Original Article

Changes in Exercise-Induced Gene Expression in 5′-AMP–Activated Protein Kinase γ3–Null and γ3 R225Q Transgenic Mice

Brian R. Barnes,[1,2] Yun Chau Long,[2] Tatiana L. Steiler,[1,2] Ying Leng,[2,3] Dana Galuska,[1] Jørgen F.P. Wojtaszewski,[4] Leif Andersson,[5,6] and Juleen R. Zierath[1,2]

5′-AMP–activated protein kinase (AMPK) is important for metabolic sensing. We used AMPKγ3 mutant–overexpressing *Tg-Prkag3*^{225Q} and AMPKγ3-knockout *Prkag3*$^{-/-}$ mice to determine the role of the AMPKγ3 isoform in exercise-induced metabolic and gene regulatory responses in skeletal muscle. Mice were studied after 2 h swimming or 2.5 h recovery. Exercise increased basal and insulin-stimulated glucose transport, with similar responses among genotypes. In *Tg-Prkag3*^{225Q} mice, acetyl-CoA carboxylase (ACC) phosphorylation was increased and triglyceride content was reduced after exercise, suggesting that this mutation promotes greater reliance on lipid oxidation. In contrast, ACC phosphorylation and triglyceride content was similar between wild-type and *Prkag3*$^{-/-}$ mice. Expression of genes involved in lipid and glucose metabolism was altered by genetic modification of AMPKγ3. Expression of lipoprotein lipase 1, carnitine palmitoyl transferase 1b, and 3-hydroxyacyl–CoA dehydrogenase was increased in *Tg-Prkag3*^{225Q} mice, with opposing effects in *Prkag3*$^{-/-}$ mice after exercise. GLUT4, hexokinase II (HKII), and glycogen synthase mRNA expression was increased in *Tg-Prkag3*^{225Q} mice after exercise. GLUT4 and HKII mRNA expression was increased in wild-type mice and blunted in *Prkag3*$^{-/-}$ mice after recovery. In conclusion, the *Prkag3*^{225Q} mutation, rather than presence of a functional AMPKγ3 isoform, directly promotes metabolic and gene regulatory responses along lipid oxidative pathways in skeletal muscle after endurance exercise. *Diabetes* 54: 3484–3489, 2005

From the [1]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; the [2]Department of Surgical Sciences, Karolinska Institutet, Stockholm, Sweden; the [3]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P.R. China; the [4]Department of Human Physiology, Copenhagen Muscle Research Centre, Institute of Exercise and Sport Sciences, University of Copenhagen, Copenhagen, Denmark; the [5]Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala Biomedical Center, Uppsala, Sweden; and the [6]Department of Medical Biochemistry and Microbiology, Uppsala University, Uppsala Biomedical Center, Uppsala, Sweden.

Address correspondence and reprint requests to Juleen R. Zierath, PhD, Karolinska Institutet, Department of Surgical Sciences, Section of Integrative Physiology, von Eulers väg 4, 4th Floor, S-171 77 Stockholm, Sweden. E-mail: juleen.zierath@fyfa.ki.se.

Received for publication 4 March 2005 and accepted in revised form 23 August 2005.

B.R.B. and Y.C.L. contributed equally to this work.

L.A. holds stock in Arexis (Gothenburg, Sweden).

ACC, acetyl-CoA carboxylase; AMPK, 5′-AMP–activated protein kinase; CPT1b, carnitine palmitoyl transferase 1b; EDL, extensor digitorum longus; HAD, 3-hydroxyacyl–CoA dehydrogenase; HKII, hexokinase II; IMTG, intramuscular triglyceride; KHBB, Krebs-Henseleit bicarbonate buffer; LPL1, lipoprotein lipase 1.

5′-AMP–activated protein kinase (AMPK) is a cellular energy sensor that responds to alterations in the AMP-to-ATP ratio. Activation of AMPK in response to metabolic stress initiates several signaling cascades aimed at restoring energy balance, including stimulation of catabolic (ATP-generating) pathways, such as fatty acid oxidation (1), glucose uptake (2,3) and glycolysis, as well as inhibition of anabolic (ATP consuming) pathways, such as synthesis of fatty acids (4) and protein (5). Several physiological consequences of exercise, including muscle contraction, hypoxia, ischemia, heat shock, glycogen catabolism, and decreased pH, are associated with AMPK activation (6). However, multiple signal transduction cascades, including mitogen-activated protein kinases (7,8), calcineurin (9), hypoxia-inducible factor-1α (10), and calmodulin-dependent protein kinase (11) are engaged in response to exercise, thus the role of AMPK in specific exercise-induced responses in skeletal muscle is incompletely resolved.

Several lines of evidence reveal that AMPK is directly involved in glucose metabolism. Overexpression of a kinase-dead AMPKα2 is associated with reduced skeletal muscle glycogen content and retarded after exercise glycogen resynthesis in mice (12,13). Moreover, a dominant missense mutation in the gene encoding AMPKγ3 isoform enhances glycogen storage in glycolytic skeletal muscle in pigs (14) and transgenic mice (*Tg-Prkag3*^{225Q}) harboring this mutation (15). *Tg-Prkag3*^{225Q} mice have similar glycogen content immediately after exercise and elevated glycogen content after recovery when compared with wild-type mice (15). Conversely, AMPKγ3 knockout mice (*Prkag3*$^{-/-}$) have severely impaired glycogenesis after exercise (15). Furthermore, expression of other isoforms of AMPK subunits in *Tg-Prkag3*^{225Q} and *Prkag3*$^{-/-}$ mice is similar to the wild-type mice (15). Therefore, AMPKγ3 appears to play a critical role in glycogen metabolism after exercise (16). However, because oxidative metabolism is important during endurance exercise, changes in lipid metabolism in response to AMPK activation may also affect glycogen metabolism in skeletal muscle.

