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Obesity impairs skeletal muscle AMPK signaling during exercise: role of AMPK α 2 in the regulation of exercise capacity *in vivo*

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Abstract

Objective—Skeletal muscle AMP-activated protein kinase (AMPK) α 2 activity is impaired in obese, insulin resistant individuals during exercise. We determined whether this defect contributes to the metabolic dysregulation and reduced exercise capacity observed in the obese state.

Design—C57BL/6J wild-type (WT) mice and/or mice expressing a kinase dead AMPK α 2 subunit in skeletal muscle (α 2-KD) were fed chow or high fat (HF) diets from 3–16 weeks (wks) of age. At 15wks mice performed an exercise stress test to determine exercise capacity. In WT mice, muscle glucose uptake and skeletal muscle AMPK α 2 activity was assessed in chronically catheterized mice (carotid artery/jugular vein) at 16wks. In a separate study, HF-fed WT and α 2-KD mice performed 5wks of exercise training (from 15–20wks of age) to test whether AMPK α 2 is necessary to restore work tolerance.

Results—HF-fed WT mice had reduced exercise tolerance during an exercise stress test, and an attenuation in muscle glucose uptake and AMPK α 2 activity during a single bout of exercise ($p < 0.05$ vs. chow). In chow-fed α 2-KD mice running speed and time were impaired ~45% and ~55%, respectively ($p < 0.05$ vs. WT chow); HF feeding further reduced running time ~25% ($p < 0.05$ vs. α 2-KD chow). In response to 5wks of exercise training, HF-fed WT and α 2-KD mice increased maximum running speed ~35% ($p < 0.05$ vs. pre-training) and maintained body weight at pre-training levels, whereas body weight increased in untrained HF WT and α 2-KD mice. Exercise training restored running speed to levels seen in healthy, chow-fed mice.

Conclusion—HF feeding impairs AMPK α 2 activity in skeletal muscle during exercise *in vivo*. While this defect directly contributes to reduced exercise capacity, findings in HF-fed α 2-KD

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CONFLICT OF INTEREST

All authors declare that there are no competing financial interests in relation to the work described.

mice show that AMPK α 2-independent mechanisms are also involved. Importantly, α 2-KD mice on a HF-fed diet adapt to regular exercise by increasing exercise tolerance, demonstrating that this adaptation is independent of skeletal muscle AMPK α 2 activity.

Keywords

obesity; muscle glucose uptake; exercise tolerance; exercise training; diet

INTRODUCTION

Obesity is a primary risk factor for disease states associated with the Metabolic Syndrome (i.e. Type 2 diabetes and cardiovascular disease), and regular physical exercise has long been recognized as an essential component in the treatment of obesity¹. A limiting factor for exercise as a therapeutic option is that obesity is characterized by impaired exercise tolerance, yet the limiting factor(s) responsible for this phenomenon is unclear. One enzyme that appears to play a significant role in the promotion of exercise tolerance is the serine-threonine AMP-activated protein kinase (AMPK), whose activation is increased in response to elevations in metabolic cellular stress². In lean otherwise healthy mice expressing a kinase-dead form of the catalytic AMPK α 2 subunit in skeletal muscle (α 2-KD), exercise tolerance is markedly impaired^{3,4}. AMPK α 2 activity is also impaired in skeletal muscle of obese individuals during moderate intensity exercise⁵; however, whether this defect directly contributes to the reduced exercise capacity observed under obese conditions is unknown.

Here we utilized wild type (WT) and α 2-KD mice to elucidate the role of AMPK α 2 in the regulation of exercise tolerance under high fat (HF) fed, obese conditions. We hypothesized that (1) diet-induced obesity would significantly impair skeletal muscle AMPK α 2 activity during exercise *in vivo*, and (2) that this impairment in skeletal muscle AMPK α 2 activity significantly contributes to exercise intolerance. Regular physical activity is a common therapeutic recommendation for obese individuals⁶. Thus, we also tested whether a five week exercise training regimen would be effective in increasing exercise tolerance in HF-fed WT and α 2-KD mice.

METHODS

Animal Maintenance

All procedures were approved by the Vanderbilt University Animal Care and Use Committee. Male and female C57BL/6J α 2-KD mice⁷ and WT littermate mice were studied. Twenty-one days after birth, littermates were separated by gender and maintained in microisolator cages. Following separation, mice were fed a standard chow (5.5% fat by weight; 5001 Laboratory Rodent Diet, Purina, USA) or HF diet (35.5% fat by weight; diet F3282, Bio-Serv Inc., Frenchtown, USA) and had access to water *ad libitum* until they were 16–20 weeks of age.

Exercise Stress Test

In chow- and HF-fed WT and α 2-KD mice, peak oxygen consumption ($\dot{V}O_{2peak}$) was assessed at 15 weeks of age using an exercise stress test protocol as previously described³.

