Genetic impairment of AMPKα2 signaling does not reduce muscle glucose uptake during treadmill exercise in mice

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Maarbjerg SJ, Jørgensen SB, Rose AJ, Jeppesen J, Jensen TE, Treebak JT, Birk JB, Schjerling P, Wojtaszewski JF, Richter EA. Genetic impairment of AMPKα2 signaling does not reduce muscle glucose uptake during treadmill exercise in mice. Am J Physiol Endocrinol Metab 297:E924–E934, 2009. First published August 4, 2009; doi:10.1152/ajpendo.90653.2008.—Some studies suggest that the 5'-AMP-activated protein kinase (AMPK) is important in regulating muscle glucose uptake in response to intense electrically stimulated contractions. However, it is unknown whether AMPK regulates muscle glucose uptake during in vivo exercise. We studied this in male and female mice overexpressing kinase-dead AMPKα2 (AMPK-KD) in skeletal and heart muscles. Wild-type and AMPK-KD mice were exercised at the same relative or the same absolute intensity. In agreement, surface membrane content of the glucose transporter GLUT4 was increased similarly in AMPK-KD and wild-type muscle in response to running. Glucose clearance was not lower in AMPK-KD muscles compared with wild-type regardless of whether animals were exercised at the same relative or the same absolute intensity. In agreement, surface membrane content of the glucose transporter GLUT4 was increased similarly in AMPK-KD and wild-type muscle in response to running. We also measured signaling of alternative exercise-sensitive pathways that might be compensatorielly increased in AMPK-KD muscles.

AMPK activity is increased in human and rodent muscles in a time- and intensity-dependent manner during exercises such as bicycling (4, 5, 39, 54, 55). The activation of AMPK in response to electrically induced contractions is retained in in situ perfused rat hindlimb muscles and in isolated ex vivo incubated muscles, suggesting that the activation is regulated mainly by local mechanisms in rodent muscle (6, 18, 20, 24). In agreement, in humans performing one-legged exercise, muscle AMPK activity increased only in the working leg and not in the contralateral resting leg, implying that activation of AMPK during exercise is regulated mainly by local mechanisms also in human muscle (29).

Several studies have investigated whether AMPK is necessary to increase glucose uptake in contracting muscle, but the data generated to date do not give a clear picture. Genetic animal models, in which a single catalytic or regulatory AMPK subunit has been knocked out (α1, α2, or γ3), reveal essentially normal glucose uptake during intermittent tetanic electrical stimulation of ex vivo incubated muscles (3, 24), although recently, AMPKα1-knockout was shown to decrease twitch-stimulated glucose uptake (21). Two transgenic models overexpressing kinase-dead AMPKα2 constructs have been studied as well (14, 37), and both of these models have substantially suppressed AMPKα2 activity and partially suppressed AMPKα1 activity in most muscle types. The study by Fujii et al. (14) suggested a normal glucose uptake in hindlimb muscles of anesthetized mice during electrical stimulation in vivo and reduced glucose uptake in isolated extensor digitorum longus muscles ex vivo. However, the latter finding was confounded by a reduced tetanic force development of the transgenic muscles. In contrast, three reports on the transgenic mouse model generated and used in studies by Jensen et al. (20), Lefort et al. (32), and Mu et al. (37) clearly suggest a partial role of AMPK signaling in the regulation of glucose uptake in both soleus and extensor digitorum longus muscle. They showed in this transgenic mouse an impaired increase in muscle glucose uptake in response to electrically induced contractions during ex vivo incubations and in anesthetized

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mice in vivo. Importantly, these decreases in glucose uptake occurred without an accompanying impairment of force production (20, 37). On the basis of these observations, it appears that AMPK plays a partial role in regulating muscle glucose uptake in response to electrically induced contractions. Moreover, when the upstream AMPK kinase LKB1 in mouse muscle is knocked out, both the covalent AMPK activation and glucose uptake are impaired in response to electrically induced contractions in ex vivo incubated muscles (26, 46). If this association between impaired AMPK activation and impaired glucose uptake is causal, it adds to the idea of an essential role for AMPK in contraction-induced muscle glucose transport.

