AuPS/ASB Meeting - Adelaide 2010

Symposium: Skeletal muscle ROS: the good, the bad and the, well it kinda depends

Wednesday 1st December 2010 - Hickinbotham Hall - 09:30

Chair: Glenn McConell

Skeletal muscle ROS and glucose uptake during contraction

G.K. McConell[1] and T.L. Merry,[2] [1]Institute of Sport, Exercise and Active Living and the School of Biomedical and Health Sciences, Victoria University, VIC 8001, Australia and [2]Department of Sport and Exercise Science, University of Auckland, New Zealand.

Exercise stimulates skeletal muscle glucose uptake by increasing GLUT-4 translocation from intracellular vesicles to the cell membrane through a mechanism(s) that differs from insulin stimulation. Although the pathway(s) through which contraction stimulates skeletal muscle glucose uptake is unclear, there is evidence for separate and collective contribution of several signalling intermediates including AMP-activated protein kinase (AMPK), nitric oxide (NO), calcium/calmodulin-dependent kinase (CaMK) and more recently, reactive oxygen species (ROS).

Exposure of isolated skeletal muscle to exogenous ROS increases glucose uptake (Toyoda *et al.*, 2004). In addition, intense contraction of isolated mouse EDL muscle increases ROS production and the antioxidant N-acetylcysteine (NAC) attenuates increases in skeletal muscle glucose uptake (Sandstrom *et al.*, 2006). Although Sandstrom *et al.* presented evidence to suggest that AMPK may play a role in ROS-mediated glucose uptake during contraction, we have recently shown that the increase in glucose uptake during *ex vivo* contraction is attenuated by NAC similarly in wild type and AMPK kinase dead mouse muscle (Merry *et al.*, 2010c). hese results indicated that ROS regulate skeletal muscle glucose uptake during *ex vivo* contraction *via* AMPK-independent mechanisms. Interestingly, we have preliminary evidence suggesting that nitric oxide and ROS may interact during *ex vivo* contractions to regulate skeletal muscle glucose uptake, potentially *via* S-glutathionylation and/or peroxynitrite signalling.

Until recently the role of ROS in the regulation of contraction-stimulated skeletal muscle glucose uptake had only been examined using these isolated muscle models. In the absence of blood flow, such models depend on diffusion gradients for substrate delivery and clearance, and result in non-uniform delivery of oxygen to all muscle fibres. Furthermore, *ex vivo* muscle preparations generally involve supra-maximal highly fatiguing stimulation protocols. Therefore, we investigated the role of ROS signalling in the regulation of skeletal muscle glucose uptake during contraction/exercise in intact preparations by infusing NAC during moderate intensity *in situ* contractions in rats (Merry *et al.*, 2010a) and during exercise in humans (Merry *et al.*, 2010b). Unlike *ex vivo* contraction, we found that NAC did not affect skeletal muscle glucose uptake during contractions *in situ* and exercise *in vivo*. These results provide evidence that under physiological contraction/exercise conditions ROS may not be involved in the regulation of skeletal muscle glucose disposal and that previous results obtained using intense *ex vivo* contractions may not be relevant to normal exercise. However, more studies are required in this emerging area of interest before definitive conclusions can be drawn.

Merry TL, Dywer RM, Bradley EA, Rattigan S and McConell GK. (2010a) *Journal of Applied Physiology* **108,** 1275-1283.

Merry TL, Wadley GD, Stathis CG, Garnham AP, Rattigan S, Hargreaves M and McConell GK. (2010b) *Journal of Physiology* **588,** 1623-1634.

Merry TL, Steinberg GR, Lynch GS and McConell GK. (2010c) *American Journal of Physiology* **298,** E577-585.

Sandstrom ME, Zhang SJ, Bruton J, Silva JP, Reid MB, Westerblad H and Katz A. (2006) *Journal of Physiology* **575,** 251-262.

Toyoda T, Hayashi T, Miyamoto L, Yonemitsu S, Nakano M, Tanaka S, Ebihara K, Masuzaki H, Hosoda K, Inoue G, Otaka A, Sato K, Fushiki T and Nakao K. (2004) *American Journal of Physiology. Endocrinology and Metabolism* **287,** E166-173.

Effects of S-glutathionylation, S-nitrosylation and oxidation on Ca-sensitivity and force: a balancing act

