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*Inactivity and exercise training differentially regulate abundance of Na<sup>+</sup> -K<sup>+</sup> -ATPase in human skeletal muscle*

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1 **Inactivity and exercise training differentially regulate the abundance of Na<sup>+</sup>, K<sup>+</sup>-ATPase in**  
2 **human skeletal muscle**

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9 **Running head:** Muscle Na<sup>+</sup>,K<sup>+</sup>-pump regulation with training and disuse

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23 **Abstract**

24 Physical inactivity is a global health risk that can be addressed through application of exercise training  
25 suitable for an individual's health and age. People's willingness to participate in physical activity is  
26 often limited by an initially poor physical capability and early onset of fatigue. One factor associated  
27 with muscle fatigue during intense contractions is an inexcitability of skeletal muscle cells, reflecting  
28 impaired transmembrane  $\text{Na}^+/\text{K}^+$  exchange and membrane depolarisation, which are regulated via the  
29 transmembranous protein,  $\text{Na}^+,\text{K}^+-\text{ATPase}$  (NKA). This short review focuses on the plasticity of NKA  
30 in skeletal muscle in humans following periods of altered usage, exploring NKA upregulation with  
31 exercise training and downregulation with physical inactivity. In human skeletal muscle, the NKA  
32 content quantified by the [ $^3\text{H}$ ]ouabain binding site content shows robust, yet tightly constrained  
33 upregulation of 8-22% with physical training, across a broad range of exercise training types. Muscle  
34 NKA content in humans undergoes extensive downregulation with injury that involves substantial  
35 muscular inactivity. Surprisingly, however, no reduction in NKA content was found in the single study  
36 which investigated short-term disuse. Despite clear findings that exercise training and injury modulate  
37 NKA content, the adaptability of the individual NKA isoforms in muscle ( $\alpha_{1-3}$  and  $\beta_{1-3}$ ) and of the  
38 accessory and regulatory protein FXYD1, are surprisingly inconsistent across studies, for exercise  
39 training, as well as for injury/disuse. Potential reasons for this are explored. Finally, we provide  
40 suggestions for future studies to provide greater understanding of NKA regulation during exercise  
41 training and inactivity in humans.

42

### 43 **Linking health, physical activity, muscle excitability and Na<sup>+</sup>,K<sup>+</sup>-ATPase**

44 Inactivity or disuse causes diverse negative health outcomes, with inactivity recognised as a  
45 contributing factor to many cardiovascular and metabolic diseases, as well as declines in mental  
46 health (21, 46, 110). Deconditioning (i.e. lack of fitness) is also recognised as a key factor adversely  
47 affecting muscular performance in many chronic diseases (16, 60, 94) and in patients receiving organ  
48 transplants (114). An important consideration of this is poor physical conditioning and associated  
49 *muscle fatigue*, which directly limit muscle function and the capability to perform repeated muscular  
50 contractions that are essential to develop or sustain muscle strength and metabolic health, as well as  
51 to prevent severity of sarcopenia (1, 115). On the other hand, physical training does improve health,  
52 muscle mass and performance in patients with chronic disease and the general population including  
53 by attenuating or delaying muscular fatigue and thereby increasing an individual's capacity to perform  
54 exercise. This differs to the intent of training in elite athletes, in which optimizing physical training  
55 protocols is critical for ensuring maximal performance of skeletal muscle during competition.  
56 Fatigue during muscle contractions is a topic of major debate still after more than a century of study  
57 and is likely to involve both central and peripheral components (1, 78, 84). Full discussion of fatigue is  
58 beyond the scope of this short review, so here we focus on one important component of fatigue  
59 occurring early in the excitation-contraction cycle, membrane excitability. Membrane excitability is  
60 linked with the release of K<sup>+</sup> from the contracting cell into the extracellular space with each action  
61 potential and is accompanied by an influx of Na<sup>+</sup> from the extracellular space into the muscle cell;  
62 repeated action potentials can lead to depolarisation of the membrane leading to inexcitability of the  
63 muscle fiber, thus contributing to fatigue (78).

64 The Na<sup>+</sup>,K<sup>+</sup>-ATPase (NKA) plays a critical role in the regulation of concentration gradients for K<sup>+</sup> and  
65 Na<sup>+</sup> ions and thus, in the maintenance of membrane potential to enable continued propagation of  
66 action potentials along the sarcolemma and into the transverse tubular system (18, 78, 101).

67 Understanding the NKA adaptability in muscle to training and downregulation to inactivity are of key  
68 interest for muscle NKA, Na<sup>+</sup>/K<sup>+</sup> regulation and fatigue. The present review extends earlier reviews  
69 which included sections on muscle NKA, but focussed on training and electrolyte regulation (77, 80)  
70 on muscle NKA regulation (18) and NKA and its contribution to fatigue at a cellular level (78). Here we  
71 explore NKA adaptability in response to increased physical activity through exercise training regimes,  
72 as well as downregulation with reduced physical activity, through injury and induced inactivity. The

73 review focusses on skeletal muscle in humans wherever possible and includes focus on NKA  
74 isoforms as well as total content. All biopsies in these studies were taken from the vastus lateralis  
75 muscle, unless otherwise stated. In order to fully understand the findings, we also briefly discuss  
76 important methodological techniques used to measure NKA in human skeletal muscle and their  
77 implications.

### 78 **NKA in skeletal muscle**

79 The NKA is a heterodimer comprized of an alpha subunit with ten transmembrane segments, and beta  
80 subunit, as well as an accessory protein from the FXYD family (18, 87). In human skeletal muscle the  
81 exact locations of NKA are not yet determined, whereas in rodent muscle the NKA are predominantly  
82 located in the plasma membrane' and within the t-tubules' (56). The  $\alpha$  subunit comprizes four isoforms  
83 ( $\alpha_{1-4}$ ), but with only  $\alpha_{1-3}$  expressed at the protein level in skeletal muscle; the  $\beta$  subunit comprizes three  
84 isoforms ( $\beta_{1-3}$ ), with each expressed in skeletal muscle (89). The specific functions of the  $\alpha$  isoforms  
85 have not yet been clarified in human muscle, but have been assumed to be similar to those identified  
86 in skeletal muscle of other species. In rodent skeletal muscle, the  $\alpha_1$  isoform is important for  $\text{Na}^+/\text{K}^+$   
87 regulation under basal conditions and has also recently been found to have an important intracellular  
88 signalling role in skeletal muscle growth, using an  $\alpha_1$ -modified murine model (63). The  $\alpha_2$  isoform, also  
89 the most abundant  $\alpha$  isoform, is primarily responsible for regulating the large  $\text{Na}^+/\text{K}^+$  fluxes that occur  
90 during muscle contractions (42, 44, 75, 98). The role for the  $\alpha_3$  isoform in skeletal muscle remains  
91 unclear. The  $\beta_1$  isoform is highly abundant in skeletal muscle (11) and is critical in NKA integration into  
92 the cell membrane (28) and plays a key role in regulating NKA enzymatic activity (64). The  $\beta_1$  isoform  
93 is highly expressed in slow muscle but is near undetectable in fast muscle, where the  $\beta_2$  isoform is  
94 heavily abundant (55, 91, 109). Thus, both the  $\beta_1$  and  $\beta_2$  isoforms must make heterodimers with both  
95  $\alpha_1$  and  $\alpha_2$  isoforms to enable NKA activity and the composition of these heterodimers differs between  
96 slow and fast muscles in the rodent. The role of the NKA  $\beta_3$  isoform in skeletal muscle is however,  
97 unclear. In human skeletal muscle, fiber-type heterogeneity is an important consideration of muscle  
98 performance, thus the expression of NKA isoforms in different fiber-types are of high interest. The  $\alpha_2$   
99 was shown to be more abundant in Type II fibers in two studies (17, 108), conversely two other studies  
100 found no difference in the abundance of  $\alpha_2$  in either fiber-types (119, 120) while the  $\beta_2$  isoform was  
101 more abundant in fast than slow twitch fibers (17, 120).

102 Phospholemman (FXYP1) is the main isoform of the FXYP family expressed in skeletal muscle, where  
103 it mainly associates with the NKA  $\alpha_1$  and  $\alpha_2$  isoforms (29, 99, 100); a further isoform, FXYP5, is also  
104 expressed in skeletal muscle (13, 72). FXYP1 binds to the  $\alpha$  subunits in an unphosphorylated state and  
105 reduces  $\alpha$  subunit Na<sup>+</sup> affinity (26), whereas when FXYP1 is phosphorylated, Na<sup>+</sup> affinity is increased  
106 (10). FXYP1 acts as a main substrate for protein kinase A and C phosphorylation in skeletal muscle  
107 (30), and it appears that FXYP1 is necessary for maximal activation of the NKA (100). FXYP1 is not  
108 expressed in a fiber type specific manner (108) but does undergo fiber-type specific phosphorylation  
109 after brief and intense acute exercise bouts (108). FXYP5 upregulation has also been shown to be  
110 responsible for increasing NKA activity (72), but nothing is known regarding its possible fiber-type  
111 specificity. Further information regarding the activation of the NKA acutely can be found in an excellent  
112 recent review (97).

### 113 **Measurement of NKA in Human Skeletal Muscle - Methodological Considerations.**

#### 114 **Outcome measures.**

##### 115 *1. [<sup>3</sup>H]ouabain binding site content*

116 The [<sup>3</sup>H]ouabain binding site content technique provides an absolute measurement of the NKA in  
117 molar units (pmol.g wet wt<sup>-1</sup> muscle). Readers are referred elsewhere to detailed discussion of the  
118 [<sup>3</sup>H]ouabain binding site content methodology and its significance (18, 19). In brief, the [<sup>3</sup>H]ouabain  
119 binds stoichiometrically to the  $\alpha$  subunit of the NKA, thereby allowing quantification of the content of  
120 these subunits, with the specific  $\alpha$  isoform detected dependent on the differing affinity to ouabain of  $\alpha$   
121 isoforms in some species, and the concentration of ouabain used. The NKA are located in both the  
122 sarcolemma and the transverse tubules in muscle (18). The [<sup>3</sup>H]ouabain binding site content in rat  
123 soleus muscles was identical when using either cut muscle pieces or intact muscles, thus this method  
124 ensures quantification of all NKA in sarcolemmal and transverse tubular membranes, at least for NKA  
125 that incorporate the  $\alpha_2$  isoform (79). Similar analyses have not been conducted on human muscles,  
126 since muscle biopsies contain cut pieces only. Due to the high affinity of ouabain binding to all  $\alpha$   
127 isoforms in human muscle (93, 111), the [<sup>3</sup>H]ouabain binding site content can also be referred to as  
128 the total NKA content in human skeletal muscle. In rat muscle the  $\alpha_1$  isoform makes up approximately  
129 20% of the NKA  $\alpha$  subunits; however the  $\alpha_1$  has a lower affinity to cardiac glycosides which doesn't  
130 allow for the  $\alpha_1$  to be detected using the standard [<sup>3</sup>H]ouabain binding site content technique (42).  
131 Thus, in rodent muscles, the standard [<sup>3</sup>H]ouabain binding site technique detects all  $\alpha_2$  but not  $\alpha_1$

132 isoforms and is not a full quantification of total content. Regardless, the  $\alpha_2$  is believed to be the major  
133 isoform in skeletal muscle (44). Thus research in rodents which showed e.g. increases in [<sup>3</sup>H]ouabain  
134 binding site content with training, represent a gain in the NKA  $\alpha_2$  isoform protein (58), whereas e.g.  
135 increases in human muscle with training, would primarily reflect increases in  $\alpha_2$ , but could also include  
136 changes in the  $\alpha_1$  or  $\alpha_3$  isoforms (82). A limitation of the standard [<sup>3</sup>H]ouabain binding site content  
137 technique for studying adaptability in human skeletal muscle is that it cannot differentiate between  
138 binding to the three  $\alpha$  isoforms, although using different concentrations of ouabain have been used for  
139 this purpose in muscle in some other species (61). A second limitation of the [<sup>3</sup>H]ouabain binding site  
140 content technique is the slow incubation time for [<sup>3</sup>H]ouabain to the muscle NKA, typically around ~2 h  
141 to saturate all sarcolemmal and t-tubular membranes, which means that impacts of processes  
142 changing within muscle on a more rapid time frame on NKA including hormonal changes, nutritional  
143 supplementation and acute exercise on e.g. translocation might not be detected (97). However, this  
144 latter limitation is not relevant to interpretation of the total NKA content in muscle, in particular with  
145 training or inactivity interventions, as biopsies are generally taken under resting conditions before and  
146 after a medium-long term intervention. Hence this long in-vitro incubation time for NKA content  
147 measurements will not affect training induced changes in resting skeletal muscle.