As previously outlined (23), electrical stimulation of skeletal muscles induces intense isometric contraction quite different from the conditions during in vivo exercise, and it is still unknown whether AMPK is important in regulating muscle glucose uptake during physiological contraction regimes. To address this question, we exercised wild-type (WT) mice and mice that overexpress a kinase-dead form of AMPKα2 in muscle by treadmill running at the same absolute and relative intensities and measured skeletal muscle glucose uptake using tracer techniques.

METHODS

Animals. Animals used were 4- to 6-mo-old C57Bl/6 mice overexpressing a muscle-specific, dominant negative, kinase-dead AMPKα2 construct, as described by Mu and colleagues (36, 37), and corresponding WT littermates. Catheterized mice, mice used for surface membrane GLUT4 content measurements, and mice used for the AICAR in vivo experiment were males, and mice used for intraperitoneal (ip) tracer injections were females. The mice used for the time course on AMPK activity were 3- to 4-mo-old C57Bl/6 males. Mice were maintained on a 10:14-h light-dark cycle and received standard rodent chow (Altromin no. 1324; Chr. Pedersen, Ringsted, Denmark) and water ad libitum. All animals were euthanized by cervical dislocation and gastrocnemius, and blood samples were collected after 27 min of running in total, except that plasma level of [14C]mannitol was kept constant by continuous infusion at 0.005 μCi/min (10 μl/min) in saline. Calculation of muscle glucose uptake was based on muscle accumulation of 1H activity corrected for extracellular space, using 14C activity as extracellular marker similarly to incubated and perfused muscles (24, 52). Specific muscle glucose uptake was calculated by using average plasma 3H activity and average plasma glucose concentration, calculated as area under curve from the last three plasma samples (15, 20, and 25 min), as described (11, 27). The specific muscle glucose uptake was expressed as glucose clearance by relating to the average plasma glucose concentration.

Exercise protocol for ip injected mice. At day −3, the maximal running capacity was determined for each mouse. During the running test, the speed was increased by 3 m/min every 2nd minute starting at 11 m/min until exhaustion, which was defined as the speed at which the mouse was incapable of running despite encouragement by air gun. On the experimental day, fed mice were randomized into either a nonexercised group or an exercised group. The exercised mice ran at 30 or 70% of their maximal running capacity for 30 min, and nonexercised/resting mice were placed in the treadmill apparatus while it was turned off. In addition, a group of WT mice ran at 50% of their maximal running capacity to obtain a group that was comparable in terms of absolute running speed (16 m/min) to the kinase-dead AMPKα2 (AMPK-KD) mice running at 70% of their maximal running capacity. Blood samples (∼35 μl) were collected from the tail at time point −2, 15, and 30 min during the exercise/rest period. During exercise, mice were quickly removed from the treadmill for <1 min at the indicated time points to collect tail blood. Blood samples were placed at 4°C and centrifuged to collect plasma. Immediately before starting exercise, mice were given an ip injection with a bolus of saline (800 μl/100 g body wt) containing 0.1 mM 2-deoxyglucose and 60 μCi/ml 2-[14C]deoxyglucose corresponding to ∼12 μCi/mouse. After 30 min, mice were euthanized by cervical dislocation, and gastrocnemius and quadriceps muscles were quickly removed, freeze clamped, and stored at −80°C until being processed further. Plasma glucose concentration and specific activity were measured as described above of sample deproteinization, using 0.1 M Ba(OH)2 and 0.1 M ZnSO4 as described (12).

AICAR protocol for ip injected mice. Fed mice were given an ip injection with a bolus of saline (800 μl/100 g body wt) with or without AICAR (500 mg/kg body wt). Fifteen minutes later, the mice were given a second ip injection with a bolus of saline (800 μl/100 g body wt)
was calculated by using average plasma 3H activity, calculated as area of the two aliquots. 2-[3H]deoxyglucose-6-phosphate was added to the supernatant after the kinase reaction, and in samples from the ip injected mice this was measured from the mixed supernatant and the beads after the kinase reaction.

Muscle glycogen. Muscle glycogen content was determined as glycosyl units after acid hydrolysis (41).

Creatine phosphate. Muscle content of creatine phosphate and creatine was measured by fluorometry in 3-M PCA extracts from wet muscles (30 min at 0°C) neutralized with 2 M KHC03, as described previously (33).