Briefly, following a 10 min acclimation period two days prior to the stress test, mice were placed in an enclosed single lane treadmill connected to Oxymax oxygen and carbon dioxide sensors (Columbus Instruments, Columbus, USA). Following a 30 min basal period, mice commenced running at $10 \text{ m} \cdot \text{min}^{-1}$ on a 0% incline. Running speed was increased by $4 \text{ m} \cdot \text{min}^{-1}$ every 3 min until mice reached exhaustion, defined as the point whereby mice remained at the back of the treadmill on an electric shock pad for 5s. Body weight was measured prior to the $\dot{V}\text{O}_{2\text{peak}}$ test, and body composition was assessed using a mq10 NMR analyzer (Bruker Optics, The Woodlands, USA). All oxygen consumption measurements were expressed per kilogram of lean body mass (kg_{LBM}).

Acute Exercise

To examine whether a HF diet impaired skeletal muscle glucose uptake and AMPK α 2 activation during acute exercise in WT mice, 5hr fasted 16 week old chow- and HF-fed WT mice either remained sedentary on a treadmill or performed a single bout of treadmill exercise for 30 min at $16 \text{ m} \cdot \text{min}^{-1}$ (0% incline). Five days prior to the exercise bout mice were chronically catheterized (carotid artery and jugular vein) as described previously^{3,8}. Fasting blood glucose was determined after the 5hr fast ($t = 0 \text{ min}$) using a hand held glucose monitor (Accu-Chek Advantage, Roche, USA). At $t = 5 \text{ min}$ mice were injected with a bolus containing $13 \mu\text{Ci}$ of 2- ^{14}C deoxyglucose (2- ^{14}C DG) into the jugular vein. Arterial blood samples were collected at $t = 10, 15, 20$ and 30 min for measurement of glucose and plasma 2- ^{14}C DG levels. At $t = 30 \text{ min}$, mice were anaesthetized with an intravenous injection of pentobarbital (3mg) and the gastrocnemius muscle was rapidly dissected, freeze-clamped in liquid nitrogen, and stored at -80°C until analysis.

Exercise Training

At 15 weeks of age, HF-fed WT and α 2-KD mice performed an exercise stress test, and then were separated into two groups. One group performed exercise training for five weeks, while the other remained sedentary for five weeks. The exercise training utilized a gradual overload protocol and ran for five days per week. Specifically, running time and speed were increased such that by the end of weeks 16, 17, 18, 19, and 20 mice were running on a treadmill for 60 min at 50, 60, 70, 80 and 90% of maximum pre-training running speed, respectively. Sedentary mice were placed on an apparatus placed just above the treadmill in an adjacent lane to exercising mice. Following the five week period, both groups repeated the exercise stress test performed at week 15.

Plasma and Tissue Analyses

Plasma 2- ^{14}C DG radioactivity was assessed following deproteinization, and accumulation of phosphorylated 2- ^{14}C DG in the gastrocnemius was determined as previously described³. Indices for glucose metabolism (R_g) and clearance (K_g) were determined in chow- and HF-fed WT mice as previously described³.

Whole cell lysates were prepared as previously described³ using a lysis buffer containing 50mM Tris-HCl (pH 7.5), 1mM EDTA, 1mM EGTA, 10% glycerol, 1% Triton X-100, 1mM DTT, 1 $\times$ HALT protease and phosphatase inhibitor cocktail (Thermo Scientific, USA). Nuclear and cytosolic fractions were prepared using the methods of McGee et al.⁹.

Gastrocnemius samples were homogenized in Buffer A (250 μ M sucrose, 10mM NaCl, 3mM MgCl, 1mM DTT, 1 $\times$ HALT protease and phosphatase inhibitor cocktail) using a hand-held pellet pestle and centrifuged at 500 g. The supernatant (cytosolic fraction) was snap frozen in liquid nitrogen and stored at -80°C . The pellet was resuspended in Buffer B (50mM Tris-HCl (pH 7.5), 1mM EDTA (pH 8.0), 1mM EGTA, 10% glycerol, 1% Triton X-100, 50mM MgCl, 1mM DTT, 1 $\times$ HALT protease and phosphatase inhibitor cocktail), kept on ice for 10 min and centrifuged at 3000 g. The resulting supernatant (nuclear fraction) was snap frozen and stored at -80°C . Characterization of the nuclear and cytosolic fraction was verified via immunoblotting, with histone 1 (Abcam, USA) being enriched in nuclear fractions, and glyceraldehydes-3-phosphate dehydrogenase (GAPDH; Abcam) being enriched in cytosolic fractions.

