Role of reactive nitrogen species in skeletal muscle glucose uptake and mitochondrial biogenesis

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Nitric oxide / nNOSμ and contraction-stimulated glucose uptake. The uptake and metabolism of glucose by skeletal muscle is a major determinant of whole body glucose homeostasis. People with type 2 diabetes have reduced insulin-stimulated skeletal muscle glucose uptake, however, muscle glucose uptake during exercise is normal. The signalling pathways associated with contraction-stimulated glucose uptake are largely undefined, but known to differ from insulin pathways. There is some evidence that AMP-activated protein kinase (AMPK), Calcium-calmodulin dependent protein kinase II (CaMKII) and nitric oxide derived from neuronal nitric oxide synthase (nNOS, expressed in skeletal muscle) may be involved. Nitric oxide donors increase skeletal muscle glucose uptake and GLUT-4 translocation and NO production / NOS activity increases in skeletal muscle during exercise/contraction. We have found that infusion of a NOS inhibitor into the femoral artery of humans attenuates the increase in leg glucose uptake during cycling exercise in normal, healthy young individuals, people with type 2 diabetes and matched controls (Bradley *et al.*, 1999; Kingwell *et al.*, 2002). Importantly, the NOS inhibitor had no effect on leg blood flow, blood pressure, arterial insulin and glucose concentration. Furthermore, we recently found that local NOS inhibition attenuated the increase in skeletal muscle glucose uptake during *in situ* contraction in rats without influencing capillary blood flow (Ross *et al.*, 2007). Rat studies from other groups have yielded conflicting results, with some studies reporting a reduction in glucose uptake during contraction/exercise with NOS inhibition and others finding no effect (review in McConell & Kingwell, 2006). The reasons for these discrepancies are unclear but are likely to include methodological differences, especially as many studies have examined glucose uptake 20 or more minutes after contraction/exercise (McConell & Kingwell, 2006). We also have preliminary evidence that the activation of skeletal muscle glucose uptake is attenuated in nNOS KO mice. It will be important to confirm these findings and also to determine the factors downstream of NO/nNOS that are associated with GLUT-4 translocation.

NO / nNOSμ and the regulation of mitochondrial biogenesis. Defective small skeletal muscle mitochondria are now recognised as a major component of the metabolic abnormalities of diabetes. Exercise increases mitochondrial volume and improves mitochondrial function. Many research groups are attempting to determine how exercise increases mitochondrial biogenesis and it appears that the same signals that may be involved in regulating glucose uptake during exercise may also be activating gene expression after exercise (AMPK, CaMK and perhaps nitric oxide). It has been shown that NO donors and cGMP analogues increase mitochondrial biogenesis in muscle cells (Nisoli *et al.*, 2004). It was important to extend this line of research to examine whole animals and also to examine whether NO was involved in the increase in skeletal muscle mitochondrial biogenesis in response to exercise. We found that ingestion of the NOS inhibitor L-NAME for 2 days reduces basal skeletal muscle mitochondrial biogenesis but has no effect on the increase in mitochondrial biogenesis in response to an acute exercise bout (Wadley & McConell, 2007). Similarly, the increase in mitochondrial biogenesis markers in response to acute exercise and exercise training is intact in nNOS KO and eNOS KO mice (Wadley *et al.*, 2007). Interestingly, however, rather than mitochondrial biogenesis being lower in nNOS KO mice muscle it was actually higher (Wadley *et al.*, 2007). Although more studies are required, these studies suggest that NO plays a role in basal mitochondrial biogenesis but not in the increase in mitochondrial biogenesis in response to exercise.

Significance. Exercise is considered the best prevention and treatment option for diabetes, but unfortunately, many people with diabetes do not or can not exercise regularly. Alternatives therapies are therefore critical to effectively manage diabetes. If skeletal muscle nNOS/nitric oxide is found to play an important role in regulating glucose uptake and/or mitochondrial biogenesis, pharmaceutical agents designed to mimic these exercise effects may improve glycaemic control.

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