## 148 2. *NKA isoform proteins*

149 Western blotting is commonly used to investigate possible changes in NKA isoform abundance and  
150 phosphorylation with inactivity and training. The immunoblot technique should detect all NKA protein  
151 for the specific isoform probed, regardless of their membrane location, or incorporation into a  
152 functional NKA dimer. Thus the technique would also be expected to detect any isoform proteins  
153 present. In contrast the [<sup>3</sup>H]ouabain binding site technique detects functional NKA dimers, being  
154 locked into a conformation by vanadate to facilitate ouabain binding. That these different techniques  
155 are detecting some differences is suggested by the considerably different responses in percentage  
156 terms to training (see later). Thus whilst immunoblotting allows investigation of relative changes in  
157 abundance (e.g. with training), this does not allow quantification with molar units (19). Different  
158 analytical techniques are used which should be considered when evaluating differences in findings  
159 between research groups. Some studies used a fractionated muscle lysate for western blotting  
160 analyses (9, 106, 107), whilst others employed whole homogenate as the preparation of the sample  
161 (6, 88, 89, 96, 118, 120). Readers are referred to two excellent methods papers regarding western

162 blotting for more detail on these issues (76, 90). This difference in sample preparation may have an  
163 effect on the yield of the isoform retrieved (76, 90). An additional issue, which is not well documented  
164 within the literature, is the heating of a sample over 60°C, which can lead to aggregation of integral  
165 membrane proteins. Interpretations may be inaccurate compared to studies where no heating was  
166 employed. As a semi-quantitative technique, western blotting probably has greater variability in the  
167 magnitude of change compared to quantitative techniques such as [<sup>3</sup>H]ouabain binding site content.  
168 The typical error of western blotting for NKA isoforms was recently reported to be 10-30% (17). Thus,  
169 western blotting may not have the sensitivity to detect small changes in NKA isoforms. Another issue  
170 with western blotting is that potential adaptations may not have been detected due to proteins being  
171 measured in a mixed-muscle homogenate sample, rather than in individual muscle fibers. It is  
172 possible that some studies failed to detect actual changes in NKA isoform proteins that occurred in  
173 one fibre type only, by not measuring NKA isoforms at the single fiber level. To overcome this,  
174 researchers have begun isolating segments of single fibers from human muscle biopsies and  
175 performing western blots. So far, single fiber analyses have been utilized to investigate effects of both  
176 training and inactivity (96, 118, 120), these changes are described in more detail within the inactivity  
177 and training sections of this manuscript.

#### 178 *Other methodological considerations*

##### 179 *1. Intervention differences*

180 Many training studies that measured isoform abundance adaptations used trained populations, such  
181 as elite cyclists, football players, as well as recreationally active participants. The varying athletic  
182 status is likely to be important, as an earlier cross sectional analysis indicated that well-trained  
183 participants had a higher abundance of NKA isoforms, relative to recreationally active participants  
184 (88). Therefore, the level of stimulus required to increase the abundance of NKA isoforms in muscle  
185 may be greater in athletes compared with recreationally active and non-trained individuals. The  
186 experimental design also varies tremendously, making it difficult to make direct comparisons between  
187 studies, or to investigate any association between upregulation of NKA isoforms with any training  
188 modality or duration. Some studies also utilized High-Intensity Interval Training (HIT), speed  
189 endurance training (SET), or sprint training (ST) in replacement of regular training (51), or to  
190 supplement training (6, 8, 41), whilst others combined multiple training modalities within the same



191 study (106, 117). This makes their findings more difficult to compare with studies that used SET, ST,  
192 repeat sprint exercise (RSE) or HIT as the sole training modality (86, 92, 118, 120).

193 Finally, the sample size studied is important and insufficient statistical power may limit the capacity to  
194 detect changes in NKA isoform abundances in a number of training studies. The typical sample size  
195 for studies ranged between 8-15 participants, however in instances where n=15 were studied, these  
196 were often divided into two different groups (51, 86). A challenge of this research is finding sufficient  
197 numbers of volunteers willing to undergo invasive procedures on multiple occasions, which explains  
198 why sample sizes are often limited. This issue of small sample size and lower power is especially  
199 prevalent in invasive training studies with humans, nonetheless, future studies should embark on  
200 larger scale, simple training interventions to minimise potential effects of insufficient statistical power.  
201 This could be achieved by multicentre trials across institutions to recruit increased numbers of  
202 participants thus generating larger data sets.

### 203 **Disuse effects on muscle NKA content**

204 Both injury and disuse models have been used to study the broad effects of inactivity within skeletal  
205 muscle (12). Common disuse models include bed rest (54) with studies extending for as long as 119  
206 days (65), or immobilisation, which typically involves a cast placed around a limb to prevent dynamic  
207 muscle contractions and movement. A less constrictive approach is that of Unilateral Lower Limb  
208 Suspension (ULLS), in which participants wear one shoe with an extended sole (~10 cm) and walk  
209 with the assistance of crutches, causing one leg to become unloaded (105), with the contralateral leg  
210 acting as a control leg (96). A model used in athletes is reduced muscular usage or detraining that  
211 occurs with cessation of training, often after completion of a competitive season (106). The literature  
212 examining the effects of injury or inactivity on NKA in human skeletal muscle is currently sparse,  
213 being limited to only six studies, likely due to the extremely difficult nature of these studies, which  
214 combined with invasive measurements involve major disruption to a participant's daily life. Thus future  
215 studies are still required to understand the effects of inactivity on muscle NKA. In lieu of these  
216 challenges surrounding human volunteers, different models of human inactivity including astronauts,  
217 as well as a multicentre approach, should be used to investigate effects on muscle NKA. Here we  
218 have reviewed findings of the current studies which have investigated inactivity and NKA in human  
219 skeletal muscle.

220 Different types of injury that induce severe localised inactivity have been found to decrease muscle  
221 NKA content, including shoulder impingement syndrome, anterior cruciate ligament injury (using a  
222 contralateral limb as a control), paraplegia and partial spinal injury compared to ambulant, age-  
223 matched controls (13, 22, 67, 95). The muscle NKA content was reduced with these injuries, with  
224 declines ranging from 20-23% in patients with ruptured anterior cruciate ligament (n=6, mean age 25  
225 years, 5-50 weeks post-injury) (95), 27% in patients with shoulder impingement syndrome (n=6, mean  
226 age 44 years, at least 11-77 months post-injury) (67), 34% in paraplegia patients (n=6, mean age 32  
227 years, 1-19 years post-injury) compared to the deltoid of the same patients (22); and as much as a  
228 45% decline in chronic cervical spinal injury patients (n=6, mean age 44 years, injured for multiple  
229 years) compared to controls (13). However, for all of these studies, it is possible that in addition to  
230 enforced muscular inactivity, effects consequent to the injury per se, or medical treatment may also  
231 have had impact on muscle NKA. Thus a preferred approach is to investigate muscle unloading per  
232 se in otherwise healthy individuals, but to date only a single study has investigated the impacts of  
233 voluntary unloading on NKA (96). A surprising finding was that 23-days of ULLS failed to cause any  
234 decrease in NKA content, despite substantial impairment of muscle mass and function, including  
235 exercise performance (96). One interpretation of the lack of NKA downregulation after ULLS  
236 compared to the marked reductions in muscle NKA content with injury, is that differences may in part  
237 be attributed to the short time frame of the ULLS intervention. In animal models, where lifespans are  
238 much shorter, short-term inactivity induced substantial reductions in muscle [<sup>3</sup>H]ouabain binding site  
239 content when expressed relative to muscle wet weight; falling by 20% in soleus muscle after 1 week  
240 limb casting in rats (58), by 23-25% in gastrocnemius muscle and 18-19% in the plantaris muscle,  
241 after 2-3 weeks partial immobilisation using a prosthesis in guinea pigs, (66) and by 39% after 9  
242 weeks limb casting in sheep (52). Substantial recovery in muscle [<sup>3</sup>H]ouabain binding site content in  
243 sheep muscle occurred after subsequent 9 weeks of remobilisation (52). Immobilisation in young rats  
244 (5 days old) for 7 days reduced the normal gain that occurred at that age in [<sup>3</sup>H]ouabain binding in  
245 soleus muscles by 33% (112). Partial immobilisation for 3 weeks also allowed eventual recovery of  
246 [<sup>3</sup>H]ouabain binding site content (66). Inactivity subsequent to training also reduced the muscle  
247 [<sup>3</sup>H]ouabain binding site content; 6 weeks of swim training induced ~41% and ~46% upregulation in  
248 soleus and extensor digitorum longus muscles, respectively, whereas 3 weeks of subsequent rest  
249 reduced NKA by ~34% and ~26%, respectively (58). Therefore the results from these inactivity

250 studies in animals suggest that either a longer duration or greater severity of unloading may be  
251 required to depress NKA content in human skeletal muscle and the balance between mRNA mediated  
252 synthesis and degradation rates of NKA proteins. Other factors concomitant with injury, such as  
253 enhanced local inflammation (69, 70) and changes to neurotrophic factors (103) may also exert  
254 effects additive to those of disuse per se, but these are untested in relation to NKA expression

#### 255 **Disuse effects on muscle NKA Isoform abundances**

256 Only three studies have investigated the effects of injury and inactivity on muscle NKA isoform  
257 abundances in humans. Patients with chronic cervical spinal injury (n=6, mean age 44 years, injured  
258 for multiple years) had 75%, 52% and 38% lower NKA  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$  abundances in the vastus lateralis  
259 muscle, respectively, compared to healthy controls (13). Interestingly, those patients who were able to  
260 perform daily activities despite partial cervical spinal injury (n=6, mean age 49 years) actually  
261 exhibited no differences in NKA isoform abundances in the paralysed vastus lateralis muscle (13).  
262 Following 3 weeks of muscular disuse induced by ULLS in healthy young adults, there were no  
263 changes in the  $\alpha_1$  or  $\alpha_2$  isoform abundances, whether measured in either whole muscle homogenates  
264 or in single muscle fibers (96). However, after ULLS, the  $\beta_1$  isoform protein abundance was lower in  
265 Type II fibers (40%) and was also restored following resistance training; no changes were detected in  
266 homogenates (96). NKA heterodimers with a  $\beta_1$  isoform have been suggested to support higher NKA  
267 activity by having a greater affinity for  $\text{Na}^+$  than the  $\alpha/\beta_2$  heterodimer (64); thus a loss of  $\beta_1$  may imply  
268 a reduced number of functional NKA heterodimers present in Type II fibres of skeletal muscle after  
269 ULLS. The functional effects of possible reduction in  $\beta$  isoforms are not clear, as skeletal muscle is  
270 thought to have an excess abundance of  $\beta$  compared to  $\alpha$  subunits (64). Similarly, no changes in the  
271  $\alpha_1$ ,  $\alpha_2$  or  $\beta_1$  isoform abundances were found after a less severe inactivity model, comprising cessation  
272 of training for two weeks following the end of a soccer season and with isoforms measured in  
273 fractionated lysates (106). These studies strongly suggest, consistent with findings in NKA content,  
274 that reductions in muscle NKA isoforms are only induced by a severe lack of physical activity over a  
275 prolonged period. This conclusion is surprising given the large and rapid reductions in NKA isoforms  
276 evidenced in animal models. In rat muscle, the marked reductions in [ $^3\text{H}$ ]ouabain binding site content  
277 with one week inactivity represent mainly a reduction in the NKA  $\alpha_2$  isoform protein (58), due to its  
278 high affinity to ouabain (18) and as the dominant  $\alpha$  isoform expressed in muscle (42, 44). Changes in  
279 NKA  $\alpha_2$  isoforms are also highly complex and time-dependent. Hindlimb suspension in rats reduced