Acute and chronic exercise promotes gene regulatory responses in skeletal muscle that may facilitate metabolic adaptations along pathways governing glycolytic and oxidative metabolism. Activation of AMPK by the adenosine analog 5′-amino-4-imidazolecarboxamide ribonucleoside increases transcription of metabolic genes in skeletal muscle that are also known to be regulated in response to

exercise (17,18). A direct role for AMPK in promoting gene regulatory responses in skeletal muscle, including mitochondrial biogenesis in response to chronic energy deprivation, has been established using kinase-dead AMPKα2 transgenic mice (19). Moreover, metabolic sensing in skeletal muscle also requires expression of the AMPKγ3 subunit, because fasting-induced transcription of enzymes involved in lipid metabolism is retarded in *Prkag3*$^{-/-}$ mice (20). Thus, AMPK plays an important role in the transcriptional regulation of multiple genes along divergent pathways controlling energy metabolism, cellular signaling, transcription, and translation (13).

Given that the metabolic requirement of fasting and long-term exercise promotes a shift from glycolytic to oxidative metabolism, we hypothesize that the AMPKγ3 isoform regulates glycogen resynthesis after exercise by altering the balance between glucose and lipid metabolism. *Tg-Prkag3*^{225Q} and *Prkag3*$^{-/-}$ mice were studied after a 2-h swimming bout or during recovery (2.5 h after swimming). Here, we provide evidence that the *Prkag3*^{225Q} mutation, rather than the presence of a functional AMPKγ3 subunit, directly promotes an enhanced reliance on lipid metabolism during endurance exercise, concomitant with a coordinated increase in expression of genes regulating lipid metabolism in skeletal muscle.

RESEARCH DESIGN AND METHODS

Three animal models were used: *Tg-Prkag3*^{225Q}, *Prkag3*$^{-/-}$, and wild-type littermates. The creation and general metabolic characteristics of these animal models have been described previously (15). Mice were cared for in accordance with regulations for the protection of laboratory animals established by the animal ethical committee at Karolinska Institutet. Mice were maintained in a temperature- and light-controlled environment and had free access to water and standard rodent diet. Mice were anesthetized with Avertin (2,2,2-tribromo ethanol 99% and tertiary amyl alcohol, at 0.015–0.017 ml/g mouse body wt), and extensor digitorum longus (EDL) and white gastrocnemius muscle were isolated.

Swimming protocol. Overnight-fasted (16 h) wild-type, *Tg-Prkag3*^{225Q}, or *Prkag3*$^{-/-}$ mice were randomly assigned to either sedentary or swimming group. The swimming protocol has been previously described (21). Six mice swam together in plastic containers measuring 45 cm in diameter. Water temperature was maintained at 32–33°C. Mice swam for four 30-min intervals separated by 5-min rest periods for a total swimming time of 2 h. After the last swim interval, mice were dried and either studied immediately or allowed to recover from the exercise bout for 2.5 h (recovery). At the onset of the recovery period, mice received an intraperitoneal glucose injection (0.5 mg/g body wt) and had free access to food and water.

Muscle incubations. All incubation media were prepared from a stock solution of Krebs-Henseleit bicarbonate buffer (KHBB) containing 0.1% BSA (radioimmunoassay grade) (21). Media were continuously gassed with 95% O_2/5% CO_2. Muscles were preincubated (15 min at 30°C) in KHBB containing 5 mmol/l glucose and 15 mmol/l mannitol. Muscles were incubated in the absence or presence of insulin (12 nmol/l) for the duration of the incubation protocol.

Glucose uptake. Muscles were transferred to KHBB containing 20 mmol/l mannitol and incubated for 10 min. Thereafter, muscles were transferred to KHBB containing 1 mmol/l 2-deoxy-[1,2-^{3}H]glucose (2.5 μCi/ml) and 19 mmol/l [^{14}C]mannitol (0.7 μCi/ml) and incubated for 20 min. Glucose transport activity is expressed as micromoles per milliliter of intracellular water per hour (22).

Glucose oxidation. Muscles were incubated (30°C for 60 min) in the absence or presence of insulin (12 nmol/l) in preincubation media supplemented with [^{14}C]glucose (0.2 mCi/ml). Thereafter, 0.2 ml Solvable (2% sodium hydroxide; Dupont, Hamburg, Germany) was injected into the center well of the incubation vial to collect liberated CO_2, and 0.5 ml 15% perchloric acid was injected into the media to lyse the muscle. Glucose oxidation was assessed by collection of liberated CO_2.

Oleate oxidation. Muscles were harvested immediately after swim exercise and incubated (30°C for 60 min) in 1 ml KHBB media supplemented with 0.3 mmol/l [1-^{14}C]oleate (0.4 μCi/ml). Thereafter, the media was acidified by 0.5 ml 15% pyrroline-5-carboxylic acid, and liberated CO_2 was collected in center wells containing 0.2 ml Protosol (DuPont NEN Research Laboratories) for 60 min. Center wells were removed for scintillation counting. Results were expressed as nanomoles of oxidized oleate per gram of wet weight per hour.