AMPK Activity Assay

AMPK α 2 was immunoprecipitated using 200 μ g of protein, 2 μ g of a rabbit AMPK α 2 polyclonal antibody (Abcam), and immobilized Recomb protein A beads (Thermo Scientific). AMPK activity in the immune complexes were measured for 20 min at 30°C (within the pre-determined linear range) in the presence of 200 μ M AMP, and calculated as picomoles of phosphate incorporated into the SAMS peptide (100 μ M; GenWay Biotech, USA) per min per mg of protein subjected to immunoprecipitation.

Immunoblotting

AMPK α 2 protein expression was determined from immunoprecipitated samples from whole cell lysates, or from 20 μ g of nuclear and 40 μ g of cytosolic fractions. Acetyl-CoA carboxylase (ACC) β expression and phosphorylation was assessed from 200 μ g of whole cell lysate that had been affinity purified overnight at 4°C using streptavidin agarose beads (Invitrogen, USA). Proteins were separated using NuPAGE 4–12% Bis-Tris Gels (Invitrogen) and transferred to PVDF membranes. Blots were probed with an anti-AMPK α 2 goat polyclonal antibody (1:100; Santa Cruz, USA), anti-AMPK α Thr¹⁷² rabbit polyclonal antibody (1:100; Cell Signaling, USA), or an anti-ACC β Ser²²¹ rabbit polyclonal antibody (1:100; Cell Signaling). Antibody binding was detected with an IRDyeTM 800-conjugated anti-goat and anti-rabbit IgG secondary antibody (Rockland, USA). ACC β protein expression was detected using IRDyeTM 800-labeled streptavidin (Rockland).

Statistical Analyses

Data are mean $\pm$ standard error of the mean (S.E.M). Statistical analysis was performed using a student t-test, one-way analysis of variance (ANOVA), or two-way repeated measures ANOVA where appropriate with the statistical software package SigmaStat. If the ANOVA was significant ($P < 0.05$), specific differences were located using Fisher's least significant difference (LSD) test.

RESULTS

Obesity impairs exercise capacity

At 16 weeks of age HF-fed WT mice weighed more than chow-fed WT mice (37 ± 2 vs. 24 ± 2 g, $p < 0.001$) due to greater fat mass (33 ± 2 vs. $9 \pm 2\%$ body weight, $p < 0.001$). In response

to an exercise stress test, HF-fed WT mice were exercise intolerant as evidenced by impairments in running speed (30 ± 1 vs. 38 ± 1 m $\cdot$ min $^{-1}$ for HF and chow, respectively; $p < 0.001$), running time (17 ± 1 vs. 23 ± 1 min; $p < 0.001$), and a ~40% impairment in $\text{VO}_{2\text{peak}}$ (77 ± 6 vs. 127 ± 5 ml $\cdot$ kg $_{\text{LBM}}^{-1}$ $\cdot$ min $^{-1}$; $p < 0.05$).

Obesity impairs the exercise-induced increase in skeletal muscle glucose uptake and AMPK α 2 activity in vivo

In the sedentary state blood glucose was higher in HF-fed mice compared to chow-fed controls (10.7 ± 0.5 vs. 9.1 ± 0.9 mM during the 30 min sedentary period; $p < 0.05$). In response to 30min of treadmill exercise blood glucose significantly increased over time in chow- and HF-fed mice, although blood glucose was consistently elevated in HF-fed mice (Figure 1A). The gastrocnemius muscle glucose metabolic index (R_g ; Figure 1B) and gastrocnemius glucose clearance (K_g ; Figure 1C) increased ~6-fold and ~4-fold, respectively, during exercise in chow-fed mice. Increases with exercise were significantly attenuated in HF-fed mice.

Under basal conditions, no differences were observed for whole cell AMPK α 2 activity or AMPK α 2 Thr 172 phosphorylation in the gastrocnemius of chow- and HF-fed WT mice. Basal levels of whole cell ACC β Ser 221 phosphorylation were slightly, but not significantly elevated in HF-fed WT mice ($p = 0.3$ vs. chow-fed WT). AMPK α 2 and ACC β protein expression were similar between groups. In chow-fed WT mice, exercise significantly increased AMPK α 2 activity (Figure 1D), AMPK α 2 Thr 172 phosphorylation (Figure 1E), and ACC β Ser 221 phosphorylation (Figure 1F). In contrast, these exercise-induced increases were all ablated in HF-fed WT mice.

We next assessed whether the impairment in whole cell AMPK α 2 activity was localized to specific subcellular compartments. Nuclear AMPK α 2 activity in response to exercise tended to increase in chow-fed mice ($p = 0.09$ vs. sedentary); nuclear AMPK α 2 activity in response to exercise was significantly reduced in HF-fed mice compared to chow-fed mice (Figure 2A). Likewise, cytosolic AMPK α 2 activity increased during exercise in chow-fed mice, whereas no increase was seen in HF-fed mice (Figure 2B). The suppressed nuclear and cytosolic AMPK α 2 activity in HF-fed mice was not due to reduced AMPK α 2 protein expression (Figure 2C and 2D). Indeed, the exercise-induced increase in nuclear AMPK α 2 activity of chow-fed WT mice occurred despite lower protein levels of AMPK α 2.

