The effects of inactivity and age on Na^+,K^+-ATPase abundance in skeletal muscle in humans

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Muscle contractile function and thus the capacity to both exercise and resist muscular fatigue requires the abundance and activation of the Na^+,K^+-ATPase (Na^+,K^+-pump, or NKA). Our research investigating NKA expression, adaptability and regulation in skeletal muscle in humans, has recently focussed on inactivity and aging, in both healthy and clinical populations. Utilising muscle biopsies, the total NKA content in muscle pieces was determined *via* the ^{3}H-ouabain binding site content, whilst the protein abundances of the six NKA isoforms expressed in human muscle (α_1-α_3, β_1-β_3) were measured using immunoblotting; these used whole muscle homogenates; in two studies, we also used single cell segments to enable comparisons between Type I and Type II muscle fibres.

The effects of inactivity *per se* in human muscle are not known. We investigated the effects of 23-days unilateral lower limb suspension (ULLS, Study 1) in six sedentary adults. After ULLS, marked impairments (P<0.05) occurred in the unloaded leg, including depressed muscle strength (22%), vertical jump (16%), thigh lean mass (4%) and earlier fatigue during one-legged cycling (23%). However, muscle NKA content and homogenate isoform abundances were unchanged with ULLS. In single muscle fibres, reductions (P<0.05) were seen after ULLS for NKA α_3 in Type I (66%) and β_1 in Type II fibres (40%). These findings indicate a remarkable resilience of NKA α_1 and α_2 isoforms to short-term inactivity. We then investigated more severe and prolonged inactivity effects on muscle NKA in six patients that had undergone knee anterior cruciate ligament repair (ACL, post-injury duration 15±17 weeks; mean±SD, Study 2). In ACL, the *quadriceps* strength (22%), thigh CSA (7%), muscle NKA content (20%) and α_2 (63%) were each reduced in the injured compared to the non-injured leg (P<0.05), with no differences detected in other NKA isoforms. Thus short-term inactivity (ULLS) depressed muscle function but not muscle NKA content or isoform abundances; whereas more severe inactivity (with severe knee injury), reduced total NKA content and the critical NKA α_2 isoform, which may contribute to impaired muscle function.

Aging is associated with reduced muscle mass, weakness and increased fatigability, but little is known about aging effects on NKA in human skeletal muscle and this was investigated in three studies. We firstly investigated the effects of age contrasting 17 Aged versus 16 Young adults (66.8±6.4 *vs* 23.9±2.2 years, Study 3). The Aged had lower (P<0.05) VO_{2peak} (37%), muscle strength (36%), NKA isoform abundance (relative to GAPDH) α_2 (24%) and β_3 (23%), with no differences for NKA content or α_1 abundance. We then compared 19 patients with osteoarthritis and 17 aged controls (69.9±6.5 *vs* 66.8±6.4 years, respectively, Study 4). The osteoarthritis patients had (P<0.05) lower muscle strength (41%), higher NKA α_2 (34%) and α_3 (100%) abundances and performed more incidental physical activity than controls but had similar NKA content and other NKA isoform abundances. Age and NKA content were negatively correlated (r=-0.47, P<0.05) with NKA content lower (26%, P<0.05) in the above-median than the below-median age groups (*i.e.* 69-81 *vs* 55-68 years). Hence older age, but not knee osteoarthritis was related to lowered muscle NKA content in older adults. Finally we compared muscle NKA content and isoforms in specific muscle fibres in 17 Aged and 14 younger adults (69.4±3.5 *vs* 25.5±2.8 years, Study 5) finding here NKA content did not differ with age. GAPDH varied with both age and fibre type (P<0.05) and thus was not a valid control protein with aging, likely explaining the NKA content and α_2 findings in Study 3. Analysis of NKA in individual muscle fibres from Young individuals, showed greater α_3 and β_2 isoforms in Type II compared with Type I fibres (P<0.05); with no fibre type differences in NKA isoforms in the Aged. In muscle from Aged compared to Young, in Type I fibres, α_1 and β_3 were greater and β_2 lower, whereas in Type II fibres, α_3 and β_2 were lower and β_3 was greater (P<0.05). In summary the effects of age on muscle NKA content, α_1 and α_2 abundances were not consistent in these studies, with the normalising control protein critical for immunoblotting. The single fibre analyses indicated that aging has complex and fibre-specific effects on NKA in human muscle.