Western blot analysis. Phosphorylation of acetyl CoA-carboxylase (ACC) was determined by Western blot analysis. White gastrocnemius skeletal muscle was lysed in ice-cold buffer (23), and an aliquot of lysate (30 μg) was separated by SDS–PAGE. Proteins were transferred to Immobilon-P membranes (Millipore, Billerica, MA) and probed with primary antibodies (described below) and secondary horseradish peroxidase–conjugated antibodies. Phosphorylation of ACC was determined using anti–phospho-ACC (Ser227; Cell Signaling Technology, Beverly, MA) antibody. Immunoreactive proteins were detected by enhanced chemiluminescence (Amersham Biosciences, Uppsala, Sweden). Immunoreactive band intensity was quantified using the Image Gauge V3.01 software (Fujifilm, Tokyo, Japan).

Intramuscular triglyceride content. Gastrocnemius muscles were removed from anesthetized mice, cleaned of fat and blood, and quickly frozen in liquid nitrogen. Triglycerides were analyzed using 15–25 mg pulverized frozen skeletal muscle. Tissue was homogenized with 0.3 ml heptan-isopropanol-Tween mixture (3:2:0.01 by volume) and centrifuged (1,500 × *g* for 15 min at 4°C). Supernatants (upper phase containing extracted triglycerides) were collected and evaporated with vacuum centrifuge. Triglyceride content was measured with a triglyceride/glycerol blanked kit (Roche, Nutley, NJ). Seronorm lipid (SERO, Billingstad, Norway) was used as a standard. Samples were measured in duplicates.

RNA purification and cDNA synthesis. Total RNA of white gastrocnemius muscle was purified using Trizol reagent (Sigma, St. Louis, MO), as specified by the manufacturer. Purified RNA was treated with DNase I using DNA-free kit (Ambion, Austin, TX), according to the manufacturer's protocol. DNase-treated RNA served as a template for cDNA synthesis using oligo(dT) primers and SuperScript First Strand Synthesis System (Invitrogen, Carlsbad, CA).

Quantitative PCR. Quantification of mRNA was performed using real-time PCR with the ABI PRISM 7700 Sequence Detector System (Applied Biosystems, Warrington, U.K.) and SYBR-green. The relative quantities of target transcripts were calculated from duplicate samples after normalization of the data against housekeeping gene 36B4 (acidic ribosomal phosphoprotein PO). Primers were selected by using PRIMER EXPRESS (Applied Biosystems). Transcript sequences obtained from ENSEMBL database were lipoprotein lipase 1 (LPL1; ENSMUST00000015715), carnitine palmitoyl transferase 1b (CPT1b; ENSMUST00000023287), 3-hydroxyacyl–CoA dehydrogenase (HAD; ENSMUST00000029610), uncoupling protein 3 (ENSMUST0–0000032958), GLUT4 (ENSMUST00000018710), and glycogen synthase (ENSMUST00000003964). Transcript sequences obtained from National Center for Biotechnology Information GenBank database were cytochrome c (NM007808), hexokinase II (HKII; Y11666), and 36B4 (BC003833).

Statistical analysis. Differences between means were analyzed using Student's *t* test or two-way ANOVA followed by Fisher's least significant differences post hoc analysis. Significance was accepted at $P < 0.05$.

RESULTS

Glucose uptake. Glucose uptake was assessed in isolated EDL muscle from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice immediately after swimming or recovery (2.5 h after swimming) (Fig. 1*A*) or in the fed condition (Fig. 1*B*). Rates of glucose uptake in isolated EDL muscle from fasted wild-type, *Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice have previously been reported (15). Acute exercise (swimming) increased glucose uptake to an equal extent in all genotypes (Fig. 1*A*). Effects of exercise and insulin (12 nmol/l) were additive on glucose uptake, with similar responses noted among the genotypes. Glucose uptake was also measured 2.5 h after exercise (recovery). The exercise effect on basal glucose transport was reversed within 2.5 h recovery (i.e., after a bolus of glucose injection and free access to food). Basal glucose uptake in *Tg-Prkag3*^{225Q} mice was lower compared with wild-type mice ($P < 0.05$). Insulin-stimulated glucose uptake was also assessed after recovery. Insulin-stimulated glucose uptake was increased more than twofold in the recovery state ($P < 0.05$ vs. without insulin stimulation), with similar effects among wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice.

Glucose oxidation. Glucose oxidation was determined in EDL muscles from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$

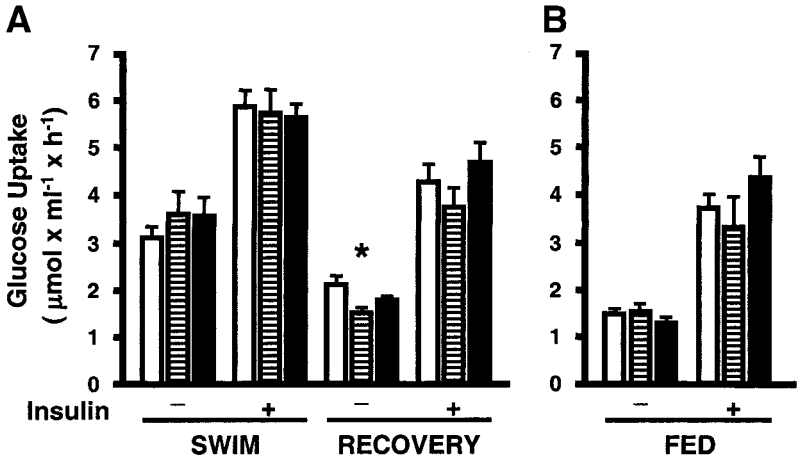

FIG. 1. Basal and insulin-stimulated 2-deoxy-glucose uptake. EDL muscle from wild-type (□, n = 9–20), *Tg-Prkag3*^{225Q} (▤, n = 6–17), and *Prkag3*$^{-/-}$ (■, n = 5–10) mice were incubated without (basal) or with insulin (12 nmol/l) immediately after exercise or recovery (2.5 h) (*A*) or in the fed state (*B*). Data are expressed as nanomoles per milliliter per hour. *P < 0.05 vs. wild type of same condition. Data are means ± SE.

