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Can an old dog learn new tricks? Novel applications of Microdialysis for detecting skeletal muscle reactive oxygen species and assessing microvascular endothelial function in hypertensive populations

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Microdialysis has predominantly been used for cerebral metabolism monitoring, a general technique facilitating the exchange of molecules between perfused solutions and extracellular fluid to assess blood-flow and molecular activity within brain tissue. However, the development and evolution of microdialysis over more than three decades has allowed for the investigation of metabolism and nutritive blood flow of a localised region within essentially any tissue, including skeletal muscle. This is particularly pertinent in studies of exercise physiology and molecular biology where more traditional methods i.e., muscle biopsies or arteriovenous methods, whilst valuable, may not be the most reasonable method in terms of cost, accessibility, or for continuous or ambulatory sampling. Therefore, using microdialysis to detect *in vivo* skeletal muscle reactive oxygen species (ROS) and assess microvascular endothelial function may provide a critically novel insight into determining the roles of ROS and endothelial dysfunction in the development of a range of cardiovascular disorders, specifically hypertension.

Using an adapted technique (La Favor et al., 2014) our research team is measuring *in vivo* skeletal muscle ROS and assessing endothelium-dependent vasodilation in adults with and without hypertension. Two microdialysis probes (CMA, Sweden), connected to Microinfusion pumps (CMA 106, Sweden) that pump perfusate (saline) through the probes at a flow rate of 2.0 µl/min, are inserted 3cm apart into the left *vastus lateralis* of human participants. The distal 10 mm of the microdialysis probes contain a semi permeable membrane allowing for the bi-directional diffusion of small molecules (< 20 kDa), as well as the interaction of known concentrations of substances mixed with the perfusate with the local tissue interstitium. Baseline nutritive blood flow rate is determined after the addition of a 5 mM ethanol-saline solution to the perfusate. As ethanol is not locally metabolised, movement out of the probe (measured as outflow[dialysate]/inflow[perfusate] ratio) is inversely related to blood flow within the local area (Wallgren et al., 1995). Following this, fluorescence spectrometry of skeletal muscle extracellular ROS is performed by adding 100 uM Amplex UltraRed reagent (Thermofisher, MA) and 1.0 U/mL horseradish peroxidase (Sigma-Aldrich, MO) to the perfusate to measure *in vivo* H_2O_2 production within the microdialysis probe, and 10 U/mL of superoxide dismutase (SOD) (Sigma-Aldrich, MO) then added to convert $O_2^{\bullet-}$ that crosses the membrane into H_2O_2. Continuous dialysate samples collected over 30 minutes indicate the difference in ROS between collection phases, which can be attributed to *in vivo* $O_2^{\bullet-}$ production. Finally, acetylcholine (ACh)-stimulated blood flow is assessed through the addition of 50 mM Acetylcholine Chloride (Sigma-Aldrich, MO) to the perfusate, using a standard curve to determine endothelium-dependent vasodilation.

Based on these observations, our research investigates the use of skeletal muscle microdialysis to provide insight into the crosslinks of microvasculature health and functional changes in hypertension that cannot be otherwise addressed. As *in vivo* monitoring of this space is largely quite limited, microdialysis can be considered the link between whole-body *in vivo* studies in humans and *in vitro* investigations.

La Favor, J. D., Anderson, E. J., & Hickner, R. C. (2014). Novel method for detection of reactive oxygen species in vivo in human skeletal muscle. *Physiol Res*. *63***(3)**:387–392.
Wallgren, F., Amberg, G., Hickner, R.C., Ekelund, U., Jorfeldt, L., & Henriksson, J. (1995). A mathematical model for measuring blood flow in skeletal muscle with the microdialysis ethanol technique. *J Appl Physiol*. 79**(2)**:648-659.