AMPK-dependent and -independent factors regulate exercise capacity in obese states

Our findings above demonstrate that a HF diet impairs exercise capacity during an exercise stress test. To determine the degree to which the impaired exercise capacity in HF-fed mice was directly dependent upon impaired activation of AMPK α 2, chow- and HF-fed α 2-KD mice performed an exercise stress test. α 2-KD mice were utilized as AMPK α 2 activity is essentially abolished in skeletal muscle of these mice both at rest and in response to moderate intensity exercise *in vivo*³. In α 2-KD mice HF-feeding resulted in similar changes in body composition to that of HF-fed WT mice (data not shown). As expected³, chow-fed α 2-KD mice had impairments in running speed and running time compared to chow-fed WT mice (~45 and ~55% reduction, respectively; $p < 0.001$). $\text{VO}_{2\text{peak}}$ during the exercise stress

test was also reduced in chow-fed $\alpha 2$ -KD vs. WT mice (Figure 3); however, $\dot{V}O_{2peak}$ for chow-fed $\alpha 2$ -KD mice was higher than that observed in HF-fed WT mice. HF feeding further reduced $\dot{V}O_{2peak}$ in $\alpha 2$ -KD mice; in fact, no significant increase in $\dot{V}O_2$ was observed during the exercise stress test (Figure 3). HF feeding also reduced running time in $\alpha 2$ -KD mice (7.6 ± 0.9 vs. 10.0 ± 0.9 min for HF-fed and chow $\alpha 2$ -KD mice, respectively; $p < 0.05$), while there was a tendency for reduced maximum running speed (18 ± 1 vs. 21 ± 1 m $\cdot$ min $^{-1}$; $p = 0.06$).

In chow- and HF-fed $\alpha 2$ -KD mice the increase in $\dot{V}O_2$ ($\dot{V}O_{2max}$) during the exercise stress test was 22 ± 4 and 5 ± 2 ml $\cdot$ kg $_{LBM}^{-1} \cdot$ min $^{-1}$, respectively. As AMPK $\alpha 2$ is not activated in these mice, the difference between these variables (17 ± 2 ml $\cdot$ kg $_{LBM}^{-1} \cdot$ min $^{-1}$) represents an AMPK $\alpha 2$ -independent component with regards to $\dot{V}O_2$ during an exercise stress test. In WT mice, $\dot{V}O_2$ was impaired by 40 ± 2 ml $\cdot$ kg $_{LBM}^{-1} \cdot$ min $^{-1}$ in response to HF feeding. Therefore, ~45% of this impairment (i.e. ~17 ml $\cdot$ kg $_{LBM}^{-1} \cdot$ min $^{-1}$) in HF-fed WT mice is due to AMPK $\alpha 2$ -independent mechanisms (i.e. the remaining ~55% of the impaired $\dot{V}O_2$ in HF-fed WT mice is AMPK $\alpha 2$ -dependent). The contributions of AMPK $\alpha 2$ -dependent and independent components are shown in Figure 4.

Exercise training improves exercise capacity of HF-fed mice independently of AMPK $\alpha 2$ in skeletal muscle

To determine whether normal AMPK $\alpha 2$ activity is necessary for a training-induced increase in exercise capacity, HF-fed WT and $\alpha 2$ -KD mice performed five weeks of exercise training or remained sedentary beginning at 15 weeks of age. Sedentary WT and $\alpha 2$ -KD mice significantly increased body weight over the five week period (Table 1), and had no improvement in maximum running speed (Figure 5). In contrast, at 20 weeks trained HF-fed WT mice maintained body weight at pre-training levels (Table 1), and increased maximum running speed by $34 \pm 6\%$ (Figure 5). Indeed, maximum running speed returned to levels observed in lean, healthy untrained WT mice. While in absolute terms running speed remained impaired in HF-fed $\alpha 2$ -KD mice post-training, trained $\alpha 2$ -KD mice had a similar relative increase in maximum running speed ($35 \pm 6\%$ vs. week 15) when compared with trained WT mice. As with HF-fed WT mice, trained HF-fed $\alpha 2$ -KD mice were able to maintain body weight at pre-training levels (Table 1). Thus, mice expressing a catalytically inactive AMPK $\alpha 2$ subunit in skeletal muscle respond normally to exercise training. The fact that $\alpha 2$ -KD mice elicited a similar relative increase in exercise capacity post-training, and were also able to maintain body weight, shows that these adaptations to training occur independently of skeletal muscle AMPK $\alpha 2$.