mice after acute swimming (Fig. 2). In *Tg-Prkag3*^{225Q} mice, a nonsignificant trend toward decreased glucose oxidation was observed under basal conditions. Glucose oxidation measured under insulin-stimulated conditions was similar among genotypes. Glucose oxidation in EDL from *Prkag3*$^{-/-}$ mice was reduced 33% ($P < 0.05$) under basal conditions and 20% under insulin-stimulated conditions after exercise (NS) when compared with the wild-type mice.

Phosphorylation of ACC. Phosphorylation of ACC (Ser227) was measured in gastrocnemius muscle from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice after swimming or recovery (Fig. 3*A*). Immediately after swimming, phosphorylation of ACC was increased in *Tg-Prkag3*^{225Q} mice compared with wild-type mice ($P < 0.05$), which is an indication of enhanced fatty acid oxidation. Phosphorylation of ACC in *Prkag3*$^{-/-}$ mice was not different from wild-type mice. After recovery from swimming, ACC phosphorylation was similar among wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice.

Intramuscular triglyceride content. Intramuscular triglyceride (IMTG) has been determined in gastrocnemius muscle from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice under fed and fasted conditions (20). IMTG content was determined in gastrocnemius muscle from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice after swimming or recovery (Fig. 3*B*). After swimming, IMTG content was reduced in *Tg-Prkag3*^{225Q} mice ($P < 0.05$) and unchanged in *Prkag3*$^{-/-}$ mice compared with wild-type mice. After recovery, IMTG content was similar to fasted levels for all genotypes (20).

Oleate oxidation. EDL muscles were excised from mice directly after the swim bout and incubated for 2 h to determine the rate of oleate oxidation after exercise. Under these in vitro conditions, similar rates of oleate oxidation were observed among genotypes (data not shown). Therefore, despite enhanced IMTG utilization during exercise, *Tg-Prkag3*^{225Q} mice maintained a similar

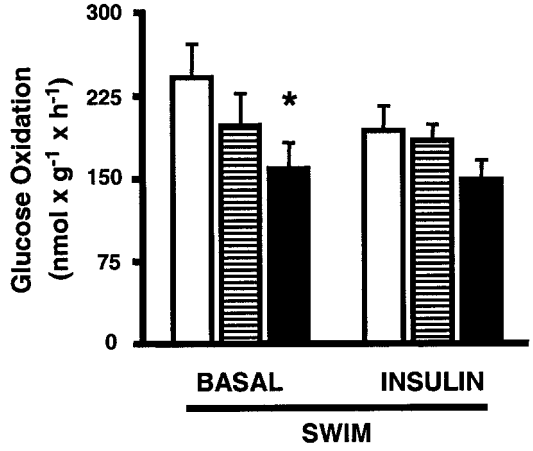

FIG. 2. Basal and insulin-stimulated glucose oxidation. EDL muscle from wild-type (□, n = 16–17), *Tg-Prkag3*^{225Q} (▤, n = 10–11), and *Prkag3*$^{-/-}$ (■, n = 8–9) mice were incubated in the absence (basal) or presence of 12 nmol/l insulin immediately after exercise. Data are expressed as nanomoles per milliliter per hour. *P < 0.05 vs. wild type of same condition. Data are means ± SE.

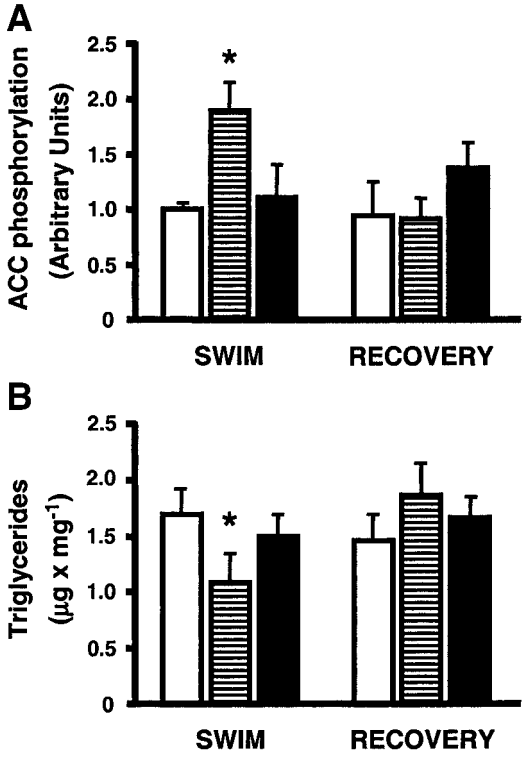

FIG. 3. Phosphorylation of ACC and IMTG content in skeletal muscle. Phosphorylation of ACC (n = 6–9 per group) (*A*) and IMTG content (n = 9–12 per group) (*B*) was determined in white gastrocnemius muscle from wild-type (□), *Tg-Prkag3*^{225Q} (▤), and *Prkag3*$^{-/-}$ (■) mice immediately after exercise or recovery (2.5 h). Data are expressed as arbitrary units for ACC phosphorylation and micrograms per milligram for triglyceride content. *P < 0.05 vs. wild type of same condition. Data are means ± SE.