280 the electrogenic activity of the  $\alpha_2$  isoform protein, measured via ouabain-suppressible activity.  
281 Surprisingly, the reduction in electrogenic  $\alpha_2$  activity was accompanied with an initial doubling in  $\alpha_2$   
282 protein abundance after 24 h and with a ~50% elevation still remaining at 72 hours post-intervention,  
283  $\beta$  subunit protein abundances were unfortunately not reported (61). This indicates that the reduction in  
284  $\alpha_2$  electrogenic activity was due to a decline in NKA enzymatic activity per se; interestingly, no  
285 changes were found in the same measures for the  $\alpha_1$  isoform in the soleus muscle (61). These  
286 changes were subsequently demonstrated in a time frame as short as 12 h post hindlimb suspension  
287 (62). These changes in NKA may also be responsive to changes in plasma  $[K^+]$ , with hypokalaemia  
288 having a profound impact on NKA content and specific isoform abundance, with particular effects on  
289  $\alpha_2$  as seen in studies with rodents. When rats were placed on  $K^+$  deficient diets over a period of 1-4  
290 weeks, the  $\alpha_2$  showed a progressive decline and disappeared after 3 weeks (48). It has been  
291 suggested that decreased  $[K^+]$  may be important in suppressing mRNA to protein translation, at least  
292 for the  $\alpha_2$  isoform (7). Conversely, hyperkalaemia typically induces increases in NKA content, as  
293 increased  $K^+$  clearance is required; in rats this was observed within 7 days of a high  $K^+$  diet (15). The  
294 link between voluntary inactivity and plasma  $[K^+]$  changes in humans are not known, however, after 23  
295 days of ULLS plasma  $[K^+]$  at rest was not altered (96). Thus, in short-term inactivity studies  
296 investigating muscle NKA content or isoform abundances, any alterations are less likely to be  
297 changes in plasma  $[K^+]$ , at least in healthy populations. Hence, the time course of these changes and  
298 the underlying mechanisms in human muscle of considerable interest for future studies to explore.

### 299 *Muscle FXYD following inactivity*

300 Despite its emerging importance in regulating NKA activity (10), few studies have investigated the  
301 regulation of FXYD with disuse in human skeletal muscle. Cervical injury patients had 52% lower  
302 muscle FXYD1 content compared to healthy controls, with no difference in phosphorylation at  
303 FXYD1<sup>ser63</sup> and FXYD1<sup>ser68</sup> (13). The amount of basal and phosphorylated FXYD1 in the cervical  
304 spinal injury patients capable of ambulation (i.e. able to perform some movements) were not different  
305 from the controls (13). There was also an increase of the FXYD5 in the spinal injury patients (13).  
306 These few studies indicate that injury and physical inactivity clearly can regulate the abundance of the  
307 FXYD1 and 5 proteins. In addition, these findings in cervical injury patients indicate that reductions in  
308 the FXYD1 due to inactivity may not be related to the abundance of phosphorylation of FXYD1. It is  
309 possible that the unchanged phosphorylation of FXYD1 and increases in FXYD5 compensated for the

310 dramatic decline in  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$  isoforms and total amount of FXVD1 in these patients, thereby  
311 assisting in maintenance of functional NKA. Thus the abundance of the FXVD1 and 5 proteins may  
312 regulate the catalytic activity of the NKA despite declines in isoform abundance associated with  
313 inactivity.

314 The effects of disuse on the abundance of FXVD1 in skeletal muscle has not been extensively studied  
315 in healthy humans. Following two weeks of cessation of training in soccer players, there was no  
316 change in the abundance of FXVD1, however, there was a decrease in the phosphorylation of  
317 FXVD1<sup>ser68</sup> by 19% and 18% at 72 h and 2 weeks after training cessation, respectively (106). Given  
318 the training status of these participants, it is likely that FXVD1 proteins were already elevated by  
319 training; this is likely to be a typical post-training reduction rather than a true disuse effect.

## 320 **Effect of exercise training on muscle NKA**

### 321 *Classification of modalities of physical training.*

322 The first investigation into adaptability of muscle NKA with longitudinal exercise training was  
323 conducted nearly three decades ago (57). Since then numerous studies have investigated exercise  
324 training effects on muscle NKA content, NKA isoforms using a broad range of training modalities,  
325 which especially for high intensity training, have adapted over time and thus require definition. For the  
326 purpose of comparison of training effects on NKA in this review, exercise training modalities have  
327 been classified into three broad categories, defined as Endurance Training (ET), High Intensity  
328 Training (HIT) and Resistance Training (RT), as described in Table 1. Each of these exercise types  
329 will likely recruit a differing proportion of both Type I and Type II fibres; Type I fibres are more heavily  
330 recruited during submaximal endurance exercise, whereas during high intensity exercise, Type II  
331 fibres are recruited in addition to Type I (24). Thus the implementation of these exercises may  
332 influence NKA isoform contribution to exercise. ET is defined as training that comprises exercise  
333 bouts performed at an intensity between 50-80% of an individual's maximum oxygen consumption  
334 ( $VO_{2max}$ ) and typically sustained for a prolonged period, therefore having a heavy reliance on aerobic  
335 metabolic pathways. High Intensity Training (HIT) is defined as training utilising repeated, short  
336 duration, intense exercise bouts, interspersed with passive or active recovery periods, requiring a  
337 heavy contribution from anaerobic metabolism. HIT typically comprises 4-10 bouts, of 10 s to 4 min  
338 duration, completed at work rates  $\geq 90\% VO_{2peak}$ , or with longer  $\sim 4$  min bouts  $\geq 80\% VO_2$  peak (31,  
339 32, 50, 82). HIT can therefore be further classified into several sub-types of training, including Aerobic

340 High Intensity Training (AHIT), Speed Endurance Training (SET), Sprint/Speed Training (ST) and  
341 Repeat Sprint Exercise (RSE). Aerobic High Intensity Training (AHIT) is defined as repeated bouts of  
342 exercise between 1-5 minutes  $\geq 80\%$   $\text{VO}_2$  peak (6, 9) or HR max (33, 106) the recovery time is  
343 between 1:0.5 up to 1:2 work rest ratio.

344 Speed Endurance Training comprizes repeated 10-40 s sprint bouts of near-maximal intensity, with a  
345 1:5 work rest ratio (50), this type of training has also previously been termed sprint training (43, 82),  
346 but for consistency we will refer to this type of training as SET. Speed training (ST) comprizes 2-10 s  
347 maximal exercise, with recovery periods up to 1:10 work rest ratio (50). Repeat-sprint exercise (RSE)  
348 comprizes multiple (4-6) high-intensity bursts, each lasting between 2-6 s, interspersed by a brief  
349 recovery period (102, 104) and are typically used to be comparable with efforts produced during  
350 intermittent team sports, such as soccer, rugby, Australian football and hockey (4, 53, 113).

351 Resistance Training (RT) is classically defined as moving limbs/ or body segments against various  
352 resistances including machines, dumbbells, body weight and cables and is utilized to improve muscle  
353 strength and power and to promote muscular hypertrophy. The performance benefits of ET, HIT and  
354 RT have been well described elsewhere (31, 33, 50, 85) and hence are not covered here.

### 355 ***Adaptations in muscle NKA content with endurance and high intensity training***

356 The findings of studies investigating training effects on muscle NKA content are indicated in Table 2.  
357 In order to summarise this literature, we searched for studies involving humans which had  
358 investigated muscle [ $^3\text{H}$ ]ouabain binding and/or muscle NKA isoforms with training or inactivity. No  
359 studies were excluded and those that measured but failed to detect any upregulation with training  
360 were also cited. The studies are broadly consistent, with 8-25% increases in NKA content elicited with  
361 training, in 10 out of 12 studies published to date. Furthermore, and importantly, these increases  
362 appear to be regardless of the type of training utilised, or the population studied. Only two of these  
363 studies did not detect an increase in NKA content; in the first neither the training modality nor fitness  
364 status of participants were detailed (57), whilst in the more recent study, the participants were already  
365 well-trained cyclists ( $\text{VO}_{2\text{peak}} 4.9 \text{ L}\cdot\text{min}^{-1}$ ) (6). Thus, it is possible that the training stimulus used was  
366 sub-optimal or that the muscle NKA content may already have been elevated before the training  
367 intervention (88). Nonetheless, upregulation of NKA content in muscle is clearly a consistent finding.  
368 To compare findings from the various studies, the 90% Confidence Interval (90%CI) was calculated  
369 utilising each of the percentage increases in NKA content, reported p values and sample size (47).

370 Where the precise p value was not presented, but rather reported as  $p < 0.05$ , we took a conservative  
371 approach, using a p-value of 0.049 for consistency across analysis. The study by (57) was not  
372 included as insufficient data were reported. The objective was to identify whether there were any  
373 apparent differences in adaptation with different training modes. The data reveals firstly that NKA  
374 content was consistently increased with training, between 8-22%, regardless of training modality,  
375 whether studied in healthy young or older adults, or in Type I diabetics (Figure 1). Furthermore, the  
376 percentage increase in NKA content was not related to either the mean training intensity or  
377 cumulative training time (Figure 2). An important additional finding was that the training duration did  
378 not affect the gain in muscle NKA content. An increase in NKA content was found after only one week  
379 of ET (39) and participants undertaking ET exhibited a 22% increase in NKA content after 3 weeks,  
380 but with no further increase after 12 weeks (34). Thus, the mean gain in NKA content did not exceed  
381 ~25%, even when training exceeded 3 months. Elderly also displayed a similar muscle NKA content  
382 upregulation with training, with an 11% increase after 12 weeks of HIT (118). An early cross sectional  
383 study demonstrated that older adults who had been active for over 10 years had higher muscle NKA  
384 content compared to sedentary older adults, which ranged between 30-40% depending on the type of  
385 training, including swimming (30%), running (32%) and RT (40%) (59). It is of interest to compare  
386 these findings in human muscles, to those with chronically stimulated muscles in animal models. Low  
387 frequency stimulation of extensor digitorum longus muscle in rabbits rapidly increased the [<sup>3</sup>H]ouabain  
388 binding site content by ~41% after only 3 days, 86% after 10 days, then plateaued, with no further  
389 increase after 50 days (37). Even larger increases were found in a subsequent study, where a gain in  
390 [<sup>3</sup>H]ouabain binding site content of 60% occurred after 6 days and by 107% after 20 days chronic low  
391 frequency stimulation (45). Apparently, there are clear differences between species in the magnitude  
392 and rate of adaptation of muscle NKA. Within humans, the importance of the NKA increasing during  
393 training has obvious implications for maintaining membrane potential and K<sup>+</sup> clearance during  
394 exercise, for improvement of exercise performance (78, 82). But the time course of adaptability in  
395 NKA in human skeletal muscle content are needed to understand why and when NKA adaptation  
396 reaches a plateau. For example in the studies listed in Table 2, the maximum NKA content increase  
397 was ~25%. There are several possible speculations on what may be limiting increases in NKA content  
398 with training. A consideration might be that the stimulus of training isn't eliciting the same 'new  
399 stimulus' and thus over time, the NKA pool is able to better cope with the demands of the training

400 session, the physiological challenge is decreased with a lesser requirement for synthesis of new NKA.  
401 Secondly, it might be dangerous to synthesize NKA beyond a particular threshold within a given  
402 individual. As muscle makes up 40% human body mass its role in clearing increases of  $K^+$  is  
403 extremely important. Thus more NKA in muscle would enable greater  $K^+$  clearance and thereby better  
404 performance; however this also has large potential effects on post-exercise plasma [ $K^+$ ].  
405 Hypokalaemia is commonly reported within the first 5-10 minutes of recovery from an acute bout of  
406 intensive exercise, in particular exercise utilizing a large muscle mass (e.g. rowing, sprint cycling) (2,  
407 3, 71); this is likely due to an highly activated NKA. Hypokalaemia has important adverse implications  
408 for cardiac muscle (71) with a recent study showing the post-exercise hypokalaemia was associated  
409 with impaired cardiac hysteresis measured via electrocardiogram (3). This lowered  $K^+$  post exercise  
410 therefore has implications for cardiac arrhythmias and sudden cardiac death after exercise (3). Thus a  
411 training plateau of the increase in NKA content may be a protective mechanism, however more  
412 research is required to determine the time point or physiological point where this plateau is reached.

