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Parallels of snipe hunting and ROS research: the challenges of studying ROS and redox signalling in response to exercise

R. A. Jacobs, E. L. Donovan and M. M. Robinson
Department of Health and Exercise Science, Colorado State University, Fort Collins, CO 80523, USA

Email: robert.jacobs@colostate.edu

The increase in reactive oxygen species (ROS) production during exercise is well established. However, the specific consequences that an increase in contraction-induced ROS has on cellular function and/or adaptation are not completely understood. An earlier notion that portrayed exercise-induced ROS formation as a necessary evil of muscular contraction, resulting in deleterious effects such as protein or lipid damage, has been transformed over the past several decades into a hormetic effect of exercise-induced ROS signifying that limited exposure to ROS is beneficial whereas a greater, uncontrolled, exposure is harmful. Recent evidence suggests that the acute and transient increase in ROS during muscular contraction is directly involved in the up-regulated expression of endogenous antioxidants, the control of redox-sensitive transcription factors, and the stimulation of mitochondrial biogenesis. Although there is ample evidence that indicates the involvement of ROS in modulation of cell signalling pathways, many questions specific to redox biology remain unanswered (Powers & Jackson, 2008).

In the August 2008 issue of *The Journal of Physiology*, the study by Brooks *et al.* (2008) attempted to examine the effect of exercise conditioning on contraction-mediated redox signalling in a murine model. The study by Brooks *et al.* utilized innovative techniques and methodology to investigate the effects of training on ROS formation and signalling. Studies of ROS formation are very difficult due to the reactivity and short half-life of ROS.

In the study, male and female C57BL/6 mice were separated and then randomly assigned to one of two possible groups: a sedentary (Sed) group and an exercise (Exer) group. The Exer mice participated in an endurance-training regimen lasting 8 weeks. Following training, all Exer mice were subjected to a maximal graded treadmill test with the intention of demonstrating the effectiveness of the 8 week training protocol. Within 24 h of the graded exercise test, both Sed and Exer mice were randomly separated into one of two groups. The first group of mice was killed and skeletal muscle was immediately collected; this muscle was qualified as quiescent muscle. The second group of mice were anaesthetized and hind limb muscles were subjected to a 15 min maximum, albeit non-damaging, isometric contraction protocol which has previously demonstrated activation of redox-sensitive transcription factors such as nuclear factor κB (NFκB) (Vasilaki *et al.* 2006). Prior to, during and following the isometric contractions, microdialysis techniques were utilized in an attempt to capture *in vivo* production of superoxide, hydrogen peroxide and nitric oxide. Fifteen minutes following cessation of stimulated contraction, these mice were then killed and their muscles collected for analysis (Brooks *et al.* 2008).

With advances such as those provided from Dr Malcolm Jackson's lab, microdialysis techniques are increasingly used to evaluate local tissue environments *in vivo*, in this case to determine local ROS production. Microdialysis probes function analogously to a capillary with equilibration of a perfused solution within the surrounding interstitial fluid across a semi-permeable membrane. Measurements of the desired analyte are derived from the fluid collected from the outlet tubing (dialysate). Microdialysis exchange characteristics are dependent on the diffusion of solutes across the probe membrane and can be affected by molecule size (restricted by pore size), exchange area (determined by membrane length, commonly 10–30 mm) and perfusion speed (typically 1–4 μl min^{-1}). Additionally, an alteration in blood flow to the tissue being perfused will lead to changes in the actual physiological milieu of the interstitial fluid and influence both equilibration of the perfusate and collection of the analyte of interest. Thus, when quantifying the concentration of substances within the dialysate, it is necessary to account for differences in probe exchange characteristics that may be altered by probe placement within the tissue, manufacturing tolerances and blood flow. A common attempt to quantify diffusion characteristics is the internal reference technique that involves perfusing a molecule that is similar to the analyte of interest and is readily distinguishable (often a radioactive tracer). The highlighted study (Brooks *et al.* 2008) did not use an internal reference or quantify differences in blood flow between exercise trained and sedentary animals during maximal stimulated contraction.