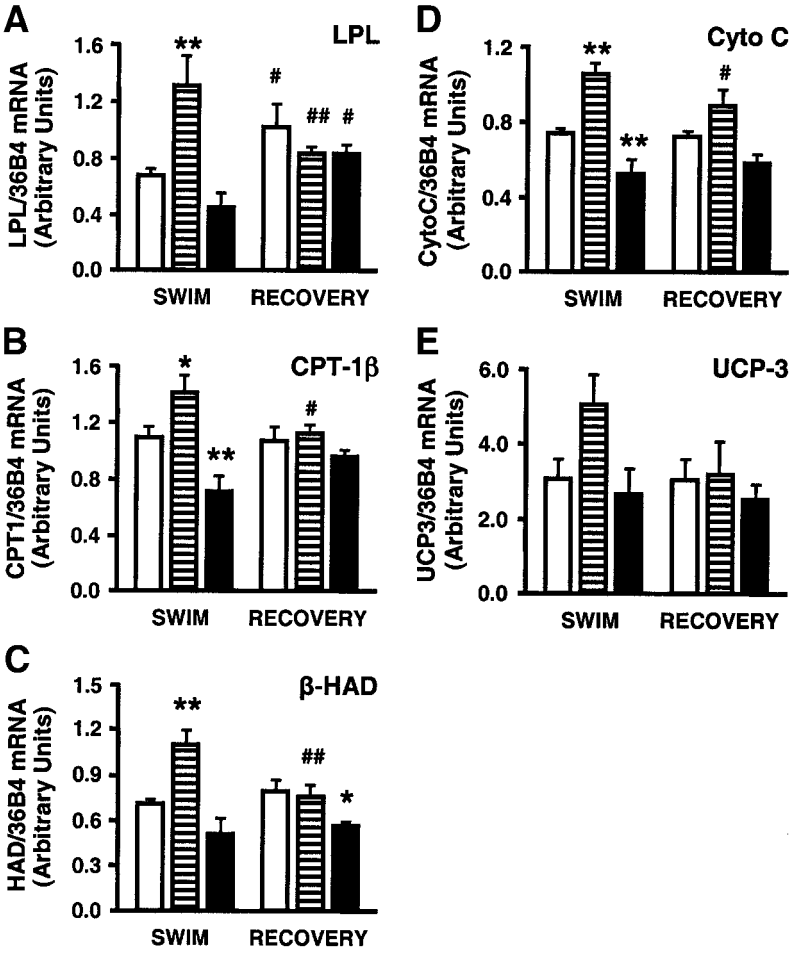

FIG. 4. mRNA expression of genes regulating lipid metabolism in skeletal muscle. mRNA expression of LPL1 (*A*), CPT1b (*B*), HAD (*C*), cytochrome c (*D*), and uncoupling protein 3 (UCP3) (*E*) was determined in white gastrocnemius muscle (n = 9–10 per group) from wild-type (□), *Tg-Prkag3*^{225Q} (▤), and *Prkag3*$^{-/-}$ (■) mice immediately after exercise or recovery (2.5 h). Results are expressed as the ratio of mRNA expression of respective gene to 36B4. **$P < 0.01$, *$P < 0.05$ vs. wild type of same condition; ##$P < 0.01$, #$P < 0.05$ vs. same genotype from swim.

rate of extracellular lipid oxidation in the state after exercise compared with wild type.

Quantitative PCR for metabolic genes. We have previously determined mRNA expression of metabolic genes in white gastrocnemius muscles from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice under fed or fasted conditions (20). mRNA expression of genes involved in lipid metabolism through quantitative real-time PCR analysis in response to swim exercise or recovery was determined (Fig. 4). In *Tg-Prkag3*^{225Q} mice, mRNA expression of LPL1 ($P < 0.01$), CPT1b ($P < 0.05$), HAD ($P < 0.01$), and cytochrome c ($P < 0.01$) was higher than the wild type after swimming exercise. In *Prkag3*$^{-/-}$ mice, mRNA expression of CPT1b and cytochrome c was lower than the wild type ($P < 0.01$) after swimming. After recovery, mRNA expression of lipid metabolic genes was similar among *Tg-Prkag3*^{225Q}, *Prkag3*$^{-/-}$, and wild-type mice, with the exception of HAD, which was reduced in *Prkag3*$^{-/-}$ mice ($P < 0.05$). When comparing mRNA expression levels after swimming and after 2.5 h of recovery in the same genotype, LPL1 was increased in wild-type ($P < 0.05$) and *Prkag3*$^{-/-}$ ($P < 0.01$) mice. However, LPL1 ($P < 0.01$), CPT1b ($P < 0.05$), HAD ($P < 0.01$), and cytochrome c ($P < 0.05$) were decreased in *Tg-Prkag3*^{225Q} mice.

Expression of genes regulating glucose metabolism in white gastrocnemius muscle was also assessed (Fig. 5). mRNA levels of GLUT4 ($P < 0.01$), HKII ($P < 0.05$), and glycogen synthase ($P < 0.01$) were higher in *Tg-Prkag3*^{225Q} mice after swimming when compared with wild-type mice. After recovery from swimming, GLUT4 and HKII mRNA were markedly increased in wild-type mice ($P < 0.05$ and $P < 0.01$, respectively) when compared with after swimming. The transcriptional induction of GLUT4 and HKII during recovery is blunted in *Prkag3*$^{-/-}$ mice. When comparing mRNA expression of genes after swimming and after 2.5 h of recovery in the same genotype, GLUT4 ($P < 0.01$), HKII ($P < 0.05$), and glycogen synthase ($P < 0.01$) mRNA were decreased in *Tg-Prkag3*^{225Q} mice.