Statistics. Data are expressed as means ± SE. Statistical evaluations were performed by either Student’s t-test or two-way ANOVA with Student-Newman-Keuls post hoc test where appropriate. Statistical evaluation of exercise performance was performed with the nonparametric Mann-Whitney test. Differences between groups were considered statistically significant if P < 0.05.

RESULTS

Muscle glucose clearance in exercising catheterized mice. We first investigated the role of AMPK in muscle glucose clearance using mice with carotid artery and jugular vein catheters for blood sampling and infusion of glucose. This model has the advantage of allowing sampling of blood and infusion of isotopes while the animal is exercising, but the model is also characterized by some impairments in exercise capacity. In WT mice, glucose clearance was increased in the quadriceps (Quad), gastrocnemius (Gast), and soleus muscle in response to treadmill running by 260, 500, and 170%, respectively (Fig. 1A). In AMPK-KD muscles, glucose clearance increased by at least the same degree as in WT muscles (Fig. 1A). In Gast and soleus, glucose clearance was similar in AMPK-KD and WT both at rest and during exercise, whereas glucose clearance in AMPK-KD Quad in general was 1.9-fold higher than WT (Fig. 1A). Thus, impairments in activation of muscle AMPK did not reduce muscle glucose clearance during treadmill running of catheterized mice. On the contrary, glucose clearance tended to be increased in the Quad of AMPK-KD mice (P = 0.07).

Muscle glycogen in exercised catheterized mice. Glycogen was decreased in response to treadmill running in the three muscle types investigated (Fig. 1B). In WT Quad, Gast, and soleus, glycogen content was decreased by 40, 60, and 70%, respectively, in response to treadmill running (Fig. 1B). In AMPK-KD muscles, glycogen content was similar to WT at rest, but the decrease in response to exercise was greater than observed for WT in Quad and Gast, although the decrease was comparable with WT in soleus (Fig. 1B).

Muscle AMPK signaling in exercised catheterized mice. Phosphorylation at Ser277 at the AMPK target ACCβ was measured as a marker of endogenous AMPK signaling. In Quad, ACCβ phosphorylation (ACCβ phos) was similar between WT and AMPK-KD muscles at rest and increased with exercise by 380% in WT muscles, whereas it remained unchanged in AMPK-KD muscles (Fig. 2). In gastrocnemius, ACCβ phos was generally ~50% lower in AMPK-KD than
WT muscles and increased by 40–50% in both genotypes with exercise (Fig. 2). In soleus, ACC\(^\beta\) phos was at rest 50% lower in AMPK-KD muscle than WT and increased only slightly in AMPK-KD muscles with exercise (Fig. 2). These data indicate that AMPK signaling was increased in WT Quad and Gast but not in soleus during treadmill running and, importantly, that AMPK-KD muscles had reduced ACC\(^\beta\) phos. A recent study has suggested the existence of alternative exercise-sensitive ACC\(^\beta\) kinases (9), and therefore, ACC\(^\beta\) phos does not necessarily only reflect AMPK signaling.

**Exercise capacity test of the AMPK-KD mouse.** In accordance with earlier reports characterizing the AMPK-KD mice as “lazy” (36), it was noticed that AMPK-KD mice were exercise intolerant, and a treadmill exercise capacity test of noncatheterized animals was conducted to examine this further. As seen in Fig. 3, the maximum speed achieved by WT

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**Fig. 1.** Glucose clearance and glycogen in muscles from exercised catheterized mice. A: glucose clearance (ml g\(^{-1}\) min\(^{-1}\)) in homogenates from quadriceps, gastrocnemius, and soleus from catheterized wild-type (WT) and AMPK-kinase-dead (KD) mice at rest and during treadmill running. B: muscle glycogen (\(\mu\)mol g\(^{-1}\) wet wt\(^{-1}\)) in homogenates from muscles from WT and AMPK-KD mice at rest and after treadmill running; \(n = 4–5\). †Significantly different from rest (\(P < 0.05\)); *significantly different from WT (\(P < 0.05\)); (\()^\ast\)tendency toward a difference from WT (\(P = 0.07\)); _____main effect. Data are presented as means ± SE.