DISCUSSION

Regular physical exercise has long been recognized as an essential component in the treatment of obesity, due to the beneficial effects on weight maintenance/reduction, and lowering the risk of developing Type 2 diabetes and cardiovascular disease^{1,6}. Here we show that skeletal muscle AMPK $\alpha 2$ plays a significant role in one's ability to fully utilize exercise as a preventative or interventional treatment option. Indeed, we show that the suppression in AMPK $\alpha 2$ activation observed during acute exercise under obese, insulin

resistant conditions plays a significant role in the impaired exercise capacity also observed under these conditions. However, the physiological adaptation to exercise training occurs regardless of whether AMPK α 2 is activated, as a catalytically inactive AMPK α 2 subunit does not attenuate the relative increase in exercise capacity seen following five weeks of exercise training. Thus, while AMPK α 2 plays a key role in the regulation of exercise capacity, factors independent of AMPK α 2 are also involved.

In the present study we utilized an exercise stress test, a non-invasive tool that has long been used to diagnose certain disease states in humans^{10,11}, to identify AMPK α 2-dependent and -independent components involved in the regulation of exercise capacity. Both diet- and genotype-specific effects were observed in the present study. Taken together with previous observations in lean healthy mice expressing a catalytically inactive AMPK α 2 subunit^{3,7,12}, as well as other genetically modified mice with impaired AMPK α 2 activation^{13,14} it can be seen that skeletal muscle AMPK α 2 clearly plays a role in the regulation of exercise capacity *in vivo*. Likewise, impaired AMPK α 2 activity in obese WT mice was also associated with a reduction in exercise capacity; however, our finding that HF feeding further reduced exercise capacity in α 2-KD mice shows that AMPK α 2-independent mechanisms also regulate exercise capacity. The relative contributions of AMPK-dependent and independent components are shown in Figure 4. Our data clearly show that the dysregulation of AMPK α 2 in skeletal muscle, whether in lean or obese states, significantly reduces exercise tolerance *in vivo*. This highlights the significant role of AMPK α 2 in the physiological response to exercise.

The finding that the increase in $\dot{V}O_2$ was fully suppressed during the exercise stress test in HF-fed α 2-KD mice is remarkable. It is unlikely that this phenomenon is due to cardiac dysfunction arising from expression of the α 2-KD transgene in cardiac muscle, as glucose uptake, heart rate, and cardiac output are normal during exercise in chow-fed α 2-KD mice³. We have shown that chow-fed α 2-KD mice exhibit vascular dysfunction, as evidenced by the inability to increase fractional cardiac output (assessed via microsphere deposition) in skeletal muscle during exercise³. It is possible that HF feeding *per se* exacerbated this impairment in vascular dysfunction in α 2-KD mice. Regardless, it is clear that these mice are heavily reliant on anaerobic sources in order to perform exercise; however, the factor(s) responsible for this phenomenon is unclear. While chow-fed α 2-KD mice also have a greater reliance on anaerobic sources during exercise – due to impairments in complex I and IV activities of the electron transport chain (ETC)³ – it has recently been shown that some of these differences are not apparent in HF-fed α 2-KD and WT mice¹⁵. Indeed, expression of different ETC complexes is actually increased in WT and α 2-KD mice following HF feeding¹⁵. Nevertheless, diet-induced obesity is characterized by enhanced release of reactive oxidative species and impaired mitochondrial function¹⁶; however, it is unclear whether AMPK α 2 plays a role in this process¹⁷. Thus, while the exact mechanism(s) accounting for the suppression of $\dot{V}O_2$ during the exercise stress test in HF-fed α 2-KD mice is unclear, it is highly likely that there is an element of vascular dysfunction.

An important finding from the present study was that AMPK α 2 activity was impaired in different subcellular compartments (i.e. nuclear and cytosolic) of HF-fed WT mice during exercise. These differences were not due to reduced AMPK α 2 expression within these

compartments. Indeed, nuclear AMPK α 2 expression was reduced in chow-fed WT mice during exercise when compared to HF-fed WT mice, yet nuclear AMPK α 2 activity tended to be higher in chow-fed WT mice under these conditions. The functional result of impaired AMPK α 2 activity in these different compartments has important implications. Cell culture studies have shown that AMPK is at least partially required for GLUT-4 transcription¹⁸. Likewise, the exercise-induced increase in PGC-1 α mediated nuclear-encoded mitochondrial genes is impaired in obese versus lean individuals, a phenomenon associated with impaired AMPK activation during exercise in obese individuals¹⁹. In contrast, evidence from global AMPK α 2 knockout mice suggests that AMPK α 2 is not required for certain metabolic adaptations to exercise training. Indeed, in *ampk α 2^{-/-}* mice key enzymes involved in glucose metabolism (i.e. GLUT4 and hexokinase (HK) II) increase in a similar manner to WT mice following 28 days of wheel running²⁰. Consistent with these findings, we show that the capacity to improve exercise performance is retained following exercise training in HF-fed mice with impaired AMPK α 2 activity.