DISCUSSION

We determined the role of AMPKγ3 in exercise-induced metabolic and gene regulatory responses in skeletal muscle. Using *Prkag3*^{225Q} and *Prkag3*$^{-/-}$ mice, we provide evidence that AMPK activation alters glucose handling and fatty acid metabolism, thereby increasing glycogen resynthesis after endurance exercise. We have previously reported that glycogen resynthesis after exercise is accelerated in *Tg-Prkag3*^{225Q} mice and blunted in *Prkag3*$^{-/-}$ mice (15). Thus, the AMPKγ3 isoform plays a major role in modulating intramuscular fuel utilization toward fat oxidation during exercise and rapid glycogen resynthesis during recovery. Furthermore, the AMPKγ3 isoform plays a critical role in transcription of genes regulating lipid and glucose metabolism after acute endurance exercise and recovery.

Glucose uptake, a rate limiting step for glycogen resynthesis, is unaltered among *Tg-Prkag3*^{225Q}, *Prkag3*$^{-/-}$, and wild-type mice either immediately after acute exercise or after 2.5 h of recovery. This was an unexpected observation because glycogen content is markedly elevated in *Tg-Prkag3*^{225Q} and reduced in *Prkag3*$^{-/-}$ mice after recov-

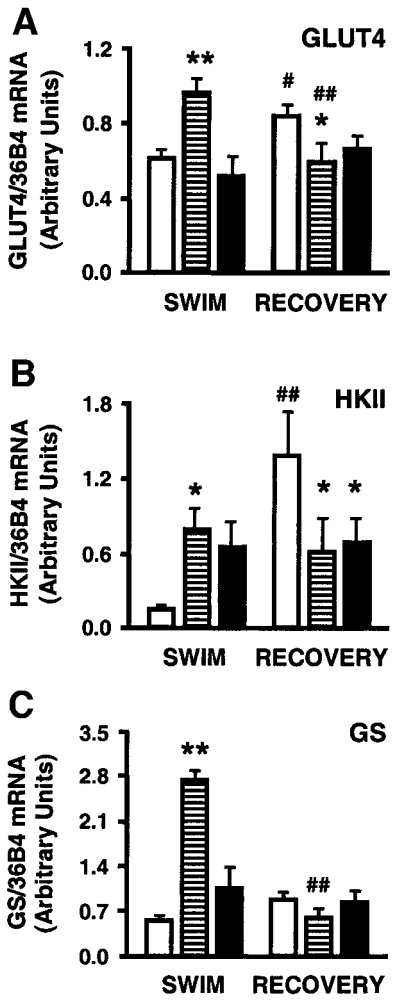

FIG. 5. mRNA expression of genes regulating glucose metabolism in skeletal muscle. mRNA expression of GLUT4 (*A*), HKII (*B*), and glycogen synthase (GS) (*C*) was determined in white gastrocnemius muscle (n = 9–10 per group) from wild-type (□), *Tg-Prkag3*^{225Q} (▤), and *Prkag3*$^{-/-}$ (■) mice immediately after exercise or recovery (2.5 h). Results are expressed as the ratio of mRNA expression of respective gene to 36B4. **$P < 0.01$, *$P < 0.05$ vs. wild type of same condition; ##$P < 0.01$, #$P < 0.05$ vs. same genotype from swim. Data are means ± SE.

ery when compared with the wild-type mice (15). However, a similar uncoupling between glucose transport and accelerated glycogen synthesis has been observed in mice overexpressing a constitutively active form of glycogen synthase in skeletal muscle (24,25), whereby the repartitioning of intracellular glucose intermediates toward glycogen synthesis after muscle contraction is enhanced (26). Glucose oxidation after intense anaerobic activity is markedly lower in *Tg-Prkag3*^{225Q} and higher in *Prkag3*$^{-/-}$ mice, respectively, (27), indicating a shift toward glucose incorporation into glycogen. However, in response to the 2-h endurance exercise bout, glucose oxidation was similar between *Tg-Prkag3*^{225Q} and wild-type mice and reduced in *Prkag3*$^{-/-}$ mice. Thus, any potential difference in glucose oxidation among the genotypes may be masked by an increased demand on lipid oxidation during steady-state endurance exercise. Collectively, these studies reveal muscle glycogen supercompensation can occur without excessive glucose transport, presumably through alterations in glucose handling between glucose oxidation and glycogenesis.

The contribution of fatty acid oxidation to total energy supply increases during long duration exercise. Thus, the metabolic shift toward utilization of fatty acids has a sparing effect on glucose utilization. Phosphorylation of ACC regulates the entry of fatty acids into the mitochondrial matrix. Immediately after swimming, ACC phosphorylation was increased in *Tg-Prkag3*^{225Q} mice, suggestive of increased fatty acid availability for oxidation. Triglyceride content was reduced in skeletal muscle from *Tg-Prkag3*^{225Q} mice directly after swimming. ACC phosphorylation and triglyceride content in skeletal muscle from *Prkag3*$^{-/-}$ mice were similar to wild-type mice. Thus, the *Prkag3*^{225Q} mutation, rather than the presence of a functional AMPKγ3 subunit, directly promotes a metabolic shift toward fatty acid utilization in response to exercise.

