High-intensity interval training in older adults does not upregulate the Na^+,K^+-ATPase isoforms measured in whole muscle homogenate, but shows fibre type specific upregulation when analysed in single fibres

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Background: Ageing is associated with decreased muscle function contributing to the inhibition of everyday activities and reduction in quality of life. The Na^+, K^+-ATPase (NKA) is a key protein in the maintenance of skeletal muscle excitability, therefore alterations of this protein with ageing may have implications for NKA activity and muscle function. Skeletal muscle is a heterogeneous tissue, comprising fast-twitch (Type II) and slow-twitch (Type I) which are distinct in their metabolic and contractile properties. Single fibre western blotting allows a comprehensive analysis of the NKA which can detect changes previously missed in human research when using whole muscle homogenate. The NKA consists of one α and β isoform to form a functional heterodimer, of which there are three α (α_{1-3}) and three β (β_{1-3}) isoforms expressed in skeletal muscle. Numerous investigations have shown the effectiveness of upregulating NKA isoform abundance with high-intensity interval training in young humans, which may have implications for increasing NKA activity. Whether older adults respond in the same way still needs to be determined.

Methods: Fourteen healthy older adults (10 male, 4 female) and seventeen healthy young participants (6 male, 9 female) volunteered for the study. Physical characteristics of older adults (mean ± SD) were 69 ± 3 years, 172 ± 10 cm, 77 ± 13 kg and symptom limited (RPE 17) VO_2 peak 24.1 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$. The young adults were 24 ± 3 years; 175 ± 11 cm; 73 ± 18 kg and VO_2 peak 43.4 ± 10.9 $mL \cdot kg^{-1} \cdot min^{-1}$. Participants underwent a VO_2 peak test and a resting muscle biopsy. Following testing eight older adults trained 3-days per week for 12 weeks. Training consisted of 4x4 minute intervals at 90-95% peak HR from the VO_2 peak test. A resting biopsy was taken 48-72 h after the final training session and VO_2 peak test performed 48 h following this. Single fibres were manually dissected from each biopsy and prepared for western blotting. Frozen pieces of whole muscle were additionally prepared for western blotting. NKA isoforms α_{1-3} and $\beta_{1-3,}$ were analysed in single fibres for old *vs* young comparison and older adults pre-post training. Myosin heavy chain isoforms I and II were analysed in all single fibres for fibre typing. Whole muscle from older adults pre-post training was analysed for NKA isoforms and mitochondrial protein COX IV.

Results: Compared to young, older adults had a 68% upregulation of α_3 in Type I fibres (0.79 ± 0.43 *vs* 1.33 ± 1.12, $P = 0.008$), 12% upregulation of β_1 in Type I (0.92 ± 0.43 *vs* 1.03 ± 0.33, $P = 0.009$), an 83% upregulation of β_3 in Type I fibres (0.96 ± 0.62 *vs* 1.76 ± 0.79, $P = 0.016$). There were no significant differences in Type II fibres. Following 12 weeks of HIT, VO_2 peak was increased by 16% (24.8 ± 5.4 *vs* 28.8 ± 5.1 $mL \cdot kg^{-1} \cdot min^{-1}$, $P = 0.026$). Whole muscle analyses showed an increased abundance of COX IV following training (0.93 ± 0.56 *vs* 1.17 ± 0.55, $P = 0.02$), but not the NKA isoforms, α_2 (Pre 0.92 ± 0.30 *vs* Post 0.95 ± 0.29 a.u) or β_1 (Pre 1.14 ± 0.043 *vs* Post 1.30 ± 0.39), which, when analysed in single fibres, were upregulated in type II fibres for α_2 (Pre 0.91 ± 0.21 *vs* Post 1.03 ± 0.20, $P = 0.002$) and type I fibres for β_1 (Pre 0.95 ± .30 *vs* Post 1.07 ± 0.30, $P = 0.030$). There was a trend for β_3 to decrease in type I fibres (Pre 2.01 ± 1.35 *vs* 1.19 ± 1.10, $P = 0.065$).

Conclusions: We show unique fibre type adaptations to the NKA with ageing, which could have important implications for NKA activity. We also demonstrate the effectiveness of improving aerobic capacity in older adults by the use of a short duration, high-intensity exercise training protocol. Finally we show in older adults, like in healthy young adults, the NKA is upregulated with training in a fibre-type specific manner, and that analyses conducted in whole muscle should be interpreted with caution.