**Fig. 2.** Acetyl-CoA carboxylase-\(^\beta\) Ser\(^{227}\) phosphorylation (ACC\(^\beta\) phos; % WT rest) in lysates from quadriceps, gastrocnemius, and soleus muscles from WT and AMPK-KD mice; \(n = 4–5\). † and ††Significantly different from rest (\(P < 0.05, P < 0.001\)); *significantly different from WT (\(P < 0.05\)); _____main effect. Data are presented as means ± SE. AU, arbitrary units.
mice was 34 m/min, and this measurement was reduced by one-third in AMPK-KD mice.

**Muscle glucose clearance in exercising ip injected mice.** Because we found that the AMPK-KD mice had a reduced exercise capacity, we conducted a second experiment investigating whether AMPK had a role in muscle glucose clearance if adjusting for this impairment. Mice were exercised at either 30 or 70% of their individual maximal running speed or at a similar absolute running speed (16 m/min). Glucose clearance was measured by injecting tracers into the peritoneal cavity of noncatheterized mice rather than by systemic infusion as above to avoid the deleterious effect of catheterization on running performance. Using this approach, we found that muscle glucose clearance at rest was comparable in the two genotypes in both muscles investigated (Fig. 4A). In WT Quad and Gast, glucose clearance increased in response to treadmill running in an intensity-dependent manner. The increase in general was similar in WT and the AMPK-KD muscle (Fig. 4A). In addition, we ran mice at the same absolute speed, which was equivalent to ~70% of the maximal running speed in AMPK-KD and ~50% in WT mice, and in accord with observations in catheterized animals (Fig. 1A) we found that, if anything, glucose clearance in Quad and Gast tended to be higher in AMPK-KD than in WT (Fig. 4, A and B). These findings collectively show that muscle glucose clearance is not reduced in AMPK-KD muscles during in vivo exercise even when correcting for impaired exercise performance of the AMPK-KD mouse.

**Plasma glucose and specific activity in exercising ip injected mice.** Blood glucose levels were similar in WT and AMPK-KD mice immediately before the onset of exercise and increased with exercise in both genotypes but with a slightly greater increase in AMPK-KD mice (Table 1). Blood glucose was also increased over time in resting animals, most likely due to stress associated with handling and sitting in the treadmill (Table 1). In rested and exercised mice, plasma activity of 2-[3H]deoxyglucose and glucose was 8.000–9.000 disintegrations·m in−1·μmol−1 15 min after injection, and the activity was reduced by ~40% 30 min after injection (Table 1).

**Muscle glycogen and creatine phosphate in exercised ip injected mice.** Muscle glycogen in the Quad was decreased in both genotypes with increasing exercise intensities, with the greatest decrease of 50% after running at 70% of maximal running speed (Fig. 4B). Although the relative decreases were similar between genotypes, the glycogen level was generally lower in AMPK-KD muscles (Fig. 4B). When exercising mice at the same absolute intensity, muscle glycogen was not significantly reduced in WT mice, but it was reduced by 50% in AMPK-KD muscle (Fig. 4B), in agreement with observations in the catheterized mice (Fig. 1B). Creatine phosphate content was measured in the Quad muscle and remained at resting levels in both genotypes after running at 30% of maximal running speed (Fig. 4C). When exercising mice at the same absolute intensity, muscle creatine phosphate content remained at resting levels in both genotypes (Fig. 4B). As glycogen and creatine phosphate in both genotypes were depleted at similar rates during exercise, the energetic challenges of the investigated muscles seem to be well matched between genotypes when they are exercised at the same relative intensities.

**Muscle AMPK signaling in exercised ip injected mice.** In WT mice, AMPKα2 activity was increased by 50 and 140% in response to running at 30 and 70% of maximal running speed, respectively (Fig. 5A). As anticipated, AMPKα2 activity was substantially reduced in AMPK-KD muscles and was not increased during exercise at either 30 or 70% of maximal running speed (Fig. 5A). In WT mice, AMPKα1 activity remained at basal levels during exercise at 30% of maximal running speed and was modestly higher during exercise at 70% of maximal running speed (Fig. 5B). AMPKα1 activity was generally slightly reduced in AMPK-KD muscles compared with WT and did not increase with exercise (Fig. 5B).