Exercise regulates transcriptional events in skeletal muscle partly through activation of AMPK. Chronic stimulation of AMPK increases protein expression of GLUT4, hexokinase (28), and the oxidative enzyme cytochrome c (19,28). We have previously determined the expression of several metabolic genes in skeletal muscle from wild-type, *Tg-Prkag3*^{225Q}, and *Prkag3*$^{-/-}$ mice under fed and fasted conditions, and we provide evidence AMPK plays a role in the coordinated expression of genes involved in lipid and glucose metabolism (20). Here, we observed a concerted upregulation of mRNA expression of genes involved in fatty acid availability (LPL1), transport into the mitochondria (CPT1b), and oxidation (cytochrome c and HAD) in *Tg-Prkag3*^{225Q} mice compared with wild-type mice. The enhanced transcriptional response and phosphorylation of ACC in *Prkag3*^{225Q} mice was associated with increased utilization of triglyceride, as was evident from the reduction in triglyceride content after swimming. In contrast, mRNA expression of genes involved in fatty acid metabolism in *Prkag3*$^{-/-}$ mice, including CPT1b and cytochrome c, was diminished, with a tendency for reduced expression of LPL and HAD after swimming. Therefore, the AMPKγ3 subunit plays a role in modulating transcription of lipid metabolic genes and, importantly, lipid metabolism during endurance exercise. Nonetheless, expression of mRNA for lipid metabolic genes in *Tg-Prkag3*^{225Q} and *Prkag3*$^{-/-}$ mice was normalized to wild-type levels after recovery. Essentially, an enhanced response in transcription of lipid metabolic genes in *Tg-Prkag3*^{225Q} mice and a diminished transcriptional response in *Prkag3*$^{-/-}$ mice compared with wild-type mice was observed after swimming.

The transition between fed and fasted conditions promotes gene regulatory responses in skeletal muscle. We have previously observed a coordinated decrease in the mRNA expression of HKII and glycogen synthase in skeletal muscle from *Prkag3*^{225Q} versus wild-type mice (20). Here, we report that mRNA expression of GLUT4, HKII, and glycogen synthase after exercise was higher in *Tg-Prkag3*^{225Q} mice compared with wild-type mice. Thus, gene regulatory changes in *Tg-Prkag3*^{225Q} mice are largely influenced by fasting and exercise. Change at the level of mRNA occurs in parallel with metabolic changes. An elevation in transcript levels of genes important for glycogen synthesis is consistent with the enhanced glycogen supercompensation of *Prkag3*^{225Q} mice (27). The elevated expression of HKII, GLUT4, and glycogen synthase in *Prkag3*^{225Q} mice during swimming was reduced after recovery, concomitant with the elevation in skeletal muscle glycogen content (15). After recovery from swimming, GLUT4 and HKII mRNA were increased in wild type when compared with the level immediately after swimming. In contrast, the transcriptional induction of GLUT4 and HKII

during recovery is blunted in Prkag3$^{-/-}$ mice. Thus, dysregulation of lipid and glucose metabolic gene expression in *Prkag3*$^{-/-}$ mice provides evidence that the AMPKγ3 subunit plays an essential role in coordinating the transcription of lipid and glucose metabolic genes in response to metabolic challenges that include fasting, exercise, and recovery in skeletal muscle. Moreover, the *Prkag3*^{225Q} mutation, rather than the presence of a functional AMPKγ3 subunit enhances the transcriptional response to metabolic challenges in skeletal muscle.

In conclusion, AMPK activation achieved by overexpression of the *Prkag3*^{225Q} mutation, rather than the presence of a functional AMPKγ 3 subunit, promotes fuel repartitioning and gene regulatory responses to facilitate lipid oxidation during endurance exercise and glycogen storage during recovery. Furthermore, the transcriptional and metabolic profile of the *Prkag3*$^{-/-}$ mice diverges from that of the wild-type mice, suggesting that the AMPKγ3 subunit plays a role in coordinating gene regulatory responses to exercise and recovery. Collectively, these results further support strategies aimed to activate AMPK in skeletal muscle as a means to improve impaired lipid and glucose homeostasis in metabolic disease.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Research Council, the Swedish Diabetes Association, the Swedish Strategic Research Foundation, the Foundation for Scientific Studies of Diabetology, Novo Nordisk, the Swedish National Center for Research in Sports, Arexis, and the Commission of the European Communities (contracts LSHM-CT-2004-005272 EXGENESIS and LSHM-CT-2004-512013). B.R.B. has received a graduate fellowship from the Swedish National Center for Research in Sports. J.W. has received a Hallas Møller Grant from the Novo Nordisk foundation.

