

A physiological drop in pH decreases mitochondrial respiration, and AMPK and Akt signalling, in L6 myotubes

A.J. Genders,¹ S.D. Martin,² S.L. McGee² and D.J. Bishop,¹ ¹*Institute of Sport, Exercise and Active Living, Victoria University, PO Box 14428, Melbourne, VIC 8001, Australia and* ²*School of Medicine, Deakin University, Geelong, VIC 3217, Australia.*

Exercise is known to stimulate mitochondrial biogenesis and to increase mitochondrial function and content, with proposed benefits for healthy aging. However, during high intensity exercise muscle pH can decrease below pH 6.8 with a concomitant increase in lactate concentration. Previously we have shown that this drop in muscle pH is associated with reduced exercise-induced mitochondrial biogenesis. Additionally, we and others have shown that when this drop in pH is minimised by prior administration of sodium bicarbonate markers of exercise-induced mitochondrial biogenesis are restored or increased. Other work has suggested that lactate, in addition to being a fuel source, acts as a signalling molecule to affect mitochondrial biogenesis. Therefore in this study we wished to determine the impact of altering pH and lactate concentration (separately and together) in L6 myotubes on genes and proteins known to be involved in and regulating mitochondrial biogenesis and metabolism. Additionally, we examined changes in mitochondrial respiration to these perturbations. Differentiated L6 myotubes were exposed to normal (pH 7.5), low (pH 7.0) or high pH (pH 8.0) media with and without 20 mM sodium L-lactate. The pH was altered in the media by altering the sodium bicarbonate content. Cells were exposed to these media combinations for 1 and 6 hours and then the cells were harvested for RNA and protein. Cell viability and cytotoxicity was measured by trypan blue staining and a commercial cytotoxicity kit respectively, and was found not to be affected by the altered pH and lactate concentration over the time period examined. Protein phosphorylation and localisation was assessed by western blotting. In conditions that mimicked high-intensity exercise, *i.e.*, low pH and additional lactate, Akt (Ser473) and AMPK (T172) phosphorylation was decreased at 1 h compared to controls. After 6 h Akt and AMPK phosphorylation had partially returned to normal and the nuclear localisation of HDAC5 was decreased. Conversely, when the pH was increased both Akt (Ser473) and AMPK (T172) phosphorylation was increased at 1 h whilst nuclear HDAC5 was unchanged. Overall increased lactate tended to decrease the nuclear localisation of HDAC5 at 6 h, but not at 1 h. PGC-1 α nuclear localisation did not appear to be altered significantly with any treatment. We next investigated the effect of altered pH and lactate on mitochondrial respiration. L6 myotubes were treated with normal, high and low pH media, with and without lactate, for six hours. They were then returned to normal media for 16 h before measurements of mitochondrial respiration were undertaken using the Seahorse XF24 Flux Analyser. Exposure to both high and low pH media significantly decreased basal mitochondrial respiration, ATP turnover and maximum mitochondrial respiratory capacity. There was no effect of lactate alone on mitochondrial respiration; however, addition of lactate to the 'high' and 'low' media appeared to return mitochondrial function to normal or at least blunt the effects of the high or low pH. These data suggest muscle pH affects several metabolic signalling pathways, including those required for mitochondrial function. Additionally, although exercise is a potent stimulus for increasing mitochondrial biogenesis, the associated decrease in muscle pH may impair signalling at the gene and protein level.