Nitric oxide and ROS regulate skeletal muscle glucose uptake during contraction independent of AMPK α2

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Introduction. The pathway(s) by which exercise regulates skeletal muscle glucose uptake are unclear, but unlike the insulin pathway, it is known to be intact in type 2 diabetics. Thus exercise is an effective treatment for type 2 diabetes, however many patients cannot or do not exercise regularly and rely heavily on blood glucose lowering medication. Currently pharmaceutical treatments for type 2 diabetes are limited in their specificity and effectiveness. Therefore, understanding how exercise simulates glucose uptake may lead to the development of new targeted pharmaceutical agents to aid in the treatment and possibly prevention of type 2 diabetes.
There is evidence that AMP-activated protein kinase (AMPK), nitric oxide (NO) and reactive oxygen species (ROS) regulate glucose uptake during exercise/contraction. Indeed, both NO and ROS have been proposed to increase glucose uptake via an AMPK-dependent mechanism, but disassociation also exist between ROS and NO stimulated glucose uptake and AMPK activation. This study investigated whether AMPKα2 is required for ROS and NO mediated glucose uptake during contraction.

Method. Male and female mice overexpressing a muscle specific AMPKα2 dominant negative transgene (AMPK DN) and wild type (WT) litter mates, aged 20-22 weeks were anesthetized and both hindlimb extensor digitorum (EDL) and soleus muscles were excised. Muscles were mounted in an incubation chamber filled with 30°C Krebs-Henseleit buffer (KHB) and oxygenated with 95% O_2 and 5% CO_2 gas. *Contracted muscles:* Following a 40 min incubation period muscles were electrically stimulated to contract for 10 min (350 ms (EDL) and 600 ms (soleus) duration, 60Hz, 12 contractions·min^{-1}) and glucose uptake was measured. During pre-contraction incubation and contraction, muscles were treated with either a NOS inhibitor (N^G-Monomethy-1-L-Arginine; L-NMMA, 100 μM), or a non-specific antioxidant (N-acetyl-L-cysteine; NAC, 20 mM). Contraction force was measured via proximal tendon suture attachment to a force transducer (PanLab, Spain). *Glucose uptake:* KHB was replaced with KHB containing radio labelled 2DG (2-Deoxy-D-glucose) and mannitol. *Muscle Analysis:* Muscles were analysed via western blot for AMPKα Thr^{172} phosphorylation, ACC-β Ser^{212} phosphorylation, total PAS-AS160 phosphorylation and nNOSμ expression.

Results. Contraction increased glucose uptake in EDL and soleus muscles of AMPK DN and WT to a similar extent (1.6-2.0-fold, $p < 0.05$ *vs* basal). In the EDL muscle, both L-NMMA and NAC attenuated the increase in glucose uptake during contraction by 50-60% ($p < 0.05$) in AMPK DN and WT muscles. NAC prevented the increase in glucose uptake in soleus muscles of AMPK DN and WT ($p > 0.05$ *vs* basal) but L-NMMA treatment had no effect ($p > 0.05$ *vs* contraction). Peak contraction force and rate of fatigue of EDL and soleus was similar between genotypes and was not affected by treatment ($p > 0.05$).

nNOSμ was expressed similarly in AMPK DN and WT muscles, with expression being greater in EDL than in the soleus ($p < 0.05$). AMPKα expression was ~2-fold greater in AMPK DN EDL and soleus muscles than in WT ($p < 0.05$ for genotype effect), however, AMPKα Thr^{172} phosphorylation relative to AMPKα expression was greater in WT than AMPK DN ($p < 0.001$ for genotype effect). Contraction increased WT (by ~4-7-fold, $p < 0.05$ *vs* basal) but not AMPK DN ($p > 0.05$ *vs* basal) EDL and soleus AMPKα Thr^{172} phosphorylation. AMPKα Thr^{172} phosphorylation was not affected by NAC or L-NMMA treatment for both muscles of both genotypes ($p > 0.05$). WT had greater ACCβ Ser^{212} phosphorylation than AMPK DN in both the EDL and soleus muscles ($p < 0.001$ for genotype effect). In the EDL contraction increased AMPK DN and WT ACCβ Ser^{212} phosphorylation (by ~1.5-fold, $p < 0.05$ *vs* basal) and NAC treatment prevented this increase in AMPK DN ($p > 0.05$ *vs* basal) but not WT. In the soleus, contraction increased WT (by ~1.5-fold, $p = 0.05$ *vs* basal) but not AMPK DN ACCβ Ser^{212} phosphorylation ($p = 0.17$ *vs* basal), and NAC treatment prevented the increase in WT ACCβ Ser^{212} phosphorylation ($p = 0.17$ *vs* basal). L-NMMA treatment did not affect AMPK DN or WT ACCβ Ser^{212} phosphorylation in either muscles ($p > 0.05$ *vs* basal). Total phosphorylation of PAS-AS160 was not affected by contraction or treatment in both the EDL and soleus muscle of AMPK DN and WT ($p > 0.05$). In the EDL, WT had a greater total phosphorylation of PAS-AS160 than AMPK DN ($p = 0.05$ for genotype effect).

Conclusion. AMPKα2 and AS160 are not essential in regulating skeletal muscle glucose uptake during contraction. NO and ROS appear to be involved in regulating skeletal muscle glucose uptake during contraction via an AMPKα2 independent mechanism.