413

#### 414 *Adaptations in NKA content with resistance training.*

415 Three studies have examined the effects of RT on skeletal muscle NKA content. In one study,  
416 participants performed RT for 12 weeks, comprising 3 sets of 6-8 repetitions of each of leg press, squat  
417 and leg extension exercises, finding that muscle NKA content was unchanged after 4 weeks (34),  
418 increased by 16% after 7 weeks, but then remained constant until 12 weeks (34). In another study, well-  
419 trained cross country skiers undertook RT comprising five series of four heavy full squat lifts, with a  
420 focus on eccentric contractions, completed either once, twice or three times per week, for 3 months  
421 (83). They found that NKA content was not significantly increased in the athletes that undertook RT only  
422 once a week, but was increased when athletes trained twice and three times per week by 15% in the  
423 pooled results (83). In the third study, the effects of 4 weeks RT on muscle NKA content were examined  
424 in six healthy participants, with RT undertaken immediately following a 23-day period of ULLS.  
425 Interestingly, RT had no effect on the NKA content, despite gains in both muscle mass and strength  
426 (96). Regardless, an unchanged NKA content in the context of an overall increased muscle mass would  
427 in fact suggest an increased NKA synthesis commensurate with the increased muscle protein content,  
428 but detailed studies are required to verify this.

#### 429 *Adaptations in muscle NKA content with exercise training in hypoxia*



430 Whilst almost all exercise training studies reported an increased muscle NKA content, undertaking  
431 training with hypoxic exposure actually induced the opposite effect of reducing NKA content, at least  
432 for ET. Participants who performed ET in normoxia exhibited a 14% increase in muscle NKA content  
433 after ET, whereas a group that trained under hypoxic conditions over 8 weeks had a decline in muscle  
434 NKA content by 14% (35). This decline was similar to the 14% reduction found after a 21-day expedition  
435 to 6,194 m in recreationally active people (36). Practically, this implies that training in hypoxia per se  
436 may not be beneficial for enhancing muscle performance. Mechanistically, this may be due to reactive  
437 oxygen species (ROS) which are generated during exercise, ROS generation is amplified when training  
438 in hypoxia (74) and ROS may inhibit NKA activity during exercise (81) and thus muscle cellular  
439 responses to chronic hypoxia may prematurely impair NKA activity and excitability during training. From  
440 a training perspective, the quality and capacity of each training session would then be compromised,  
441 with athletes' therefore not reaching required training load and reflective in a lack of NKA responses  
442 (5). Regarding chronic hypoxic exposure that caused a reduction in NKA content, although it was  
443 hypothesised exposure to hypoxia may result in greater protein breakdown and thus a loss of NKA was  
444 seen after 21 days reaching 6,194 m (36), there is little evidence to directly support this explanation. An  
445 alternative approach to training in hypoxia, that allows athletes to receive the beneficial adaptations of  
446 altitude exposure has been termed Live-High, Train-Low (LHTL) (68). When well-trained endurance  
447 athletes continued their normal training whilst undertaking 23 consecutive nights of hypoxic exposure,  
448 no change in muscle NKA content occurred (5) thereby intermittent exposure to hypoxia may be more  
449 beneficial to NKA and allows athletes are able to train at appropriate intensities while obtaining  
450 haematological benefit (27, 49).

#### 451 **Muscle NKA isoform adaptability to training**

452 Over the past decade, there has been considerable interest in determining the malleability of NKA  
453 isoforms in human skeletal muscle with training. These studies show highly variable responsiveness  
454 of specific NKA isoforms to various training modalities (Table 2). The percent change  $\pm 90\%$  CI (47) for  
455 most commonly measured NKA isoforms,  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$  with training is presented in Figure 3. Only  
456 around one-half of the studies published to date reported increases in these isoforms, with increases  
457 found for  $\alpha_1$  in 6 of 13 studies, for  $\alpha_2$  in 6 of 13 studies and for  $\beta_1$  isoforms in 7 of 13 studies. It is  
458 surprising that less than one-half of studies utilizing western blotting detected an increase in the  $\alpha_2$

459 isoform with training. There is no apparent consistency regarding the upregulation of any isoform with  
460 a particular type of training. This may be due to methodological considerations, as outlined on page's  
461 5-7. It is also unclear whether any particular training modality consistently increased one isoform more  
462 than another. Only three of these 13 studies detected an increase for each of the  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$   
463 isoforms (9, 20, 38). One study utilizing high-intensity single leg cycling reported an increase in both  
464 the  $\alpha_1$  and  $\alpha_2$  isoforms (92); while SET (running) increased in both the  $\alpha_2$  and  $\beta_1$  isoforms (86); two  
465 studies which incorporated either regular football (soccer) training or repeated small sided soccer  
466 drills (8x2 min) in conjunction with SET running training found increases in  $\alpha_2$  (8, 106); another study  
467 found sprint training (running) exclusively increased  $\alpha_1$  (51); while a combination of mixed RT and  
468 SET training found an increase only in  $\beta_1$  (117).

#### 469 *NKA isoform measurements within single muscle fibers*

470 There have been a handful of studies conducted within single fibers to elucidate how the NKA works  
471 during exercise and adapts to training. The first study examined acute exercise responses primarily  
472 focusing on FXYD1 phosphorylation (108). Following a 5-min bout of intense exercise, corresponding  
473 to ~95% of maximal oxygen uptake on a cycle ergometer, there was an increase in phosphorylation of  
474 FXYD1<sup>ser68</sup> in Type II fibers and increased unspecified FXYD1 phosphorylation in both Type I and II  
475 fibers (108). Following 4 weeks of RSE training, which comprised 3 sets of 5 x 4 s sprints performed  
476 on a non-motorised treadmill, there was a 42% increase in  $\beta_1$  isoform protein abundance in Type II  
477 fibers, with no changes found for other isoforms (120). A 12-week training protocol comprising four 4-  
478 min bouts at 95% peak heart rate, performed 3 times per week in adults aged over 65 years, showed  
479 a 30%-increase in  $\alpha_2$  in Type II fibers with no other isoforms being upregulated (118). In adults aged  
480 between 18-35 years, six weeks of High-Intensity Training (HIT) comprising 4x 30s sprints, with 4  
481 minutes recovery between sprints, induced increases in the  $\alpha_1$  and  $\beta_3$  isoforms in both Type I and II  
482 fibers,  $\beta_1$  in Type II fibers, and decreases in FXYD1 in Type I fibers (17).

483 Despite a lack of consistency around training and isoform upregulation, one observation is the studies  
484 that found increases in  $\alpha_2$  utilized training that comprized either exercise of high intensity, ranging  
485 between 90-150%of  $VO_2$  max or running speed (8, 9, 86, 92, 106), or of high volume, with training  
486 sessions lasting between 60-120 minutes (9, 38). One RT study also found increases in the  $\alpha_2$  isoform  
487 (20), which might relate to the repeated highly intense contractions performed in RT. It is likely that

488 intense quadriceps contractions during these high-intensity or high-volume running and cycle training  
489 studies (5, 6, 71, 76, 90), as well as during RT (14) also indicate heavy recruitment of the vastus  
490 lateralis muscle, hence accounting for the consistent elevations in the NKA  $\alpha_2$  isoform in the vastus  
491 lateralis, thus explaining why different modes of exercise training induced similar outcomes for  $\alpha_2$ .

#### 492 *Muscle FXYD1 and training*

493 Ten days of training which incorporated both ET at  $\sim 75\%$   $VO_{2\text{ peak}}$  for 45-90 min and AHIT (comprising  
494 6x5 min intervals at 90-100%  $VO_{2\text{ peak}}$ ), had no effect on total FXYD1 content or phosphorylation at  
495 Ser<sup>63</sup>, Ser<sup>68</sup> or Thr<sup>69</sup>, despite upregulation of each of the NKA  $\alpha_1$ ,  $\alpha_2$  and  $\beta_1$  isoforms (9). In contrast,  
496 after 2 weeks combined SET and AHIT, FXYD1 phosphorylation on site Ser<sup>68</sup> relative total FXYD1 was  
497 increased by 27% (106). Similarly, in well trained endurance cyclists, subsequent to a reduction in  
498 training volume by  $\sim 70\%$  and then replaced with SET and AHIT, there was a 30% increase in FXYD1  
499 protein abundance and an increase in non-specific FXYD1 phosphorylation, suggested to be attained  
500 through phosphorylation at Ser<sup>68</sup> (107). An interesting observation is when there was a heavy ET  
501 component during 10 d of one-legged cycling training, there were no changes in FXYD1  
502 phosphorylation on sites Ser<sup>63</sup>, Ser<sup>68</sup> or Thr<sup>69</sup> or the total FXYD1 abundance (9). Conversely, when  
503 intermittent intense exercise training was predominantly used, both FXYD1 abundance and  
504 phosphorylation were increased (106, 107). Together this suggests a higher intensity of training may  
505 be required to induce FXYD1 phosphorylation adaptations.