ACCβ phosphorylation remained at basal levels when exercising took place at 30% of maximal running speed but was increased similarly by ~60% in both genotypes in response to exercising at 70% (Fig. 5C). The overall phosphorylation level of ACCβ was 20–25% lower in AMPK-KD muscles compared with WT (Fig. 5C). When exercised at the same absolute running intensity, ACCβ phos was increased to the same level in WT and AMPK-KD muscles (Fig. 5C).

**Time course-dependent activation of AMPK with exercise.** To verify that AMPK was activated when muscle glucose clearance during treadmill running was measured, we conducted a time course experiment in WT mice and estimated AMPK activity as AMPKα Thr172 phosphorylation (35). AMPKα Thr172 phosphorylation remained at basal levels in Quad and Gast after 1 min of treadmill running (Fig. 5D) and was increased by 85 and 50% after 5 min and by 100 and 70%, respectively, after 30 min of running (Fig. 5D).

**GLUT4 surface membrane content.** Translocation of GLUT4 to the surface membrane is essential to facilitate muscle glucose uptake during exercise (13, 59). The total content of muscle GLUT4 was measured in lysates and was reduced by ~30% in AMPK-KD muscles compared with WT (Fig. 6A). We subsequently measured the GLUT4 content in a surface membrane fraction (PM) and found that the PM GLUT4 enrichment was increased by ~50% with exercise and that the increase was similar in both genotypes (Fig. 6B). When correcting for the total content of muscle GLUT4, the relative increase in PM
GLUT4 content with exercise was still similar between the two genotypes (Fig. 6C).

CaMKII and MAPK signaling in ip injected mice. CaMKII, Trisk95, p38 MAPK, and ERK1/2 phosphorylation were measured to assess possible compensatory increases of these exercise-sensitive kinases in response to lack of AMPK activation.

(see Fig. 7E for representative blots). In WT mice, CaMKII Thr287 and Trisk95 phosphorylation, a target of CaMKII (43), were increased by ~40 and 700%, respectively, in response to running at 70% of maximal running speed, and these responses were maintained in AMPK-KD muscles (Fig. 7, A and B). There was a tendency toward a general reduction in CaMKII Thr287 phosphorylation in AMPK-KD muscle but no genotype effect on Trisk95 phosphorylation (Fig. 7, B). When exercised at the same absolute running intensity, phosphorylation of CaMKII and Trisk95 still increased similarly in the two genotypes (Fig. 7, A and B).

MAPK kinase signaling, expressed as activating phosphorylation of p38 and ERK1/2 isoforms, was increased with exercise. In WT mice, p38 Thr180/Tyr182 and ERK1/2 Thr202/Tyr204 phosphorylations were increased by 330 and 90%, respectively, in response to running at 70% of maximal running speed (Fig. 7, C and D). Although p38 phosphorylation was increased to nearly the same extent in AMPK-KD as in WT muscles, no significant increase in ERK1/2 phosphorylation was detected in AMPK-KD muscle but no genotype effect on Trisk95 phosphorylation (Fig. 7, A and B). When exercised at the same absolute running intensity, phosphorylation of CaMKII and Trisk95 still increased similarly in the two genotypes (Fig. 7, A and B).
response to in vivo exercise in the AMPK-KD mouse is in contrast to the previous reports of partially reduced muscle glucose uptake in response to electrically induced contractions ex vivo in the same mouse model (20, 32, 37). Therefore, we performed an in vivo AICAR experiment to validate that AMPK-regulated muscle glucose clearance was compromised in AMPK-KD muscles using our in vivo glucose uptake method. We found that AICAR in this in vivo setting increased muscle glucose clearance, with \( \frac{95}{100} \) in both WT Quad and Gast, and importantly, AICAR failed to increase glucose clearance in AMPK-KD muscles (Fig. 8), in accord with previous observations ex vivo (14, 24, 37). This finding shows that AMPK-regulated muscle glucose uptake in vivo is abolished in AMPK-KD muscles.

DISCUSSION

AMPK is activated during muscle contraction and is an important protector of energy homeostasis (22, 23). Previous studies have shown that a substantial impairment of AMPK signaling results in a partial reduction of muscle glucose uptake in response to electrically induced contractions ex vivo in the same mouse model (20, 32, 37). Therefore, we performed an in vivo AICAR experiment to validate that AMPK-regulated muscle glucose clearance was compromised in AMPK-KD muscles using our in vivo glucose uptake method. We found that AICAR in this in vivo setting increased muscle glucose clearance, with \( \sim 95\% \) in both WT Quad and Gast, and importantly, AICAR failed to increase glucose clearance in AMPK-KD muscles (Fig. 8), in accord with previous observations ex vivo (14, 24, 37). This finding shows that AMPK-regulated muscle glucose uptake in vivo is abolished in AMPK-KD muscles.