REFERENCES

1. Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB: Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. *J Biol Chem* 272:13255–13261, 1997
2. Koistinen HA, Galuska D, Chibalin AV, Yang J, Zierath JR, Holman GD, Wallberg-Henriksson H: 5-Amino-imidazole carboxamide riboside increases glucose transport and cell-surface GLUT4 content in skeletal muscle from subjects with type 2 diabetes. *Diabetes* 52:1066–1072, 2003
3. Bergeron R, Russell RR III, Young LH, Ren JM, Marcucci M, Lee A, Shulman GI: Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am J Physiol* 276:E938–E944, 1999
4. Henin N, Vincent MF, Gruber HE, Van den Berghe G: Inhibition of fatty acid and cholesterol synthesis by stimulation of AMP-activated protein kinase. *FASEB J* 9:541–546, 1995
5. Bolster DR, Crozier SJ, Kimball SR, Jefferson LS: AMP-activated protein kinase suppresses protein synthesis in rat skeletal muscle through down-regulated mammalian target of rapamycin (mTOR) signaling. *J Biol Chem* 277:23977–23980, 2002
6. Hardie DG, Hawley SA: AMP-activated protein kinase: the energy charge hypothesis revisited. *Bioessays* 23:1112–1119, 2001
7. Aronson D, Violan MA, Dufresne SD, Zangen D, Fielding RA, Goodyear LJ: Exercise stimulates the mitogen-activated protein kinase pathway in human skeletal muscle. *J Clin Invest* 99:1251–1257, 1997
8. Widegren U, Jiang XJ, Krook A, Chibalin AV, Bjornholm M, Tally M, Roth RA, Henriksson J, Wallberg-Henriksson H, Zierath JR: Divergent effects of exercise on metabolic and mitogenic signaling pathways in human skeletal muscle. *FASEB J* 12:1379–1389, 1998
9. Wu H, Rothermel B, Kanatous S, Rosenberg P, Naya FJ, Shelton JM, Hutcheson KA, DiMaio JM, Olson EN, Bassel-Duby R, Williams RS: Activation of MEF2 by muscle activity is mediated through a calcineurin-dependent pathway. *EMBO J* 20:6414–6423, 2001
10. Mason SD, Howlett RA, Kim MJ, Olfert IM, Hogan MC, McNulty W, Hickey RP, Wagner PD, Kahn CR, Giordano FJ, Johnson RS: Loss of skeletal muscle HIF-1alpha results in altered exercise endurance. *PLoS Biol* 2:e288, 2004
11. Wright DC, Hucker KA, Holloszy JO, Han DH: Ca^{2+} and AMPK both mediate stimulation of glucose transport by muscle contractions. *Diabetes* 53:330–335, 2004
12. Mu J, Brozinick JT Jr, Valladares O, Bucan M, Birnbaum MJ: A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol Cell* 7:1085–1094, 2001
13. Mu J, Barton ER, Birnbaum MJ: Selective suppression of AMP-activated protein kinase in skeletal muscle: update on 'lazy mice.' *Biochem Soc Trans* 31:236–241, 2003
14. Milan D, Jeon JT, Looft C, Amarger V, Robic A, Thelander M, Rogel-Gaillard C, Paul S, Iannuccelli N, Rask L, Ronne H, Lundstrom K, Reinsch N, Gellin J, Kalm E, Roy PL, Chardon P, Andersson L: A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. *Science* 288:1248–1251, 2000
15. Barnes BR, Marklund S, Steiler TL, Walter M, Hjalm G, Amarger V, Mahlapuu M, Leng Y, Johansson C, Galuska D, Lindgren K, Abrink M, Stapleton D, Zierath JR, Andersson L: The 5′-AMP-activated protein kinase gamma3 isoform has a key role in carbohydrate and lipid metabolism in glycolytic skeletal muscle. *J Biol Chem* 279:38441–38447, 2004
16. Andersson L: Identification and characterization of AMPK gamma 3 mutations in the pig. *Biochem Soc Trans* 31:232–235, 2003
17. Ren JM, Semenkovich CF, Gulve EA, Gao J, Holloszy JO: Exercise induces rapid increases in GLUT4 expression, glucose transport capacity, and insulin-stimulated glycogen storage in muscle. *J Biol Chem* 269:14396–14401, 1994
18. Holmes BF, Kurth-Kraczek EJ, Winder WW: Chronic activation of 5′-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol* 87:1990–1995, 1999
19. Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, Shulman GI: AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci U S A* 99:15983–15987, 2002
20. Long YC, Barnes BR, Mahlapuu M, Steiler TL, Martinsson S, Leng Y, Wallberg-Henriksson H, Andersson L, Zierath JR: Role of AMP-activated protein kinase in the coordinated expression of genes controlling glucose and lipid metabolism in mouse white skeletal muscle. *Diabetologia*. In press (DOI 10.1007/s00125-005-1962-5)
21. Ryder JW, Kawano Y, Galuska D, Fahlman R, Wallberg-Henriksson H, Charron MJ, Zierath JR: Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT4-deficient mice. *FASEB J* 13:2246–2256, 1999
22. Wallberg-Henriksson H, Zetan N, Henriksson J: Reversibility of decreased insulin-stimulated glucose transport capacity in diabetic muscle with in vitro incubation: insulin is not required. *J Biol Chem* 262:7665–7671, 1987
23. Mahlapuu M, Johansson C, Lindgren K, Hjalm G, Barnes BR, Krook A, Zierath JR, Andersson L, Marklund S: Expression profiling of the gamma-subunit isoforms of AMP-activated protein kinase suggests a major role for gamma3 in white skeletal muscle. *Am J Physiol Endocrinol Metab* 286:E194–E200, 2004
24. Azpiazu I, Manchester J, Skurat AV, Roach PJ, Lawrence JC Jr: Control of glycogen synthesis is shared between glucose transport and glycogen synthase in skeletal muscle fibers. *Am J Physiol Endocrinol Metab* 278:E234–E243, 2000
25. Manchester J, Skurat AV, Roach P, Hauschka SD, Lawrence JC Jr: Increased glycogen accumulation in transgenic mice overexpressing glycogen synthase in skeletal muscle. *Proc Natl Acad Sci U S A* 93:10707–10711, 1996
26. Fogt DL, Pan S, Lee S, Ding Z, Scrimgeour A, Lawrence JC Jr, Ivy JL: Effect of glycogen synthase overexpression on insulin-stimulated muscle glucose uptake and storage. *Am J Physiol Endocrinol Metab* 286:E363–E369, 2004
27. Barnes BR, Glund S, Long YC, Hjälm G, Andersson L, Zierath JR: 5′-AMP–activated protein kinase regulates skeletal muscle glycogen content and ergogenics. *FASEB J* 19:773–779, 2005
28. Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO: Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 88:2219–2226, 2000