Mitochondrial ROS and muscle glucose uptake during exercise in transgenic mice

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Much research has examined the factors regulating skeletal muscle glucose uptake during exercise. Part of the impetus for this work has been to describe and understand the effects of regular physical activity (i.e., exercise training) on muscle glucose uptake and energy substrate partitioning, whereas another part of the impetus results from attempts to explain why people with the insulin resistance and glucose intolerance of type 2 diabetes have normal glucose uptake during exercise (4, 5). These observations of normal working muscle glucose uptake and insulin resistance at rest appear paradoxical and reflect on our incomplete knowledge of the mechanisms regulating muscle glucose uptake and how we might improve it in disease states. Kang et al. (3) utilize transgenic mouse models, dietary manipulation, and a variety of techniques to test a hypothesis on the role of mitochondrial reactive oxygen species (ROS) generation in determining muscle glucose uptake (MGU) during exercise.

Potential regulators of skeletal muscle glucose uptake during exercise include Ca\textsuperscript{2+}/calmodulin-dependent protein kinase (CaMK), nitric oxide (NO), and AMP-activated protein kinase (AMPK). Interestingly, there is also some evidence that ROS produced during exercise may play a role. Over the past few years there have been ex vivo mouse contraction studies that suggested, at least under these conditions, that ROS production during contraction is required for normal increases in glucose uptake during contraction. Sandstrom et al. (11) showed that the nonspecific antioxidant N-acetyl-l-cysteine (NAC) attenuated increases in skeletal muscle glucose uptake during contractions of mouse muscle. They also demonstrated that mice overexpressing Mn\textsuperscript{2+}-dependent superoxide dismutase (mitochondrial isoform; SOD2), which converts superoxide to hydrogen peroxide, had 25% greater skeletal muscle glucose uptake during contractions. Ebselen, a glutathione peroxidase mimetic, which converts hydrogen peroxide to water, inhibited contraction-mediated 2-DG uptake in murine muscles contracting ex vivo (11).

It needs to be appreciated, however, that although ex vivo muscle preparations have advantages such as circumventing the confounding influence of blood flow, nervous input, or hormones, lack of the same render ex vivo preparations nonphysiological. In addition, bubbling with 95% oxygen, along with the existence of a hypoxic core and the overriding effect of intense fatiguing muscle contractions required likely result in supra-physiological ROS production during contraction that are amenable to the effects of antioxidants. Therefore, we (8, 9) and now Kang et al. (3) in the this volume of the Journal of Applied Physiology examined the effects of ROS on glucose uptake during contraction and exercise using more physiological models. Previously, we found NAC had no effect on glucose uptake during in situ contractions in rats (8) or during exercise in humans (9). NAC would be expected to decrease all ROS production by the muscle cell. It therefore appeared from our studies that ROS might only be playing a role in skeletal muscle glucose uptake during very intense contractions ex vivo and not during more physiological contractions/exercise.

The current authors conducted a state-of-the-art very ambitious study with four different genotypes of C57BL6 mice (3 transgenic) and surgeries on the carotid arteries and jugular veins of ~150 mice and tracer infusions to enable thorough investigation during treadmill exercise in a chronically catheterized conscious mice. What was surprising, given the above discussion, was that Kang et al. (3) hypothesized that MGU during exercise in vivo in mice would be augmented by increasing mitochondrial ROS (mtROS) scavenging capacity (rather than reduced or no effect). It must be noted here that Kang et al. (3) targeted mitochondrial ROS rather than all ROS. Mice overexpressing SOD2 (sod2\textsuperscript{m}) mitochondria-targeted catalase (mcat\textsuperscript{m}), and combined SOD2 and mCAT (mtAO) to increase mtROS scavenging were placed on either a chow or high-fat diet and then run. The idea of the high-fat feeding was to increase mtROS production. In general, the mtROS scavenging increased MGU during exercise, especially in the high fat mice, which supports Kang et al.’s hypothesis. However, as the authors concede, the observed increases in muscle glucose uptake by overexpressing the mitochondrial antioxidant enzymes is possibly attributable to changes in other gene products that regulate muscle glucose uptake through yet undefined regulation of signaling/substrate transport. It was unfortunate that the authors did not measure blood flow during exercise in this study unlike in their other recent similar studies.

At first it seemed to go against their hypothesis that high fat-fed (HFF) mice had higher MGU during exercise given that HFF increases mitochondrial ROS production. However, as the authors point out, the running speed of 12 m/min (for 30 min) represented only ~30% of V\textsubscript{max} of the chow-fed mice but ~50% of V\textsubscript{max} of the HFF mice. Therefore, the increased MGU in HFF mice was probably attributable to the increased relative exercise intensity. Ideally the authors would have examined exercise at both the same absolute and the same relative workload but this would have made an already very large study massive. However, perhaps conducting the study at the same relative exercise intensity would have been more informative and allowed comparisons between the chow and high fat groups. Arguments can be made for and against both approaches.
Because of methodological limitations inherent in the currently available techniques, the authors were only able to measure mtROS production (H$_2$O$_2$) at rest, and the methods used were also unphysiological (isolated muscle fibers, permeabilized and then incubated with oligomycin and >1 mM succinate in the absence of ADP). Therefore, the authors could only provide indirect experimental evidence, by use of transgenic mice for mitochondrial genes, to support their hypothesis. However, it is important to note that, for this reason, the schematic in Fig. 6 relates to sedentary animals, although the focus of the paper was on exercise.

Although this is elegant work, it is not immediately clear the relevance of the results as they suggest that mtROS decreases skeletal muscle glucose uptake during exercise. However, people with type 2 diabetes have normal MGU or, if anything, increased (not lower) during exercise (4, 5) despite having increased mtROS production (at rest at least). Furthermore, people with type 2 diabetes had normal MGU when studied at both the same absolute power output and relative exercise intensity (4). In addition, exercise training in humans increases skeletal muscle antioxidant enzymes and GLUT4 expression, but rather than augmenting MGU during exercise, as would be suggested by the results of Kang et al. (3), MGU is reduced, especially during exercise after training at a given absolute power output (2, 6) when ROS production is also reduced. Similarly, unlike human studies, there is evidence in rodents that exercise training increases rather than decreases MGU during contraction/exercise (1, 7). Therefore, there appear to be species differences that place in question the rodent results and the current results in transgenic mice do not appear to fit at all with observations in humans.

In summary, Kang et al. (3) have tested a contemporary hypothesis on the effect of mtROS generation on muscle glucose uptake by conducting a technically demanding set of experiments on genetically modified and wild-type mice subjected to exercise and dietary challenges. The work is necessary and an important contribution to understanding how muscle glycogen is regulated in resting and working mammalian muscles. However, readers should consider that the results are contrary to those obtained on exercising humans either following endurance training (2) or antioxidant supplementation (9).

In part, the unexpected results may be attributable to species differences, methodological difficulties, and/or assumptions on the importance of mitochondrial, as opposed to cytosolic, muscle ROS generation during contraction (10). The work of Kang is a technological tour de force that produces an unexpected result. The paper and resulting discussion contain important messages to be conveyed through the literature.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author.

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**REFERENCES**