G.D. Lamb, J.P. Mollica, T.L. Dutka, G.S. Posterino and R.M. Murphy, Department of Zoology, La Trobe University, Melbourne, VIC 3086, Australia.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important for skeletal muscle function both in physiological and pathological conditions. These agents are generated in active muscle and can induce both acute and long term effects on muscle function. Exposure of intact fast-twitch muscle fibres to the oxidant hydrogen peroxide (H_2O_2) affects force principally by altering myofibrillar Ca^{2+} sensitivity, initially producing increased sensitivity, followed by a decrease with more prolonged exposure (Andrade *et al.*, 1998). Experiments on skinned fibres show that these effects can be attributed to H_2O_2 interacting with glutathione and myoglobin, causing S-glutathionylation and oxidation of the contractile apparatus respectively (Lamb & Posterino, 2003; Murphy *et al.*, 2008). H_2O_2 can also oxidize the sarcoplasmic reticulum Ca^{2+} release channels, the ryanodine receptors, and studies on isolated channels show that this oxidation has a large stimulatory effect on Ca^{2+}-induced Ca^{2+} release. However, experiments on skinned and intact fibres show that acute H_2O_2-induced oxidation has little or no effect on action potential-induced Ca^{2+} release, the normal physiological process governing Ca^{2+} release (Posterino *et al.*, 2003). Application of nitric oxide donors, on the other hand, produce a decrease in submaximal force in skinned muscle fibres, due primarily to a decrease in myofibrillar Ca^{2+} sensitivity (Spencer & Posterino, 2009), brought about by S-nitrosylation of the contractile apparatus. More extensive exposure of muscle to oxidants or nitrosylating agents can also lead to a decrease in both maximum force and Ca^{2+}-sensitivity of the contractile apparatus, likely due primarily to oxidation of reactive sulphydryls in the myosin heads. The overall effect on muscle function of ROS and RNS generated in physiological and pathological conditions is determined by the balance of these conflicting actions of S-glutathionylation, S-nitrosylation and oxidation.

Andrade FH, Reid MB, Allen DG & Westerblad H. (1998) *Journal of Physiology* **509,** 565-575.
Lamb GD & Posterino GS. (2003) *Journal of Physiology* **546,** 149-163.
Murphy RM, Dutka TL & Lamb GD. (2008) *Journal of Physiology* **586,** 2203-2216.
Posterino GS, Cellini MA & Lamb GD. (2003) *Journal of Physiology* **547,** 807-823.
Spencer T & Posterino GS. (2009) *American Journal of Physiology. Cell Physiology* **296,** C1015-1023.

Skeletal Muscle H2O2 and Insulin Sensitivity

T. Tiganis, Department of Biochemistry & Molecular Biology, School of Biomedical Sciences, Monash University, VIC 3800, Australia.

Reactive oxygen species (ROS) are thought to contribute to the progression of various human diseases. In type 2 diabetes, ROS are generated by mitochondria, as a by-product of oxidative phosphorylation and as a consequence of inflammation. There is direct evidence for ROS serving to suppress the insulin response and contribute to the development of insulin resistance, a key pathological feature of type 2 diabetes. Paradoxically, ROS generated by NADP(H) oxidases at the plasma membrane and endomembranes may also be required for normal intracellular signaling. A wide variety of physiological stimuli including growth factors, cytokines and hormones such as insulin promote the generation of ROS for the coordinated inactivation of protein tyrosine phosphatases (PTPs) and the promotion of tyrosine phosphorylation, as well as phosphatidylinositol 3-kinase and mitogen-activated protein kinase signaling. Thus, ROS have the potential to both promote and attenuate the insulin response. Our recently published studies (Loh *et al.*, 2009) have focused on the capacity of ROS to promote muscle insulin sensitivity through the inactivation of the PTP superfamily member PTEN, a lipid phosphatase that terminates signals generated by phosphatidylinositol-3-kinase.

Loh, K., Deng, H., Fukushima, A., Cai, X., Boivin, B., Galic, S., Bruce, C., Shields, B.J., Skiba B., Ooms L., Stepto, N., Wu, B., Mitchell, C.A., Tonks, N.K., Watt, M.J., Febbraio, M.A., Crack, P.J., Andrikopoulos, S., & Tiganis, T. (2009) Reactive oxygen species enhance insulin sensitivity. *Cell Metabolism* **10,** 260-272.

The role of ROS in insulin resistance

D.E. James, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia.
(Introduced by Glenn McConell)

A great deal is known about the cellular response to starvation *via* AMP-activated protein kinase (AMPK), but less is known about the adaptation to nutrient excess. Insulin resistance is one of the earliest responses to nutrient excess, but the cellular sensors that link these parameters remain poorly defined. It has been suggested that defects in the early elements of the insulin signalling cascade constitute the major cause of insulin resistance. However, we have recently described evidence in cell and animal models as well as in insulin resistant humans that this is not the case. On the other hand, mitochondrial superoxide production is a common feature of many different models of insulin resistance in adipocytes, myotubes, and mice. Moreover, insulin resistance was reversed by agents that act as mitochondrial uncouplers, ETC inhibitors, or mitochondrial superoxide dismutase (MnSOD) mimetics. Similar effects were observed with overexpression of mitochondrial MnSOD. Furthermore, acute induction of mitochondrial superoxide production using the complex III antagonist antimycin A caused rapid attenuation of insulin action independently of changes in the canonical PI3K/Akt pathway. These results were validated *in vivo* in that MnSOD transgenic mice were partially protected against HFD induced insulin resistance and MnSOD+/− mice were glucose intolerant on a standard chow diet. These data place mitochondrial superoxide at the nexus between intracellular metabolism and the control of insulin action potentially defining this as a metabolic sensor of energy excess.