#### 506 ***Association between muscle NKA, performance and fatigue***

507 The increases of  $\alpha_2/\beta_1$  isoforms in skeletal muscle with training reported in a number of studies may  
508 have considerable implications for NKA activity and exercise performance, but it is important to  
509 acknowledge that these changes have not been consistently reported. The fact that the [<sup>3</sup>H]ouabain  
510 binding sites are increased suggests that the  $\alpha_2$  isoform at least should also be elevated and points to  
511 methodological reasons underpinning the inconsistent findings. Both  $\alpha_2$  and  $\beta_1$  isoforms are believed  
512 to be the major isoforms employed during muscle contractions/exercise (64, 98). The  $\alpha_2$  isoform  
513 abundance was correlated to high-intensity running during soccer. Importantly, the  $\alpha_2$  and  $\beta_1$  isoforms  
514 are each expressed in Type I versus II muscle fibers with no fibre-type dominance being reported  
515 (119, 120). This suggests that both isoforms can exert an effect on the whole muscle, rather than  
516 being constrained to a dominant effect in one fiber-type only, as is the case for other enzymes and  
517 proteins that are expressed specifically in one fiber-type only in skeletal muscle. The use of co-

518 immunoprecipitation of  $\alpha$ ,  $\beta$  and  $\gamma$  subunit isoforms would be particularly valuable in identifying fiber-  
519 type specific heterodimers. The same could be said for improvements in the NKA  $\alpha_1$  isoform, which  
520 was observed to adapt as often as the  $\alpha_2$  isoform, but just as inconsistently, and which also showed  
521 the largest reported increase in any isoform, of up to ~80% (9) (Figure 3). Given we do not yet know  
522 the relative composition or respective roles of the  $\alpha$  subunit isoforms in human skeletal muscle, it is  
523 possible that adaptations in the  $\alpha_1$  may play an equally important role as those for  $\alpha_2$ , since  
524 improvements in performance and  $K^+$  regulation were also seen with increases only in  $\alpha_1$  (51). The  $\alpha_2$   
525 key role is to regulate  $Na^+/K^+$  gradients during contractions, and thus it would be expected to be  
526 increased in most training studies. However, this review demonstrates that this is not always the case.  
527 In training protocols utilising short bouts of only a few seconds duration, the rise in interstitial  $[K^+]$  and  
528 intracellular  $[Na^+]$  may not be as pronounced, in particular, intercellular  $Na^+$  is a potentially important  
529 regulator which may trigger the synthesis of new NKA as demonstrated in myotubes (14, 116). Thus if  
530 these sprints are too short, there might be insufficient stimulus for complete  $\alpha_2$  activation and or  $\alpha_2$   
531 synthesis. The lack of consistency among training studies and the mechanistic research conducted  
532 thus far makes speculation difficult. For these reasons, it should not be a surprise that both  $\alpha_1$  and  $\alpha_2$   
533 display large adaptability to longer periods of both intense (9, 92) and long endurance exercise (9,  
534 39). It is likely that FXYP1 also plays an important role in skeletal muscle function, since a reduction  
535 in phosphorylation of FXYP1<sup>ser68</sup> were associated with declines in physical tests related to team sport  
536 performance, namely a repeat sprint test and Yo-Yo IR2 performance, (106, 107).

### 537 ***Conclusions and perspectives***

538 Exercise training has been demonstrated to robustly increase NKA content with most training types,  
539 however individual isoform responses are much more varied. More studies need to be undertaken to  
540 determine which isoforms are changed with various types of training inclusive of changes in FXYP1  
541 and its phosphorylation. These investigations will need to calibrate the potentially differing impacts of  
542 training intensity, duration and training modalities. Studying both exercise intensity and duration as  
543 differing regulators of NKA, would provide valuable understanding whether specific isoforms have a  
544 particular threshold of physical activity for upregulation, whether one specific isoform is upregulated in  
545 preference to, in concert with, in sequence with, or independent of other isoforms during training and  
546 may reveal the mechanisms behind training induced NKA upregulation.

547 The limited available evidence with voluntary disuse in humans suggests that NKA content is  
548 surprisingly resilient to change with short-term inactivity. However, severe injury, which promotes long-  
549 term inactivity, such as observed with spinal injury, ACL injury and shoulder impingement clearly reduce  
550 skeletal muscle NKA content. These conclusions are all drawn however, from a limited number of  
551 studies, so further research is needed to better understand the NKA response to disuse. An important  
552 component of this should be a focus on the time course of responses in NKA isoforms with both training  
553 and inactivity, focusing on specific adaptations to disuse as well as their implications for muscle NKA  
554 activity and overall muscle function. Finally, molar quantification of each of the NKA  $\alpha$  and  $\beta$  isoforms  
555 in human skeletal muscle is essential, particularly in the context of heterodimers, which determine NKA  
556 function. Understanding the relative distribution of these isoforms in muscle, in specific fiber-types,  
557 including through co-IP studies, could uncover their specific contributions to changes in muscle function  
558 and adaptability. Detailed understanding of the functional roles of the different NKA isoforms will enable  
559 the implications of their adaptability for understanding human musculoskeletal function, as well as  
560 exercise limitation through peripheral and respiratory muscles.

561

562 **LIST OF FIGURES.**

563 **Figure 1. Percentage changes in [<sup>3</sup>H]ouabain binding site content (NKA content) in human**  
564 **skeletal muscle with A) Injury, inactivity and chronic disease and B) exercise training**

565 Panel A, the data shows difference from pre and post inactivity and is presented as calculated percent  
566 change  $\pm$  90% CI. Data is presented from four studies, of which two were models of injury, one of  
567 paraplegia and one study comprized inactivity. Specifically, three references show percentage  
568 compared to a control limb (67, 95, 96) while two others compared to control participants (13, 22).  
569 Panel B, the data shows difference from pre and post training, calculated percent change  $\pm$  90% CI.

570

571 **Figure 2. Neither training intensity nor volume is specifically related to upregulation of**  
572 **[<sup>3</sup>H]ouabain binding site content in human skeletal muscle with training.**

573 Data is presented as percentage increases in [<sup>3</sup>H]ouabain binding site content (NKA content) in  
574 human skeletal muscle, plotted against A) training intensity, B) minutes trained per week and C) total  
575 training minutes. Training intensity was expressed as percentage of maximum, using measures  
576 utilized in differing studies, which included maximum HR, maximum running speed and VO<sub>2 max</sub>. In  
577 studies where training minutes or exercise intensity were gradually increased during the training  
578 period, the average over the duration of the study was used and plotted.

579

580 **Figure 3. Inconsistent training adaptions of NKA isoforms measured in homogenates in human**  
581 **skeletal muscle.**

582 Data for isoforms are compared to 'pre-training' and presented as calculated percent change  $\pm$  90%  
583 CI for A)  $\alpha_1$ , B)  $\alpha_2$  C)  $\beta_1$

584 Isoforms not indicated were not measured, or reported in that study. Significance levels were  $p < 0.05$ .

585

586 **LIST OF TABLES.**

587 **Table 1. Table 1. General characteristics of different training types**

588 ET, Endurance Training; HIT, High Intensity Training; AHIT, Aerobic High-Intensity Training, RT,  
589 Resistance Training; SET, Speed Endurance Training; ST, Sprint/Speed Training; RSE, Repeat  
590 Sprint Exercise.

591

592 **Table 2. Adaptations in exercise performance and skeletal muscle [<sup>3</sup>H]ouabain binding site**  
593 **content (NKA content) to intense exercise training in healthy young humans**

594 NR not reported in that study. n.c= no significant difference pre-post training. ↑ = increase compared  
595 to pre-training. Significance levels were p<0.05. References. 1. Kjeldsen et al., 1990 (57) 2. McKenna  
596 et al., 1993 (82) 3. Green et al., 1993 (39) 4. Madsen et al., 1994 (73) 5. Green et al., 1999a (34) 6.  
597 Evertsen et al., 1997 (25) 7. Medbø et al., 2001 (83) 8. Harmer et al., 2006 (43) 9. Aughey et al.,  
598 2007 (6) 10. Green et al., 2008 (38) 11. Edge et al., 2013 (23) 12. Wyckelsma et al., 2017 (118).

599 **Table 3. NKA isoform abundance in human homogenates and exercise performance changes**  
600 **following intense exercise training in healthy young humans**

601 n.s = no significant difference pre-post training. ↑ = increase compared to pre-training. Significance  
602 levels were p<0.05. References.1. Dela et al., 2004 (20) (CON group) 2. Nielsen et al., 2004 (92) 3.  
603 Aughey et al., 2007 (6) 4. Mohr et al., 2007 (86) 5. Green et al., 2008 (38) 6. Iaia et al., 2008 (51) 7.  
604 Bangsbo et al., 2009 (8) 8. Thomassen et al., 2010 (106) 9. Gunnarsson et al., 2012 (41) 10.  
605 Gunnarsson & Bangsbo, 2012 (40) 11. Thomassen et al., 2016 (107) 12. Benziene et al., 2011 (9) 13.  
606 Vorup et al., 2016 (117).

607

608 **References**

- 609 1. **Allen DG, Lamb GD, and Westerblad H.** Skeletal muscle fatigue: cellular mechanisms. *Physiol*  
610 *Rev* 88: 287-332, 2008.
- 611 2. **Atanasovska T, Petersen AC, Rouffet DM, Billaut F, Ng I, and McKenna MJ.** Plasma K<sup>+</sup>  
612 dynamics and implications during and following intense rowing exercise. *J Appl Physiol* 117: 60-68,  
613 2014.
- 614 3. **Atanasovska T, Smith R, Graff C, Tran CT, Melgaard J, Kanters JK, Petersen AC, Tobin A,**  
615 **Kjeldsen KP, and McKenna MJ.** Protection against severe hypokalemia but impaired cardiac  
616 repolarization after intense rowing exercise in healthy humans receiving salbutamol. *J Appl Physiol*  
617 125: 624-633, 2018.
- 618 4. **Aughey RJ.** Australian Football Player Workrate: Evidence of fatigue and pacing? *Int J Sport*  
619 *Physiol* 5: 394-405, 2010.
- 620 5. **Aughey RJ, Gore CJ, Hahn AG, Garnham AP, Clark SA, Petersen AC, Roberts AD, and**  
621 **McKenna MJ.** Chronic intermittent hypoxia and incremental cycling exercise independently depress  
622 muscle in vitro maximal Na<sup>+</sup>-K<sup>+</sup>-ATPase activity in well-trained athletes. *J Appl Physiol* 98: 186-192,  
623 2005.
- 624 6. **Aughey RJ, Murphy KT, Clark SA, Garnham AP, Snow RJ, Cameron-Smith D, Hawley JA, and**  
625 **McKenna MJ.** Muscle Na<sup>+</sup>-K<sup>+</sup>-ATPase activity and isoform adaptations to intense interval exercise  
626 and training in well-trained athletes. *J Appl Physiol* 103: 39-47, 2007.
- 627 7. **Azuma KK, Hensley CB, Putnam DS, and McDonough AA.** Hypokalemia decreases Na<sup>+</sup>-K<sup>+</sup>-  
628 ATPase alpha 2- but not alpha 1-isoform abundance in heart, muscle, and brain. *Am J Physiol Cell*  
629 *Physiol.*260: C958-C964, 1991.
- 630 8. **Bangsbo J, Gunnarsson TP, Wendell J, Nybo L, and Thomassen M.** Reduced volume and  
631 increased training intensity elevate muscle Na<sup>+</sup>-K<sup>+</sup> pump alpha2-subunit expression as well as short-  
632 and long-term work capacity in humans. *J Appl Physiol* 107: 1771-1780, 2009.
- 633 9. **Benziane B, Widegren U, Pirkmajer S, Henriksson J, Stepto NK, and Chibalin AV.** Effect of  
634 exercise and training on phospholemman phosphorylation in human skeletal muscle. *Am J Physiol*  
635 *Endocrinol Metab* 301: E456-466, 2011.
- 636 10. **Bibert S, Roy S, Schaer D, Horisberger JD, and Geering K.** Phosphorylation of  
637 phospholemman (FXD1) by protein kinases A and C modulates distinct Na,K-ATPase isozymes. *J Biol*  
638 *Chem* 283: 476-486, 2008.
- 639 11. **Blanco G, and Mercer RW.** Isozymes of the Na-K-ATPase: heterogeneity in structure,  
640 diversity in function. *Am J Physiol-Renal* 275: F633-F650, 1998.
- 641 12. **Bogdanis GC.** Effects of physical activity and inactivity on muscle fatigue. *Front Physiol* 3:  
642 142, 2012.
- 643 13. **Boon H, Kostovski E, Pirkmajer S, Song M, Lubarski I, Iversen P, Hjeltnes N, Widegren U,**  
644 **and Chibalin A.** Influence of chronic and acute spinal cord injury on skeletal muscle Na<sup>+</sup>/K<sup>+</sup>-ATPase  
645 and phospholemman expression in humans. *Am J Physiol Endocrinol Metab* 302: E864-871, 2012.
- 646 14. **Brodie C, and Sampson SR.** Regulation of the sodium-potassium pump in cultured rat  
647 skeletal myotubes by intracellular sodium ions. *J Cell Physiol* 140: 131-137, 1989.
- 648 15. **Bundgaard H, Schmidt TA, Larsen JS, and Kjeldsen K.** K<sup>+</sup> supplementation increases muscle  
649 Na<sup>+</sup>-K<sup>+</sup>-ATPase and improves extrarenal K<sup>+</sup>-homeostasis in rats. *J Appl Physiol* 82: 1136-1144, 1997.
- 650 16. **Chambers MA, Moylan JS, and Reid MB.** Physical inactivity and muscle weakness in the  
651 critically ill. *Crit Care Med* 37: S337-346, 2009.
- 652 17. **Christiansen D, Bishop DJ, Broatch JR, Bangsbo J, McKenna MJ, and Murphy RM.** Cold-  
653 water immersion after training sessions: effects on fiber type-specific adaptations in muscle K<sup>+</sup>  
654 transport proteins to sprint-interval training in men. *J Appl Physiol* 125: 429-444, 2018.
- 655 18. **Clausen T.** Na<sup>+</sup>-K<sup>+</sup> Pump Regulation and Skeletal Muscle Contractility. *Physiol Rev* 83: 1269-  
656 1324, 2003.
- 657 19. **Clausen T.** Quantification of Na<sup>+</sup>,K<sup>+</sup> pumps and their transport rate in skeletal muscle:  
658 Functional significance. *J Gen Physiol* 142: 327-345, 2013.