It has been proposed for many years that AMPK is an essential signaling factor regulating contraction-stimulated muscle glucose uptake during ex vivo conditions and during more physiological conditions, and in many cases AMPK signaling corresponds well with glucose uptake (17, 18, 38). However, correlations do not necessarily prove causality, and in some cases, clear dissociation of AMPK activity and glucose uptake has been reported. For instance, in muscle from exercising humans, changes in AMPK signaling and glucose uptake do not correlate well (54). Furthermore, in humans undertaking low-intensity bicycle exercise, muscle glucose uptake

![Fig. 5. AMPK activity and ACCβ phos.](image-url)
increases (51) but AMPK activity is unaltered (5, 49, 50). Also, models of isolated muscles from animals have reported cases of dissociations. Studies of the red oxidative rat soleus have shown that if AMPK activation is suppressed by glycogen loading prior to electrically stimulated contraction, glucose uptake is still increased despite no increase in AMPK activity (6). The latter observation is in accord with the observations in soleus muscle in the present study, where AMPK signaling remained at basal levels despite a more than twofold increase in glucose clearance during exercise (Fig. 1). It could be argued that AMPK has more profound roles in glycolytic muscle types since AMPK in general is activated more robustly in response to AICAR and contraction in glycolytic muscles than in oxidative muscles (1, 6, 24, 52). Both quadriceps and gastrocnemius muscle are to a large degree composed of glycolytic fibers (2), and in the present study,
AMPKα2 activity and glucose clearance were greatly increased with exercise in WT muscles. In muscle from AMPK-KD mice, however, no increases in AMPKα2 and AMPKα1 activity were observed, but glucose uptake was still increased normally. This was true regardless of whether the comparison was based on exercise at the same relative intensities of 30 and 70% or the same absolute intensity. In fact, if any phenotype was detected, this was an enhanced glucose clearance in AMPK-KD muscle (Figs. 1 and 4). Thus, these observations may suggest that despite AMPK being activated during running in glycolytic muscle types, it does not have a significant role in regulating glucose uptake during low and moderate intensities.

It is well established that muscle glucose uptake is negatively related to the muscle glycogen content (7, 42, 52, 54), and AMPK-KD muscles had a somewhat lower glycogen level than WT, especially after exercise at the same absolute intensity was completed, and the further enhancement of glucose clearance in AMPK-KD muscles may be related to the lower level of muscle glycogen. Regardless, although muscle glycogen may have contributed to a greater responsiveness of glucose clearance to exercise in AMPK-KD muscle, it does not impinge on the finding that muscles deficient in AMPK were well capable of regulating muscle glucose uptake during exercise. This notion is supported by measurements of GLUT4 (Fig. 6), which showed that, although AMPK-KD muscles had a lower expression level of GLUT4 protein, the ability to maintain GLUT4 content in the surface membranes during exercise was not compromised despite no increases in AMPK activity.

The maintained increase in AMPK-KD muscle glucose uptake is in contrast to previous findings using the same or a comparable mouse model, but in these studies muscle contraction was stimulated electrically ex vivo in incubated muscle and in vivo in hindlimb muscles via electrodes attached to the sciatic nerve (14, 20, 32, 37). It should be noted that the metabolic and mechanical stress imposed on the muscle fiber by electrical stimulation and treadmill running may be quite different. Electrical stimulation induces isometric contractions of all muscle fibers within the muscle simultaneously, and this results in severe muscle fatigue and a pronounced depletion of muscle glycogen in response to a cumulative contraction period of only a few minutes (14, 24, 48). On the other hand, during treadmill running, recruitment of muscle fibers to contract is intermittent, muscle glycogen is depleted at a much slower rate (Ref. 25 and Figs. 1B and 4B), and the animal can be exercised for hours before exhaustion. Thus, perhaps the reliance on AMPK in contraction-stimulated glucose uptake is evident only during very intense contraction protocols, such as that induced by electrical stimulation, and not significant during more physiological conditions such as the treadmill exercise protocols applied in the present study.

