# Fibre-type specific changes to skeletal muscle $Na^+$-$K^+$-ATPase and FXYD1 following post-exercise cold-water immersion in humans

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**Introduction.** Activation of the $Na^+$-$K^+$-ATPase (NKA), mediated in part by post-translational modification of its adjoining, regulatory protein, phospholemman (FXYD1), counteracts the contraction-stimulated rundown of trans-sarcolemmal potassium and sodium gradients, and constitutes a decisive defence mechanism against loss of myocellular excitability and the occurrence of fatigue (McKenna *et al.*, 2008). Understanding which strategies may promote expressional upregulation of muscle NKA and FXYD1 would therefore seem of great relevance. On the basis of our recent observation that post-exercise cold-water immersion (CWI) stimulates whole-muscle NKA α-isoform gene transcription (Christiansen *et al.*, article in preparation), and considering the fibre-type specificity of NKA-isoform protein abundance in regularly active human muscle (Thomassen *et al.*, 2013, Wyckelsma *et al.*, 2015), our current study evaluated the hypothesis that regular post-exercise CWI enhances skeletal muscle NKA α-isoform protein abundance in a fibre-type dependent manner following repeated-intense exercise training in humans.

**Methods.** Seventeen males with an age and $\dot{V}O_{2peak}$ (mean ± SD) of 24 ± 6 y and 47 ± 8 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively, performed 2-4 training sessions per week for 6 weeks. Each session consisted of four to six, 30s, maximal-intensity efforts on a cycle ergometer, interspersed by 4 min of passive recovery, which was followed by one of two 15 min recovery treatments: rest at room temperature (23°C, CON) or 10°C water immersion up to the umbilicus (CWI). After being matched on their pre-training $\dot{V}O_{2peak}$, participants were randomly assigned to one of the two recovery treatments. Following 1% Xylocaine injected in the skin and fascia, muscle was sampled from the *vastus lateralis* muscle at rest before and after training. Single-fibre segments were collected and fibre-typed based on MHC-isoform expression. Fibre segments with similar MHC expression were pooled to create samples of type I and II fibres. These pools were used to quantify NKA $\alpha_{1\text{-}3}$ and $\beta_{1\text{-}3}$ isoforms and FXYD1 protein abundance by western blotting (Wyckelsma *et al.*, 2015).

**Results.** In CON, $\alpha_1$ abundance increased by 2.1 ± 1.2 fold in type I and by 2.5 ± 0.8 fold in type IIa fibres with training ($P$<0.05). In CWI, the increase with training in $\alpha_1$ was of similar magnitude, being 2.3 ± 0.8 fold in type I and 2.3 ± 0.4 fold in type IIa fibres ($P$<0.05). In CON, $\alpha_2$ remained unaltered in type I ($P$=0.32), but tended to increase by 0.3 ± 0.6 fold ($P$=0.055) in type IIa fibres post training. In CWI, $\alpha_2$ remained statistically unchanged in both fibre types ($p \geq 0.39$), with the mean fold change in type IIa fibres being of similar magnitude as per CON (0.3 ± 0.4). Based on data from both groups, $\beta_1$ was 0.6 ± 0.8 fold higher in type II, relative to type I, fibres after training ($P$<0.05). In CON, $\beta_3$ increased by 2.2 ± 1.4 fold in type I and by 2.4 ± 1.6 fold in type IIa fibres post training ($P$<0.05). In CWI, the increase with training in $\beta_3$ was 3.8 ± 3.9 fold in type I and 2.3 ± 2.5 fold in type IIa fibres ($P$<0.05). Based on pooled group data, FXYD1 protein abundance decreased significantly by 0.3 ± 0.4 fold in type I ($P$<0.05), but not in type IIa ($p > 0.05$), fibres. From the same data, FXYD1 protein was 0.4 ± 0.3 fold more abundant in type I, relative to type IIa, fibres before ($P$<0.05), but not after ($P$>0.05), training. No statistically significant changes occurred for the remaining isoforms in any group and fibre type, and no group interactions were found ($P$>0.05).

**Discussion and Conclusion.** This is the first study to demonstrate that FXYD1 is more abundant in type I, relative to type II, fibres in untrained, but not in trained, human skeletal muscle. We also provide novel evidence that post-exercise CWI does not affect training-induced changes in muscle NKA and FXYD1 abundance. Our findings are likely to have important functional consequences for muscle ion regulation and function, as well as practical application, for humans *in vivo*.

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