The short half-lives and high reactivity of ROS cause methodological difficulties when attempting to determine *in vivo* production. Therefore reducible agents, such as cytochrome c, are included as part of the perfusate. The collected dialysate concentration of reduced cytochrome c is used as an estimation of superoxide concentration. The study by Brooks *et al.* (2008) used cytochrome c reduction to quantify superoxide production during electrical stimulation of skeletal muscle. However, the pore size (35 kDa) in the membrane utilized in the study allowed for unrestricted diffusion of cytochrome c into the tissue, an issue for which an internal reference may provide valuable information. The use of an internal reference would help to correct analyte recovery for possible confounding effects such as differences in blood flow.

As a result of exercise training, skeletal muscle capillarization increases. The increase in capillarization functions to increase total skeletal muscle blood flow during exercise or, in the case of this study, maximal stimulated contraction. When using microdialysis to make comparisons between trained and untrained animals, differences in total blood flow must be accounted for. A greater amount of skeletal muscle blood flow following muscle contraction in the exercise trained mice compared to sedentary controls could result in findings of decreased rates of ROS generation because of a more rapid dilution of the ROS. An increase in muscle capillarization and blood flow due to exercise training supports the null findings for the reported ROS produced during the stimulated muscle contraction. The results of Brooks *et al.* (2008) do not indicate any difference in collected reduced cytochrome c concentrations in the exercise-trained mice compared to the

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sedentary controls, which is reported as being due to an increase in antioxidant capabilities in response to training. This conclusion may be appropriate, but it does not rule out the possibilities that the results were influenced by differences in blood flow.

Additionally, when evaluating the results, the role of the previous acute bout of exercise should be considered. The paper reports that the quiescent muscle from the Exer male mice displayed greater NFκB–DNA binding than Sed male quiescent muscle. Moreover, following isometric contractions, NFκB–DNA binding was reported to increase in Sed male muscle whereas the Exer male muscle NFκB–DNA binding did not change. These observations were interpreted as a dampened response to contraction-mediated stimulation and transduction of redox-sensitive pathways in the exercise-trained animals. However, these results may be attributed to an acute bout of intense exercise and not the training state of the animal. Maximal graded treadmill tests were administered within 24 h of all muscle collection. Previous studies have demonstrated that exercise-induced increases in NFκB binding can last 24 h, if not longer, following a single bout of exercise (25 m min^{-1}, 5% grade, until exhaustion) (Ji *et al.* 2004). We wonder if it would have been more appropriate to allow a greater time period after the bout of exercise so that the cellular signalling events following each specific physiological perturbation, specific either to the maximal graded exercise test or the electrically stimulated maximal isometric contraction, can be identified and interpreted separately. Measurements made in the muscle of the exercise-trained mice, such as the NFκB–DNA binding, cannot be traced to one specific physiological source, therefore interpreting the data as a dampening of contraction-mediated redox signalling in exercise-trained muscle may be difficult.

This work (Brooks *et al.* 2008) will serve as a foundation and framework for future research regarding the effect of training on ROS production and signalling as well as studies utilizing microdialysis to detect ROS. Consideration of microdialysis recovery and confounding effects of time between exercise bouts may be of interest in future studies. If the conclusions of this paper were to hold true in humans, the subsequent question to ask is what are the implications? Does this indicate a potential mechanism for training plateaus? If exercise reduces ROS generation, oxidative stress and redox signalling pathways, does this imply a possible reduction in ROS damage, or is this indicative of a decrease in redox-stimulated exercise adaptations? Further method development and research into ROS generation and exercise adaptations will help to answer questions regarding redox signalling and relevance to human exercise.

References

Brooks SV, Vasilaki A, Larkin LM, McArdle A & Jackson MJ (2008). Repeated bouts of aerobic exercise lead to reductions in skeletal muscle free radical generation and nuclear factor κB activation. *J Physiol* **586**, 3979–3990.

Ji LL, Gomez-Cabrera MC, Steinhafel N & Vina J (2004). Acute exercise activates nuclear factor (NF)-κB signaling pathway in rat skeletal muscle. *FASEB J* **18**, 1499–1506.

Powers SK & Jackson MJ (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiol Rev* **88**, 1243–1276.

Vasilaki A, McArdle F, Iwanejko LM & McArdle A (2006). Adaptive responses of mouse skeletal muscle to contractile activity: The effect of age. *Mech Ageing Dev* **127**, 830–839.

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