- 659 20. **Dela F, Holten M, and Juel C.** Effect of resistance training on Na,K pump and Na<sup>+</sup>/H<sup>+</sup>  
660 exchange protein densities in muscle from control and patients with type 2 diabetes. *Pflugers Arch*  
661 447: 928-933, 2004.
- 662 21. **Ding D, Lawson KD, Kolbe-Alexander TL, Finkelstein EA, Katzmarzyk PT, van Mechelen W,**  
663 **Pratt M, and Lancet Physical Activity Series 2 Executive C.** The economic burden of physical  
664 inactivity: a global analysis of major non-communicable diseases. *Lancet* 388: 1311-1324, 2016.
- 665 22. **Ditor DS, Hamilton S, Tarnopolsky MA, Green HJ, Craven BC, Parise G, and Hicks AL.**  
666 Na<sup>+</sup>,K<sup>+</sup>-ATPase concentration and fiber type distribution after spinal cord injury. *Muscle Nerve* 29:  
667 38-45, 2004.
- 668 23. **Edge J, Eynon N, McKenna MJ, Goodman CA, Harris RC, and Bishop DJ.** Altering the rest  
669 interval during high-intensity interval training does not affect muscle or performance adaptations.  
670 *Exp Physiol* 98: 481-490, 2013.
- 671 24. **Edström L, and Grimby L.** Effect of exercise on the motor unit. *Muscle Nerve* 9: 104-126,  
672 1986.
- 673 25. **Evertsen F, Medbo JJ, Jebens E, and Nicolaysen K.** Hard training for 5 mo increases Na<sup>+</sup>-K<sup>+</sup>  
674 pump concentration in skeletal muscle of cross-country skiers. *Am J Physiol* 272: R1417-1424, 1997.
- 675 26. **Galuska D, Kotova O, Barrès R, Chibalina D, Benziane B, and Chibalin AV.** Altered  
676 expression and insulin-induced trafficking of Na<sup>+</sup>-K<sup>+</sup>-ATPase in rat skeletal muscle: effects of high-fat  
677 diet and exercise. *Am J Physiol Endocrinol Metab* 297: E38-E49, 2009.
- 678 27. **Garvican-Lewis LA, Clark SA, Polglaze T, McFadden G, and Gore CJ.** Ten days of simulated  
679 live high:train low altitude training increases Hbmass in elite water polo players. *Br J Sports Med* 47  
680 Suppl 1: i70-73, 2013.
- 681 28. **Geering K.** The functional role of beta subunits in oligomeric P-type ATPases. *J Bioenerg*  
682 *Biomembr* 33: 425-438, 2001.
- 683 29. **Geering K.** FXYP proteins: new regulators of Na-K-ATPase. *Am J Physiol-Renal* 290: F241-  
684 F250, 2006.
- 685 30. **Geering K, BÉGuin P, Garty H, Karlish S, FÜZesi M, Horisberger J-D, and Crambert G.** FXYP  
686 Proteins: New Tissue- and Isoform-Specific Regulators of Na,K-ATPase. *Ann N Y Acad Sci.* 986: 388-  
687 394, 2003.
- 688 31. **Gibala MJ, Little JP, Macdonald MJ, and Hawley JA.** Physiological adaptations to low-  
689 volume, high-intensity interval training in health and disease. *J Physiol* 590: 1077-1084, 2012.
- 690 32. **Gibala MJ, and McGee SL.** Metabolic adaptations to short-term high-intensity interval  
691 training: a little pain for a lot of gain? *Exerc Sport Sci Rev.* 36: 58-63, 2008.
- 692 33. **Gliemann L, Gunnarsson TP, Hellsten Y, and Bangsbo J.** 10-20-30 training increases  
693 performance and lowers blood pressure and VEGF in runners. *Scand J Med Sci Sports* 25: e479-489,  
694 2015.
- 695 34. **Green H, Dahly A, Shoemaker K, Goreham C, Bombardier E, and Ball-Burnett M.** Serial  
696 effects of high-resistance and prolonged endurance training on Na<sup>+</sup>-K<sup>+</sup> pump concentration and  
697 enzymatic activities in human vastus lateralis. *Acta Physiol Scand* 165: 177-184, 1999.
- 698 35. **Green H, MacDougall J, Tarnopolsky M, and Melissa NL.** Downregulation of Na<sup>+</sup>-K<sup>+</sup>-ATPase  
699 pumps in skeletal muscle with training in normobaric hypoxia. *J Appl Physiol* 86: 1745-1748, 1999.
- 700 36. **Green H, Roy B, Grant S, Burnett M, Tupling R, Otto C, Pipe A, and McKenzie D.**  
701 Downregulation in muscle Na<sup>+</sup>-K<sup>+</sup>-ATPase following a 21-day expedition to 6,194 m. *J Appl Physiol* 88:  
702 634-640, 2000.
- 703 37. **Green HJ, Ball-Burnett M, Chin ER, Dux L, and Pette D.** Time-dependent increases in Na<sup>+</sup>-K<sup>+</sup>-  
704 ATPase content of low-frequency-stimulated rabbit muscle. *FEBS Lett* 310: 129-131, 1992.
- 705 38. **Green HJ, Bombardier E, Duhamel TA, Stewart RD, Tupling AR, and Ouyang J.** Metabolic,  
706 enzymatic, and transporter responses in human muscle during three consecutive days of exercise  
707 and recovery. *Am J Physiol Regul Integr Comp Physiol* 295: R1238-1250, 2008.
- 708 39. **Green HJ, Chin ER, Ball-Burnett M, and Ranney D.** Increases in human skeletal muscle Na<sup>+</sup>-  
709 K<sup>+</sup>-ATPase concentration with short-term training. *Am J Physiol Cell Physiol* 264: C1538-1541, 1993.

- 710 40. **Gunnarsson TP, and Bangsbo J.** The 10-20-30 training concept improves performance and  
711 health profile in moderately trained runners. *J Appl Physiol* 113: 16-24, 2012.
- 712 41. **Gunnarsson TP, Christensen PM, Holse K, Christiansen D, and Bangsbo J.** Effect of  
713 additional speed endurance training on performance and muscle adaptations. *Med Sci Sports Exerc*  
714 44: 1942-1948, 2012.
- 715 42. **Hansen O.** The  $\alpha 1$  isoform of  $\text{Na}^+, \text{K}^+$ -ATPase in rat soleus and extensor digitorum longus.  
716 *Acta Physiol Scand* 173: 335-341, 2001.
- 717 43. **Harmer AR, Ruell PA, McKenna MJ, Chisholm DJ, Hunter SK, Thom JM, Morris NR, and**  
718 **Flack JR.** Effects of sprint training on extrarenal potassium regulation with intense exercise in Type 1  
719 diabetes. *J Appl Physiol* 100: 26-34, 2006.
- 720 44. **He S, Shelly DA, Moseley AE, James PF, James JH, Paul RJ, and Lingrel JB.** The  $\alpha 1$ - and  $\alpha 2$ -  
721 isoforms of Na-K-ATPase play different roles in skeletal muscle contractility. *Am J Physiol Regul*  
722 *Integr Comp Physiol* 281: R917-R925, 2001.
- 723 45. **Hicks A, Ohlendieck K, Gopel SO, and Pette D.** Early functional and biochemical adaptations  
724 to low-frequency stimulation of rabbit fast-twitch muscle. *Am J Physiol* 273: C297-305, 1997.
- 725 46. **Hoare E, Milton K, Foster C, and Allender S.** The associations between sedentary behaviour  
726 and mental health among adolescents: a systematic review. *Int J Behav Nutr Phys Act* 13: 108, 2016.
- 727 47. **Hopkins WG.** A spreadsheet to compare groups.
- 728 48. **Hsu YM, and Guidotti G.** Effects of hypokalemia on the properties and expression of the  
729  $\text{Na}^+, \text{K}^+$ -ATPase of rat skeletal muscle. *J Biol Chem* 266: 427-433, 1991.
- 730 49. **Humberstone-Gough CE, Saunders PU, Bonetti DL, Stephens S, Bullock N, Anson JM, and**  
731 **Gore CJ.** Comparison of live high: train low altitude and intermittent hypoxic exposure. *J Sports Sci*  
732 *Med* 12: 394-401, 2013.
- 733 50. **Iaia FM, and Bangsbo J.** Speed endurance training is a powerful stimulus for physiological  
734 adaptations and performance improvements of athletes. *Scand J Med Sci Sports* 20 Suppl 2: 11-23,  
735 2010.
- 736 51. **Iaia FM, Thomassen M, Kolding H, Gunnarsson T, Wendell J, Rostgaard T, Nordsborg N,**  
737 **Krustrup P, Nybo L, Hellsten Y, and Bangsbo J.** Reduced volume but increased training intensity  
738 elevates muscle  $\text{Na}^+ - \text{K}^+$  pump 1-subunit and NHE1 expression as well as short-term work capacity in  
739 humans. *Am J Physiol Regul Integr Comp Physiol.* 294: R966-R974, 2008.
- 740 52. **Jebens E, Steen H, Fjeld TO, Bye E, and Sejersted OM.** Changes in  $\text{Na}^+, \text{K}^+$ -  
741 adenosinetriphosphatase, citrate synthase and  $\text{K}^+$  in sheep skeletal muscle during immobilization  
742 and remobilization. *Eur J Appl Physiol Occup Physiol* 71: 386-395, 1995.
- 743 53. **Jennings D, Cormack SJ, Coutts AJ, and Aughey RJ.** GPS analysis of an international field  
744 hockey tournament. *Int J Sport Physiol* 7: 224-231, 2012.
- 745 54. **Jost PD.** Simulating human space physiology with bed rest. *Hippokratia* 12 Suppl 1: 37-40,  
746 2008.
- 747 55. **Juel C.**  $\text{Na}^+ - \text{K}^+$ -ATPase in rat skeletal muscle: muscle fiber-specific differences in exercise-  
748 induced changes in ion affinity and maximal activity. *Am J Physiol Regul Integr Comp Physiol.* 296:  
749 R125-R132, 2009.
- 750 56. **Juel C, Grunnet L, Holse M, Kenworthy S, Sommer V, and Wulff T.** Reversibility of exercise-  
751 induced translocation of  $\text{Na}^+ - \text{K}^+$  pump subunits to the plasma membrane in rat skeletal muscle.  
752 *Pflugers Arch* 443: 212-217, 2001.
- 753 57. **Kjeldsen K, Norgaard A, and Hau C.** Human skeletal muscle Na, K-ATPase concentration  
754 quantified by  $^3\text{H}$ -ouabain binding to intact biopsies before and after moderate physical conditioning.  
755 *Int J Sports Med.* 11: 304-307, 1990.
- 756 58. **Kjeldsen K, Richter EA, Galbo H, Lortie G, and Clausen T.** Training increases the  
757 concentration of [ $^3\text{H}$ ]ouabain-binding sites in rat skeletal muscle. *Biochim Biophys Acta* 860: 708-  
758 712, 1986.
- 759 59. **Klitgaard H, and Clausen T.** Increased total concentration of Na-K pumps in vastus lateralis  
760 muscle of old trained human subjects. *J Appl Physiol* 67: 2491-2494, 1989.

