 2006) approaches.
Depending on the model, changes in the GS activity ratios may result from more adaptive changes,
such as GS protein content, or by acute mechanisms
involving regulation of glycogen synthase. For example,
exercise training in rats promotes glycogen storage and
coincides with a decrease in GS activity ratio, which is due
almost exclusively to elevated total GS activity and
increases in GS content (Manabe et al. 2013). On the other
hand, in rats exposed to a fasting/refeeding approach, GS
activity ratio was inversely related to glycogen content, but
there were no significant differences in GS content (Jensen
et al. 2006).

Elevated glycogen content in AMPK$\gamma^2$R200Q and
AMPK$\gamma^3$R200Q + RyR1R615C muscle parallels total GS activ-
ity, suggesting that capacity to stimulate glycogen synthase
plays a major role in glycogen storage in our model. In turn,
glycogen synthase activity is also a function of the metabo-
lites and enzymatic activity in the steps preceding glycogen
synthesis. Traditionally, other enzymes in the glycogen syn-
thesis pathway, including phosphoglucosemutase and UGP2,
are not considered rate-limiting. Overexpression of UGP2
in muscle does not affect glycogen content, glucose tolerance,
or insulin-stimulated rates of glucose incorporation
into glycogen (Reynolds et al. 2005). Yet, various genetic
models used to manipulate glycogen content indicate a link
between UGP2 expression and glycogen level. In models
manipulating GS, glycogen content is inversely related to
UGP2 expression; UGP2 mRNA is increased in GS knock-
out mice lacking muscle glycogen, but decreased in mice
overexpressing glycogen synthase (Parker et al. 2006). On
the other hand, in models that manipulate AMPK, glycogen
is positively related to UGP2 expression. AMPK$\gamma^3$R225Q
mice exhibit greater UGP2 expression and deposit more
muscle glycogen, whereas AMPK$\gamma^3$ knockout mice have
decreased UGP2 expression and possess less muscle glycogen.
Increased UGP2 protein content in AMPK$\gamma^3$R200Q pig
muscle reported herein and elsewhere (Hedegaard et al.
2004) is consistent with gene expression patterns in AMPK
mice. Interestingly, UGP2 levels are strongly upregulated in
hearts of mice with a mutation in AMPK$\gamma^2$, the
predominant cardiac isoform (Luptak et al. 2007); and upregulation of UGP2 precedes glycogen accumulation. These authors suggested that UGP2 helps “pull” glucose toward glycogen in cardiac muscle with mutated γ2. The equilibrium of the phosphoglucosemutase reaction (G6P ↔ glucose 1-phosphate) favors G6P; however, increasing utilization of glucose 1-phosphate by UGP2 would help promote flux toward glucose 1-phosphate, and in turn, UDP-glucose. Moreover, Manchester et al. (Manchester et al. 1996) showed that GS overexpression decreases UDP-glucose concentrations and this lack of substrate likely restricts glycogen accumulation. Therefore, increasing UGP2 content in skeletal muscle may be an important means of enhancing flux to glycogen synthase and promoting glycogen accumulation in AMPKγ3R200Q and AMPKγ3R200Q + RyR1K613C muscle under some conditions.

Glycogen content in muscle is the net result of glycogen synthesis and degradation. Although certain glycogen storage diseases are caused by defects in glycogen degradation and/or glycosylation, mutations in the γ3 and γ2 subunits of AMPK have not been linked to compromised glycogenolysis (Estrade et al. 1994; Luptak et al. 2007). In fact, elevated glycogen in AMPKγ3R225Q mouse muscle enhances capacity for anaerobic exercise compared to wild type (Barnes et al. 2005). In our hands, AMPKγ3R200Q increases glycogen phosphorylase activity; Granlund (Granlund et al. 2005). In our hands, AMPKγ3R200Q increases glycogen phosphorylase activity; Granlund (Granlund et al. 2011) also reported elevated GP activity in longisimus muscle of AMPKγ3R200Q pigs compared to wild type. Clearly, glycogen phosphorylase activity does not help explain elevated glycogen content in our pig model; however, it does indicate that glycogen turnover is increased. Manchester et al. (Manchester et al. 1996) speculated that glycogen content influences GS and GP levels, but other models are not consistent with this concept.

Altogether, we have shown that AMPKγ3R200Q and AMPKγ3R225Q + RyR1K613C muscle exhibit differences in GLUT4 as well as AMPK and GS activation; however, both clearly possess elevated total GS activity and high muscle glycogen. Our model demonstrates that the capacity for glycogen storage is more closely related to the AMPK mutation than activity. Additionally, we show that UGP2, previously considered a minor player in glycogen storage pathway, may be an important contributor to glycogen storage by promoting flux toward glycogen synthase.

**Conflict of Interest**

None declared.

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