As mentioned above, both HF-fed WT and α 2-KD mice are able to increase exercise capacity in response to exercise training. Given that AMPK α 2 activity is essentially abolished in α 2-KD mice³, this training-induced increase in exercise capacity clearly occurs via AMPK α 2-independent mechanisms. This is evidenced by the almost identical relative increase in maximum running speed between genotypes (~35%) when compared to respective pre-training levels. This is the first study to show that obese mice lacking a catalytically active AMPK α 2 subunit can increase exercise capacity in response to exercise training. In the present study, the training protocol employed involved mice exercising for the same amount of time at the same relative intensity. This meant that WT mice were constantly exercising at a greater absolute running speed than α 2-KD mice. We chose to exercise α 2-KD mice at the same relative work intensity as WT mice, since exercising at the same relative intensity elicits similar increases in muscle cellular stress between the two genotypes, whereas at the same absolute running speed cellular stress is lower in WT mice³. It should be noted that in the present study we assessed the extreme condition of HF fed mice with and without a mutated AMPK α 2 subunit. It is reasonable to assume that under the less extreme condition of chow feeding, lean mice would adapt to regular exercise as well. Importantly, we show that HF-fed obese mice were able to obtain a normal capacity to perform physical exercise after five weeks of training, despite reduced and even absent AMPK activity. This phenomenon is likely to be at least partially due to increases in mitochondrial markers, GLUT-4, and HKII, all of which increase in an AMPK α 2-independent manner following exercise training²⁰.

In skeletal muscle AMPK α 2 has been hypothesized as a key regulator of muscle glucose uptake. Here we demonstrate that in HF-fed WT mice a partial impairment in the exercise-induced increase in R_g occurred concomitantly with impaired activation of AMPK α 2. It is notable that the impaired AMPK α 2 activity in HF-fed WT mice occurred even though these mice exercised at a higher percentage of maximum running speed. HF-fed WT mice were exercising at ~50% of maximum running speed whereas chow-fed WT mice were exercising at ~40% of maximum running speed, further highlighting the inhibitory effect of HF feeding on skeletal muscle AMPK α 2 activation during exercise *in vivo*. Despite the impairment in

AMPK α 2 activity in HF-fed mice during exercise, it is unlikely that this phenomenon directly accounted for the reduction in the exercise-induced increase in R_g . Previous studies from our laboratory³ and others²¹ have demonstrated that the tissue extraction of glucose and GLUT-4 translocation during exercise in healthy α 2-KD mice occur at levels similar to that of WT mice. Rather, the attenuation of muscle glucose uptake during exercise in α 2-KD mice is due to an impaired fraction of cardiac output to skeletal muscle, and thus reduced glucose delivery, to skeletal muscle. It is notable that HF-fed WT mice, that have impaired AMPK α 2 activation during exercise, are also characterized by vascular dysfunction²².

In conclusion, principles of exercise testing were uniquely applied to mice expressing a kinase-dead AMPK α 2 protein in skeletal muscle to determine the mechanisms of exercise intolerance in response to HF feeding. The exercise intolerance arising from HF feeding was due to AMPK α 2-dependent and -independent dysfunction. The AMPK α 2-dependent exercise-intolerance in HF-fed mice was due to diminished AMPK α 2 activity in both nuclear and cytosolic fractions of skeletal muscle during exercise. Nevertheless, ablation of AMPK α 2 activity in skeletal muscle did not prevent the restoration of normal work tolerance in HF-fed mice in response to exercise training. This emphasizes the value of regular physical activity even in subjects in whom muscle AMPK α 2 activity is reduced, such as it is in obesity.

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REFERENCES

1. Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C, White RD. Physical Activity/Exercise and Type 2 Diabetes: A consensus statement from the American Diabetes Association. *Diabetes Care*. 2006; 29:1433–1438. [PubMed: 16732040]
2. Steinberg GR, Kemp BE. AMPK in Health and Disease. *Physiol Rev*. 2009; 89:1025–1078. [PubMed: 19584320]
3. Lee-Young RS, Griffie SR, Lynes SE, Bracy DP, Ayala JE, McGuinness OP, et al. Skeletal Muscle AMP-Activated Protein Kinase is Essential for the Metabolic Response to Exercise In Vivo. *J Biol Chem*. 2009; 284:23925–23934. [PubMed: 19525228]
4. Mu J, Barton ER, Birnbaum MJ. Selective suppression of AMP-activated protein kinase in skeletal muscle: update on 'lazy mice'. *Biochem Soc Trans*. 2003; 31:236–241. [PubMed: 12546693]
5. Sriwijitkamol A, Coletta DK, Wajcberg E, Balbontin GB, Reyna SM, Barrientes J, et al. Effect of Acute Exercise on AMPK Signaling in Skeletal Muscle of Subjects With Type 2 Diabetes: A Time-Course and Dose-Response Study. *Diabetes*. 2007; 56:836–848. [PubMed: 17327455]
6. Blaak EE, Saris WHM. Substrate oxidation, obesity and exercise training. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2002; 16:667–678. [PubMed: 12468414]
7. 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*. 2001; 7:1085–1094. [PubMed: 11389854]
8. Ayala JE, Bracy DP, McGuinness OP, Wasserman DH. Considerations in the Design of Hyperinsulinemic-Euglycemic Clamps in the Conscious Mouse. *Diabetes*. 2006; 55:390–397. [PubMed: 16443772]