761 60. **Knight JA.** Physical Inactivity: Associated Diseases and Disorders. *Annals of Clinical &*  
762 *Laboratory Science* 42: 320-337, 2012.

763 61. **Kravtsova VV, Matchkov VV, Bouzinova EV, Vasiliev AN, Razgovorova IA, Heiny JA, and**  
764 **Krivoi, II.** Isoform-specific Na,K-ATPase alterations precede disuse-induced atrophy of rat soleus  
765 muscle. *Biomed Res Int* 2015: 720172, 2015.

766 62. **Kravtsova VV, Petrov AM, Matchkov VV, Bouzinova EV, Vasiliev AN, Benziane B, Zefirov AL,**  
767 **Chibalin AV, Heiny JA, and Krivoi II.** Distinct  $\alpha 2$  Na,K-ATPase membrane pools are differently  
768 involved in early skeletal muscle remodeling during disuse. *J Gen Physiol* 147: 175-188, 2016.

769 63. **Kutz LC, Mukherji ST, Wang X, Bryant A, Larre I, Heiny JA, Lingrel JB, Pierre SV, and Xie Z.**  
770 Isoform-specific role of Na/K-ATPase  $\alpha 1$  in skeletal muscle. *Am J Physiol Endocrinol Metab* 314:  
771 E620-E629, 2018.

772 64. **Lavoie L, Levenson R, Martin-Vasallo P, and Klip A.** The molar ratios of alpha and beta  
773 subunits of the Na<sup>+</sup>-K<sup>+</sup>-ATPase differ in distinct subcellular membranes from rat skeletal muscle.  
774 *Biochemistry* 36: 7726-7732, 1997.

775 65. **LeBlanc AD, Schneider VS, Evans HJ, Pientok C, Rowe R, and Spector E.** Regional changes in  
776 muscle mass following 17 weeks of bed rest. *J Appl Physiol* 73: 2172-2178, 1992.

777 66. **Leivseth G, Clausen T, Everts ME, and Bjordal E.** Effects of reduced joint mobility and  
778 training on Na,K-ATPase and Ca-ATPase in skeletal muscle. *Muscle Nerve* 15: 843-849, 1992.

779 67. **Leivseth G, and Reikeras O.** Changes in muscle fiber cross-sectional area and concentrations  
780 of Na,K-ATPase in deltoid muscle in patients with impingement syndrome of the shoulder. *J Orthop*  
781 *Sports Phys Ther* 19: 146-149, 1994.

782 68. **Levine BD, and Stray-Gundersen J.** A practical approach to altitude training: where to live  
783 and train for optimal performance enhancement. *Int J Sports Med* 13 Suppl 1: S209-212, 1992.

784 69. **Levinger I, Levinger P, Trenerry MK, Feller JA, Bartlett JR, Bergman N, McKenna MJ, and**  
785 **Cameron-Smith D.** Increased inflammatory cytokine expression in the vastus lateralis of patients  
786 with knee osteoarthritis. *Arthritis Rheum* 63: 1343-1348, 2011.

787 70. **Levinger P, Caldow MK, Feller JA, Bartlett JR, Bergman NR, McKenna MJ, Cameron-Smith**  
788 **D, and Levinger I.** Association between skeletal muscle inflammatory markers and walking pattern in  
789 people with knee osteoarthritis. *Arthritis Care Res (Hoboken)* 63: 1715-1721, 2011.

790 71. **Lindinger MI.** Potassium regulation during exercise and recovery in humans: Implications for  
791 skeletal and cardiac muscle. *J Mol Cell Cardiol.* 27: 1011-1022, 1995.

792 72. **Lubarski I, Pihakaski-Maunsbach K, Karlsh SJD, Maunsbach AB, and Garty H.** Interaction  
793 with the Na,K-ATPase and Tissue Distribution of FXD5 (Related to Ion Channel). *J Biol Chem.* 280:  
794 37717-37724, 2005.

795 73. **Madsen K, Franch J, and Clausen T.** Effects of intensified endurance training on the  
796 concentration of Na,K-ATPase and Ca-ATPase in human skeletal muscle. *Acta Physiol Scand* 150: 251-  
797 258, 1994.

798 74. **Magalhaes J, Ascensao A, Soares JMC, Ferreira R, Neuparth MJ, Marques F, and Duarte JA.**  
799 Acute and severe hypobaric hypoxia increases oxidative stress and impairs mitochondrial function in  
800 mouse skeletal muscle. *J Appl Physiol* 99: 1247-1253, 2005.

801 75. **Manoharan P, Radzyukevich TL, Hakim Javadi H, Stiner CA, Landero Figueroa JA, Lingrel JB,**  
802 **and Heiny JA.** Phospholemman is not required for the acute stimulation of Na<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha 2$ -  
803 activity during skeletal muscle fatigue. *Am J Physiol Cell Physiol* 309: C813-C822, 2015.

804 76. **McDonough AA, Veiras LC, Minas JN, and Ralph DL.** Considerations when quantitating  
805 protein abundance by immunoblot. *Am J Physiol-Cell Ph* 308: C426-C433, 2015.

806 77. **McKenna MJ.** Effects of training on potassium homeostasis during exercise. *J Mol Cell*  
807 *Cardiol* 27: 941-949, 1995.

808 78. **McKenna MJ, Bangsbo J, and Renaud J-M.** Muscle K<sup>+</sup>, Na<sup>+</sup>, and Cl<sup>-</sup> disturbances and Na<sup>+</sup>-K<sup>+</sup>  
809 pump inactivation: implications for fatigue. *J Appl Physiol* 104: 288-295, 2008.

810 79. **McKenna MJ, Gissel H, and Clausen T.** Effects of electrical stimulation and insulin on Na<sup>+</sup>-K<sup>+</sup>-  
811 ATPase ([<sup>3</sup>H]ouabain binding) in rat skeletal muscle. *J Physiol* 547: 567-580, 2003.

- 812 80. **McKenna MJ, Harmer AR, Fraser SF, and Li JL.** Effects of training on potassium, calcium and  
813 hydrogen ion regulation in skeletal muscle and blood during exercise. *Acta Physiolo Scand* 156: 335-  
814 346, 1996.
- 815 81. **McKenna MJ, Medved I, Goodman CA, Brown MJ, Bjorksten AR, Murphy KT, Petersen AC,**  
816 **Sostaric S, and Gong X.** N-acetylcysteine attenuates the decline in muscle Na<sup>+</sup>,K<sup>+</sup>-pump activity and  
817 delays fatigue during prolonged exercise in humans. *J Physiol* 576: 279-288, 2006.
- 818 82. **McKenna MJ, Schmidt TA, Hargreaves M, Cameron L, Skinner SL, and Kjeldsen K.** Sprint  
819 training increases human skeletal muscle Na<sup>+</sup>-K<sup>+</sup>-ATPase concentration and improves K<sup>+</sup> regulation. *J*  
820 *Appl Physiol* 75: 173-180, 1993.
- 821 83. **Medbø IJ, Jebens E, Vikne H, Refsnes EP, and Gramvik P.** Effect of strenuous strength  
822 training on the Na-K pump concentration in skeletal muscle of well-trained men. *Eur J Appl Physiol*  
823 84: 148-154, 2001.
- 824 84. **Meeusen R, and Roelands B.** Fatigue: Is it all neurochemistry? *Eur J Sport Sci.* 18: 37-46,  
825 2018.
- 826 85. **Milanović Z, Sporiš G, and Weston M.** Effectiveness of High-Intensity Interval Training (HIT)  
827 and Continuous Endurance Training for VO<sub>2</sub>max Improvements: A Systematic Review and Meta-  
828 Analysis of Controlled Trials. *Sports Med* 45: 1469-1481, 2015.
- 829 86. **Mohr M, Krstrup P, Nielsen JJ, Nybo L, Rasmussen MK, Juel C, and Bangsbo J.** Effect of  
830 two different intense training regimens on skeletal muscle ion transport proteins and fatigue  
831 development. *Am J Physiol Regul Integr Comp Physiol* 292: R1594-R1602, 2007.
- 832 87. **Morth JP, Pedersen BP, Toustrup-Jensen MS, Sørensen TLM, Petersen J, Andersen JP,**  
833 **Vilsen B, and Nissen P.** Crystal structure of the sodium–potassium pump. *Nature* 450: 1043, 2007.
- 834 88. **Murphy KT, Aughey RJ, Petersen AC, Clark SA, Goodman C, Hawley JA, Cameron-Smith D,**  
835 **Snow RJ, and McKenna MJ.** Effects of endurance training status and sex differences on Na<sup>+</sup>,K<sup>+</sup>-pump  
836 mRNA expression, content and maximal activity in human skeletal muscle. *Acta Physiol* 189: 259-  
837 269, 2007.
- 838 89. **Murphy KT, Snow RJ, Petersen AC, Murphy RM, Mollica J, Lee JS, Garnham AP, Aughey RJ,**  
839 **Leppik JA, Medved I, Cameron-Smith D, and McKenna MJ.** Intense exercise up-regulates Na<sup>+</sup>,K<sup>+</sup>-  
840 ATPase isoform mRNA, but not protein expression in human skeletal muscle. *J Physiol* 556: 507-519,  
841 2004.
- 842 90. **Murphy RM, and Lamb GD.** Important considerations for protein analyses using antibody  
843 based techniques: down-sizing Western blotting up-sizes outcomes. *J Physiol* 591: 5823-5831, 2013.
- 844 91. **Ng Y-C, Nagarajan M, Jew KN, Mace LC, and Moore RL.** Exercise training differentially  
845 modifies age-associated alteration in expression of Na<sup>+</sup>-K<sup>+</sup>-ATPase subunit isoforms in rat skeletal  
846 muscles. *Am J Physiol Regul Integr Comp Physiol* 285: R733-R740, 2003.
- 847 92. **Nielsen JJ, Mohr M, Klarskov C, Kristensen M, Krstrup P, Juel C, and Bangsbo J.** Effects of  
848 high-intensity intermittent training on potassium kinetics and performance in human skeletal  
849 muscle. *J Physiol* 554: 857-870, 2004.
- 850 93. **Norgaard A, Kjeldsen K, and Clausen T.** A method for the determination of the total number  
851 of 3H-ouabain binding sites in biopsies of human skeletal muscle. *Scand J Clin Lab Invest* 44: 509-518,  
852 1984.
- 853 94. **Parry SM, and Puthuchery ZA.** The impact of extended bed rest on the musculoskeletal  
854 system in the critical care environment. *Extrem Physiol Med* 4: 16, 2015.
- 855 95. **Perry BD, Levinger P, Morris HG, Petersen AC, Garnham AP, Levinger I, and McKenna MJ.**  
856 The effects of knee injury on skeletal muscle function, Na<sup>+</sup>, K<sup>+</sup>-ATPase content, and isoform  
857 abundance. *Physiol Rep* 3: 2015.
- 858 96. **Perry BD, Wyckelsma VL, Murphy RM, Steward CH, Anderson M, Levinger I, Petersen AC,**  
859 **and McKenna MJ.** Dissociation between short-term unloading and resistance training effects on  
860 skeletal muscle Na<sup>+</sup>,K<sup>+</sup>-ATPase, muscle function, and fatigue in humans. *J Appl Physiol* 121: 1074-  
861 1086, 2016.

