Inhibition of NADPH Oxidase May Promote Exercise Endurance by Suppressing Oxidant Stress in Skeletal Muscle

Repetitive muscle contraction is associated with increased superoxide production in skeletal muscle.[1-8] There is considerable evidence that muscle fatigue during prolonged exercise is associated with oxidant stress in muscle fibers, and that this stress is indeed partially responsible for the fatigue. Thus, pharmacological strategies which depress the antioxidant defenses of muscle fibers accelerate the onset of fatigue, whereas certain agents which scavenge oxidants, such as N-acetylcysteine and pegylated superoxide dismutase, have been shown to postpone muscle fatigue in vivo and in isolated muscle preparations.[1;2;6] Peroxynitrite may be a prominent mediator of this muscle dysfunction, inasmuch as muscle fibers also produce nitric oxide.[4;8;9] While it is still unclear which targets of oxidant stress are the primary mediators of oxidant-induced fatigue, studies indicate that function of the sarcoplasmic reticulum (the calcium release channel and the calcium-ATPase), of the plasma membrane Na^+/K^+-ATPase, and of mitochondria can be disrupted by excessive oxidant exposure.[1]

The chief source or sources of superoxide in contracting muscle remain undefined. Leading candidates include the mitochondrial respiratory chain, xanthine oxidase, and NADPH oxidase. There are several reasons to suspect that the latter makes an important contribution in this regard:

1. A phagocyte-type NADPH oxidase has been characterized in skeletal muscle fibers. $p22^{phox}$, $gp91^{phox}$, $p47^{phox}$, and $p67^{phox}$ have all been detected in the diaphragm and gastrocnemius of rats, and incubation with DPI (a potent inhibitor of NADPH oxidase) lessens the basal production of superoxide in these muscle, suggesting a certain level of NADPH oxidase activity in unstimulated muscle.[10] An apocynin-inhibitable NADPH/NADH oxidase activity has been documented in transverse tubules, which contain p22phox and gp91phox subunits, as well as variable amounts of $p47^{phox}$ and $p67^{phox}$.[11]

2. Repeated contractions induce activation of PKC-zeta in skeletal muscle fibers (whereas the classical and novel isoforms of PKC are not activated).[12-14] $p47^{phox}$ is an excellent substrate for PKC-zeta, such that PKC-zeta activity efficiently promotes the membrane assembly and activation of NADPH oxidase.[15-17] Thus, it is logical to expect that contraction induces NADPH oxidase activation.

3. One of the activators of PKC-zeta is arachidonic acid.[18] There is evidence that activation of phospholipase A2 (PLA2) – which releases arachidonic acid from membrane phospholipids – is crucial for the generation of contraction-induced oxidant stress.[19] Thus, it is reasonable to speculate that contraction promotes superoxide generation, at least in part, by sequential activation of PLA2, PKC-zeta, and NADPH oxidase.

4. A very recent study demonstrates that electrical stimulation as well as potassium-induced depolarization of cultured rat skeletal muscle cells (myotubes) results in oxidant

generation that is wholly inhibited by apocynin, a potent and specific inhibitor of NADPH oxidase activation.[20] Furthermore, these myotubes were shown to express both $gp92^{phox}$ and $p47^{phox}$.

5. A recent controlled clinical study concludes that daily supplementation with spirulina (7.5-15 g daily – the report is unclear on this point) is associated with a significant increase in endurance time - from 713 seconds to 765 seconds - in individuals subjected to exhaustive treadmill exercise (the Bruce intermittent protocol); this supplementation was also associated with a significant decrease in post-exercise systemic oxidant stress, as quantified by plasma malondialdehyde.[21] Spirulina is a rich source of phycocyanobilin (PCB), a homolog of biliverdin that, like biliverdin, has recently been found to inhibit NADPH oxidase in human cell cultures in low micromolar concentrations. (Inoguchi T, personal communication) The true proximal inhibitor of NADPH oxidase is likely to be phycocyanorubin, a bilirubin homolog that can be generated intracellular from PCB by biliverdin reductase activity.[22] In light of many previous Cuban studies demonstrating that oral ingestion of phycocyanin (the Spirulina holoprotein which contains PCB as a covalently-bound chromophore) exerts numerous anti-inflammatory actions in rodents,[23-25] it is reasonable to expect that orally administered PCB, even when bound to phycocyanin, is absorbable and physiologically active. Thus, the impact of spirulina ingestion on exercise endurance may reflect PCB-mediated inhibition of NADPH oxidase in contracting muscle fibers.

Intriguingly, the authors of the recent spirulina study state that "The Chinese and Cuban Olympic teams eat spirulina daily during training and before competition."[21]

If activation of NADPH oxidase does indeed account for a high proportion of the excess superoxide production associated with muscle contraction, future studies may confirm that ingestion of PCB or biliverdin has a favorable impact on endurance during prolonged exercise. However, there is evidence that contraction-evoked superoxide production may play a useful physiological role, boosting calcium release through ryanodine receptor channels.[20;26;26] Hence, complete inhibition of NADPH oxidase might prove counterproductive for physical performance – and in any case would have an adverse impact on immune defenses and other physiological systems. Moderate partial inhibition of this complex should thus be the goal, and presumably would be most beneficial in endurance events associated with oxidant-induced fatigue.

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