9. McGee SL, Howlett KF, Starkie RL, Cameron-Smith D, Kemp BE, Hargreaves M. Exercise increases nuclear AMPK α 2 in human skeletal muscle. *Diabetes*. 2003; 52:926–928. [PubMed: 12663462]
10. Wasserman, K.; Hansen, JE.; Sue, DY.; Casaburi, R.; Whipp, BJ. Principles of exercise testing and interpretation. 3rd edn.. Baltimore: Lippincott Williams and Wilkins; 1999.
11. Wasserman K, Sietsema KE. Assessing cardiac function by gas exchange. *Cardiology*. 1988; 75:307–310. [PubMed: 3048668]
12. Fujii N, Seifert MM, Kane EM, Peter LE, Ho RC, Winstead S, et al. Role of AMP-activated protein kinase in exercise capacity, whole body glucose homeostasis, and glucose transport in skeletal muscle - insight from analysis of a transgenic mouse model. *Diabetes Res Clin Pract*. 2007; 77:S92–S98. [PubMed: 17452058]
13. Lee-Young RS, Ayala JE, Hunley CF, James FD, Bracy DP, Kang L, et al. Endothelial Nitric Oxide Synthase is Central to Skeletal Muscle Metabolic Regulation and Enzymatic Signaling during Exercise In Vivo. *Am J Physiol*. 2010; 298:R1399–R1408.
14. Sakamoto K, McCarthy A, Smith D, Green KA, Grahame Hardie D, Ashworth A, et al. Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J*. 2005; 24:1810–1820. [PubMed: 15889149]
15. Beck Jørgensen S, O'Neill H, Hewitt K, Kemp B, Steinberg G. Reduced AMP-activated protein kinase activity in mouse skeletal muscle does not exacerbate the development of insulin resistance with obesity. *Diabetologia*. 2009; 52:2395–2404. [PubMed: 19688337]
16. Anderson EJ, Lustig ME, Boyle KE, Woodlief TL, Kane DA, Lin C-T, et al. Mitochondrial H₂O₂ emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *J Clin Invest*. 2009; 119:573–581. [PubMed: 19188683]
17. Merry TL, Steinberg GR, Lynch GS, McConell GK. Skeletal muscle glucose uptake during contraction is regulated by nitric oxide and ROS independently of AMPK. *Am J Physiol*. 2010; 298:E577–E585.
18. McGee SL, van Denderen BJW, Howlett KF, Mollica J, Schertzer JD, Kemp BE, et al. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes*. 2008; 57:860–867. [PubMed: 18184930]
19. De Filippis E, Alvarez G, Berria R, Cusi K, Everman S, Meyer C, et al. Insulin-resistant muscle is exercise resistant: evidence for reduced response of nuclear-encoded mitochondrial genes to exercise. *Am J Physiol*. 2008; 294:E607–E614.
20. Jorgensen SB, Treebak JT, Viollet B, Schjerling P, Vaulont S, Wojtaszewski JF, et al. Role of AMPK α 2 in basal, training-, and AICAR-induced GLUT4, hexokinase II, and mitochondrial protein expression in mouse muscle. *Am J Physiol*. 2007; 292:E331–E339.
21. Maarbjerg SJ, Jorgensen SB, Rose AJ, Jeppesen J, Jensen TE, Treebak JT, et al. Genetic impairment of α 2-AMPK signaling does not reduce muscle glucose uptake during treadmill exercise in mice. *Am J Physiol*. 2009; 297:E924–E934.
22. Kim F, Pham M, Maloney E, Rizzo NO, Morton GJ, Wisse BE, et al. Vascular Inflammation, Insulin Resistance, and Reduced Nitric Oxide Production Precede the Onset of Peripheral Insulin Resistance. *Arterioscler Thromb Vasc Biol*. 2008; 28:1982–1988. [PubMed: 18772497]

Figure 1.

