Influence of training intensity on adaptations in acid/base transport protein abundance and non-bicarbonate muscle buffer capacity in active men

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Regulation of pH in skeletal muscle comprises intracellular buffering of hydrogen ions (H^+) and acid/base transport across the sarcolemma (Juel, 2008). During high-intensity exercise H^+ transport is primarily lactate-coupled through the monocarboxylate transporters (MCT)1/4, with non-lactate-coupled transport provided by the sodium/hydrogen exchanger (NHE) system. The chaperone protein basigin is essential for MCT functioning (Wilson *et al.*, 2005). In addition, sodium-coupled bicarbonate transport proteins enhance intracellular buffering and H^+ efflux, while the cytosolic and sarcolemmal carbonic anhydrase (CA) isozymes may enhance activity of each transport system through physical or functional interactions (Deitmer & Becker, 2013). Yet, despite their importance for muscle pH regulation, the response to exercise training of most of these acid/base transport proteins has not been investigated.

This study sought to provide the first comprehensive analysis of the acid/base transport protein response to exercise training and to test the assumption that training intensity is a key factor in provoking upregulation of these proteins and intracellular buffers in skeletal muscle. Using a two-group parallel design, 16 active men [23 (5) y, mean (SD)] undertook 4 weeks of work-matched, high-intensity interval training (HIT), 3 days per week. HIT comprised 2-min work intervals interspersed with 1 min of passive recovery, performed at either 20% (HITΔ20; n = 8) or 90% (HITΔ90; n = 8) of the difference between the lactate threshold and peak aerobic power. Muscle biopsies were taken from the *vastus lateralis* before and after 2 and 4 weeks of HIT, and also 6 weeks after stopping HIT to ascertain potential detraining. Protein content of MCT1, MCT4, basigin, NHE1, electrogenic sodium-bicarbonate cotransporter (NBCe)1, and CAII, CAIII, CAIV, and CAXIV were measured by quantitative western blotting. Coomassie blue staining of total protein was used as a loading control. Non-bicarbonate muscle buffer capacity ($\beta m_{in\ vitro}$) was measured by titration of homogenate. Data were analysed using linear mixed models and standardized effect sizes (ES) are reported as (ES; 90% confidence interval) of the between-group difference scores. Where no between-group differences were seen, pooled (ES; 90% CI) of the within-group difference scores are reported.

The first two weeks (six sessions) of HIT induced little change in any variable. After 4 weeks of HIT, MCT4 protein content only increased for HITΔ20 (ES; 90% CI: 1.06; 0.29 to 1.83), whereas in contrast, basigin content only increased for HITΔ90 (ES; 90% CI: 1.49; 0.07 to 2.91). Otherwise, training intensity did not discriminate between adaptations for all other pH-regulatory proteins, with abundance of MCT1, NHE1, NBCe1, CAII, and CAXIV increasing after 4 weeks of HIT, while there was either no change or a decrease in CAIII and CAIV abundance. There were no group differences in $\beta m_{in\ vitro}$ (ES: 0.07; −0.64 to 0.78). Pooled $\beta m_{in\ vitro}$ decreased by 5.7 mmol $H^+ \cdot kg\ dm^{-1} \cdot pH^{-1}$ after 4 weeks (ES: −0.41; −0.74 to −0.07), but this was less than the typical error of measurement (9.8 mmol $H^+ \cdot kg\ dm^{-1} \cdot pH^{-1}$). Detraining was evident from an almost complete loss of adaptations for all of the proteins 6 weeks after removing the stimulus of HIT. Notably, detraining was not total for those proteins showing the largest training effect, *viz.* MCT1 and NHE1.

In summary, measurement of the adaptation to training of a comprehensive selection of proteins involved in muscle pH regulation has been undertaken for the first time. Increased abundance of most proteins was achieved after 4 weeks of HIT, but 6 sessions over the first 2 weeks provided an insufficient stimulus. And rapid physiological detraining was evident 6 weeks after removal of the HIT stimulus. Finally, contrary to our hypothesis, an ~40% difference in training intensity did not discriminate between adaptations for most proteins, with the exception of MCT4 and basigin.

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