It could be envisaged that alternative mechanisms were able to substitute for AMPK by a compensatory increase in signaling, a view also of relevance when using genetically modified animals with the potential risk of secondary adaptations to the congenital transgenic intervention. Both the CaMKII (43, 44) and MAPK (28, 53) kinases have been reported to be activated in skeletal muscle by exercise, and activation of CaMKII in particular has been suggested to be an important signaling factor in contraction-stimulated glucose uptake (19, 40, 56, 57). We estimated CaMKII activation by measuring Thr287 phosphorylation of CaMKII and threonine phosphorylation of its substrate Trisk95 (43) and found that CaMKII signaling was increased similarly in WT and AMPK-KD muscles. As for MAPK kinase signaling, we did not observe any compensatory increases in p38 and ERK1/2 phosphorylation in AMPK-KD muscles. In fact, ERK was increased only in WT and not in AMPK-KD muscles when exercise at the same relative intensity occurred, likely explained by a higher ERK phosphorylation at rest in the AMPK-KD mice. The absence of compensation in these signaling pathways could be argued to support that increased AMPK activity in muscle is not necessary for increasing glucose uptake during exercise of a moderate intensity.

While this study was in review, Lee-Young et al. (31) published an article in which exercise metabolism in AMPK-KD mice originating from the same founder mice as in the present study was studied. Although they reported similar running impairment in the KD mice as in the present study, in contrast to our findings they reported decreased muscle glucose uptake in KD mice when the mice exercised at the same relative exercise intensity as the WT mice. They attributed this primarily to decreased glucose delivery during exercise due to impaired muscle perfusion rather than to decreased fractional uptake, likely indicating that the GLUT4 translocation process was normal in their AMPK-KD mice, as in the present study. The discrepancy between the two studies is not easily explained, but in their study Lee-Young et al. (31) used catheterized mice, whereas in the present study we initially used similarly catheterized mice but in the second part of the study switched to noncatheterized mice because we observed some running impairment due to the catheterization procedure. Another apparent difference is that, although in the study by Lee-Young et al. (31) both AMPKα1 and AMPKα2 activity in skeletal muscle were barely detectable, in our KD mice AMPKα2 activity was also barely detectable, but AMPKα1 activity was not markedly reduced. Still, AMPKα1 activity did not increase with exercise as it did in the WT. It cannot be excluded that this apparent difference in AMPKα1 activity in conjunction with ablated

**Fig. 8.** Glucose clearance in homogenates from quadriceps and gastrocnemius muscles from WT and AMPK-KD mice at basal or in response to 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) stimulation; n = 6–10. †Significantly different from basal (P < 0.05). Data are presented as means ± SE.
AMPKa2 activity in the study by Lee-Young et al. (31) might lead to vascular abnormalities to a greater extent than in our mice. In this light the impairment in glucose uptake during exercise in the study by Lee-Young et al. (31) is likely due to chronic alterations in muscle perfusion, whereas there are no indications of compromised glucose delivery in the present study.

In summary, we showed that increases in glucose clearance in oxidative and glycolytic muscle types were maintained during treadmill exercise in mouse muscle specifically expressing a kinase-dead AMPKa2 construct in heart and skeletal muscle. Because increases in surface membrane GLUT4 content were also normal in AMPK-KD muscle with exercise, it appears that mechanisms regulating GLUT4 trafficking in response to exercise were intact in AMPK-KD muscles. In addition, we measured the CaMKII, p38, and ERK pathway to assess whether these pathways had adapted to the lack of AMPK activation by enhanced signaling, but neither of these signaling factors were activated more in AMPK-KD than in WT muscle. Collectively, these findings suggest that AMPKa2 is not important in regulating glucose uptake in muscle during exercise of a moderate intensity and that other mechanisms must have a central role. These findings do not exclude that AMPK may be required to increase muscle glucose uptake during intense exercise, but they do contrast reports of decreased glucose uptake in the AMPK-KD muscles ex vivo following electrically stimulated contraction. This emphasizes the importance of supplementing ex vivo studies with in vivo exercise studies, since signaling components deemed necessary ex vivo may not be required in the integrated in vivo exercise setting.

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GRANTS

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