Blood glucose levels during exercise (A), and gastrocnemius glucose uptake (R_g ; B), glucose clearance (K_g ; C), AMP-activated protein kinase (AMPK) α 2 activity (D), AMPK α 2 Thr¹⁷² phosphorylation (E), and acetyl-CoA carboxylase (ACC) β Ser²²¹ phosphorylation at rest (SED) and in response to a 30 min bout of exercise (EX) in 16 week old C57BL/6J mice fed a chow or high-fat (HF) diet. Data are mean $\pm$ S.E.M for N=5–7 per group. In B–F data are expressed as fold-change from corresponding SED. ‡ main effect for chow vs. HF, $p < 0.05$; † $p < 0.05$ vs. corresponding SED; * $p < 0.05$ vs. corresponding chow.

Figure 2.

Nuclear AMP-activated protein kinase (AMPK) α2 activity (A), cytosolic AMPKα2 activity (B), nuclear AMPKα2 protein expression (C), and cytosolic AMPKα2 protein expression in gastrocnemius muscle of 16 week old C57BL/6J mice fed a chow or high-fat (HF) diet. Mice either remained sedentary (SED) or performed 30 min of treadmill exercise (EX). Data are mean ± S.E.M for N=6–8 per group. † $p < 0.05$ vs. corresponding SED; * $p < 0.05$ vs. corresponding chow.

Figure 3.

Oxygen consumption ($\dot{V}O_2$) at rest or exhaustion in 16 week old C57BL/6J mice. Wild-type (WT) and AMP-activated protein kinase $\alpha 2$ kinase-dead ($\alpha 2$ -KD) mice fed a chow or high-fat (HF) diet performed an exercise stress test on a treadmill (see *Methods*). Data are mean $\pm$ S.E.M for N=8–9 per group. † $p < 0.05$ vs. corresponding basal; * $p < 0.05$ vs. WT chow; ** $p < 0.05$ vs. WT chow and WT HF; *** $p < 0.05$ vs. WT chow, WT HF, and $\alpha 2$ -KD chow. kg_{LBM}, kilograms of lean body mass.

Figure 4.

Oxidative capacity during exercise is regulated via AMP-activated protein kinase (AMPK) $\alpha 2$ -dependent and -independent components. $\dot{V}O_2$, change in oxygen consumption; WT, wild-type; $\alpha 2\text{-KD}$, AMPK $\alpha 2$ kinase-dead; HF, high fat. kg_{LBM} , kilograms of lean body mass.

Figure 5.

Effect of 5wk exercise training on maximum running speed of high-fat (HF) fed wild-type (WT) and HF fed AMP-activated protein kinase $\alpha 2$ kinase-dead ($\alpha 2$ -KD) mice. Mice performed an exercise stress test at week 15, and then either remained sedentary or performed treadmill exercise training (see *Methods*) for 5wk. An exercise stress test was performed at week 20. Data for Chow (untrained) was taken from the acute exercise studies. Data are mean $\pm$ S.E.M for N=4–5 per group. † $p < 0.001$ vs. corresponding Week 15 group; ** $p < 0.001$ vs. corresponding sedentary Week 20 group; * $p < 0.001$ vs. HF WT.

Table 1

Body weight and body composition measurements of high-fat (HF)-fed C57BL/6J wild-type (WT) mice, and HF-fed C57BL/6J mice expressing a kinase-dead AMP-activated protein kinase $\alpha 2$ subunit ($\alpha 2$ -KD) in skeletal muscle. Mice either remained sedentary or performed treadmill exercise training from 15–20 weeks of age (see *Methods*).

	Body weight (g)	Fat mass (g)	Lean mass (g)
WT Sedentary			
Week 15	39 $\pm$ 5	12.8 $\pm$ 2.8	24.1 $\pm$ 1.6
Week 20	42 $\pm$ 5*	15.3 $\pm$ 2.9*	24.1 $\pm$ 2.1
WT Trained			
Week 15	35 $\pm$ 2	12.2 $\pm$ 1.6	21.0 $\pm$ 0.4
Week 20	32 $\pm$ 2	10.4 $\pm$ 1.6	19.8 $\pm$ 0.7
$\alpha 2$ -KD Sedentary			
Week 15	41 $\pm$ 5	14.6 $\pm$ 3.6	23.7 $\pm$ 1.6
Week 20	44 $\pm$ 5*	17.6 $\pm$ 3.8*	23.9 $\pm$ 1.8
$\alpha 2$ -KD Trained			
Week 15	36 $\pm$ 3	12.3 $\pm$ 2.5	21.9 $\pm$ 1.2
Week 20	36 $\pm$ 2	13.4 $\pm$ 1.8	20.9 $\pm$ 0.9

Data are mean $\pm$ S.E.M for N=4–5 per group.

*
 $p < 0.05$ vs. corresponding Week 15 value.