862 97. **Pirkmajer S, and Chibalin AV.** Na,K-ATPase regulation in skeletal muscle. *Am J Physiol*  
863 *Endocrinol Metab* 2016.

864 98. **Radzyukevich TL, Neumann JC, Rindler TN, Oshiro N, Goldhamer DJ, Lingrel JB, and Heiny**  
865 **JA.** Tissue-specific role of the Na,K-ATPase alpha2 isozyme in skeletal muscle. *J Biol Chem* 288: 1226-  
866 1237, 2013.

867 99. **Rasmussen MK, Kristensen M, and Juel C.** Exercise-induced regulation of phospholemman  
868 (FXD1) in rat skeletal muscle: implications for Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. *Acta Physiol*194: 67-79, 2008.

869 100. **Reis J, Zhang L, Cala S, Jew KN, Mace LC, Chung L, Moore RL, and Ng Y-C.** Expression of  
870 phospholemman and its association with Na<sup>+</sup>-K<sup>+</sup>-ATPase in skeletal muscle: effects of aging and  
871 exercise training. *J Appl Physiol* 99: 1508-1515, 2005.

872 101. **Sejersted OM, and Sjøgaard G.** Dynamics and Consequences of Potassium Shifts in Skeletal  
873 Muscle and Heart During Exercise. *Physiol Rev* 80: 1411-1481, 2000.

874 102. **Serpiello F, McKenna M, Stepto N, Bishop D, and Aughey R.** Performance and physiological  
875 responses to repeated-sprint exercise: a novel multiple-set approach. *Eur J Appl Physiol* 111: 669-  
876 678, 2011.

877 103. **Shields RK.** Muscular, skeletal, and neural adaptations following spinal cord injury. *J Orthop*  
878 *Sports Phys Ther* 32: 65-74, 2002.

879 104. **Spencer M, Bishop D, Dawson B, and Goodman C.** Physiological and metabolic responses of  
880 repeated-sprint activities: specific to field-based team sports. *Sports Med* 35: 1025-1044, 2005.

881 105. **Tesch PA, Lundberg TR, and Fernandez-Gonzalo R.** Unilateral lower limb suspension: From  
882 subject selection to “omic” responses. *J Appl Physiol* 120: 1207-1214, 2016.

883 106. **Thomassen M, Christensen PM, Gunnarsson TP, Nybo L, and Bangsbo J.** Effect of 2-wk  
884 intensified training and inactivity on muscle Na<sup>+</sup>-K<sup>+</sup> pump expression, phospholemman (FXD1)  
885 phosphorylation, and performance in soccer players. *J Appl Physiol* 108: 898-905, 2010.

886 107. **Thomassen M, Gunnarsson TP, Christensen PM, Pavlovic D, Shattock MJ, and Bangsbo J.**  
887 Intensive training and reduced volume increases muscle FXD1 expression and phosphorylation at  
888 rest and during exercise in athletes. *Am J Physiol Regul Integr Comp Physiol* 310: R659-669, 2016.

889 108. **Thomassen M, Murphy RM, and Bangsbo J.** Fibre type-specific change in FXD1  
890 phosphorylation during acute intense exercise in humans. *J Physiol* 591: 1523-1533, 2013.

891 109. **Thompson CB, and McDonough AA.** Skeletal Muscle Na,K-ATPase  $\alpha$  and  $\beta$  Subunit Protein  
892 Levels Respond to Hypokalemic Challenge with Isoform and Muscle Type Specificity. *J Biol Chem.*  
893 271: 32653-32658, 1996.

894 110. **Wahid A, Manek N, Nichols M, Kelly P, Foster C, Webster P, Kaur A, Friedemann Smith C,**  
895 **Wilkins E, Rayner M, Roberts N, and Scarborough P.** Quantifying the Association Between Physical  
896 Activity and Cardiovascular Disease and Diabetes: A Systematic Review and Meta-Analysis. *J Am*  
897 *Heart Assoc* 5: 2016.

898 111. **Wang J, Velotta JB, McDonough AA, and Farley RA.** All human Na<sup>+</sup>-K<sup>+</sup>-ATPase  $\alpha$ -subunit  
899 isoforms have a similar affinity for cardiac glycosides. *Am J Physiol Cell Physiol* 281: C1336-C1343,  
900 2001.

901 112. **Ward KM, Manning W, and Wareham AC.** Effects of denervation and immobilisation during  
902 development upon [<sup>3</sup>H]ouabain binding by slow- and fast-twitch muscle of the rat. *J Neurol Sci* 78:  
903 213-224, 1987.

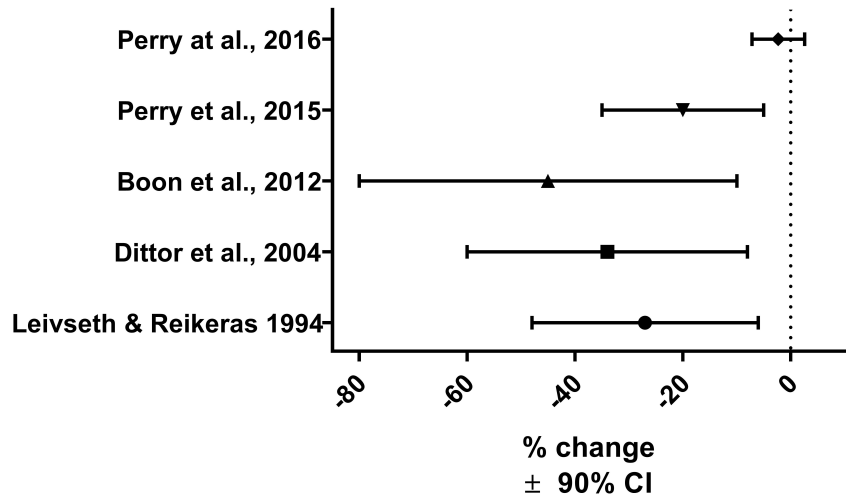
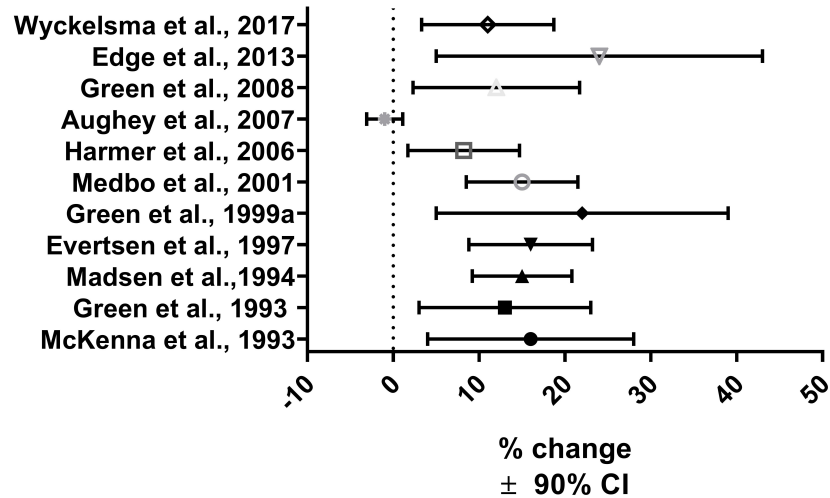
904 113. **Varley MC, Gabbett T, and Aughey RJ.** Activity profiles of professional soccer, rugby league  
905 and Australian football match play. *J Sports Sci* 1-9, 2013.

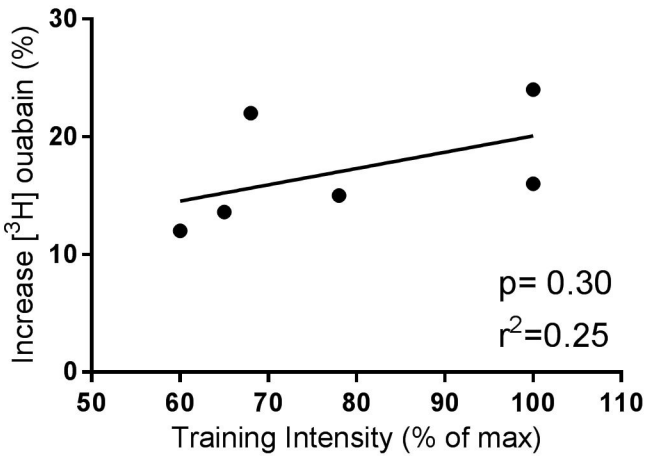
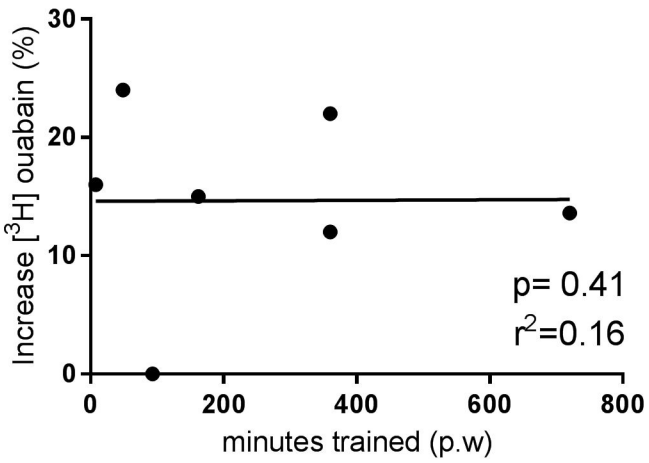
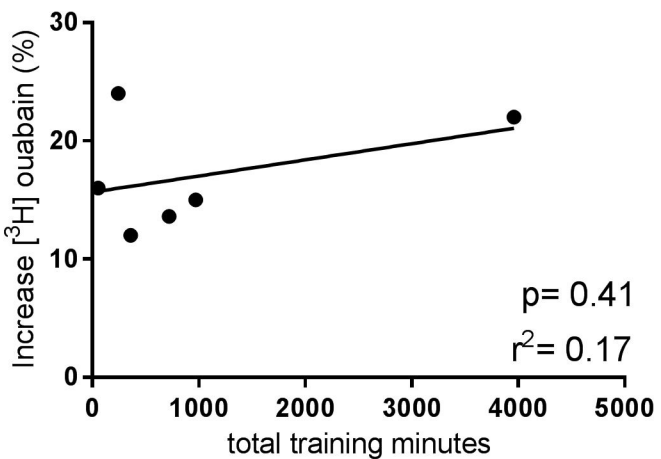
906 114. **Williams TJ, and McKenna MJ.** Exercise limitation following transplantation. *Compr Physiol*  
907 2: 1937-1979, 2012.

908 115. **Wolfe RR.** The underappreciated role of muscle in health and disease. *Am J Clin Nutr* 84:  
909 475-482, 2006.

910 116. **Wolitzky BA, and Fambrough DM.** Regulation of the Na<sup>+</sup> K<sup>+</sup>-ATPase in cultured chick skeletal  
911 muscle. Modulation of expression by the demand for ion transport. *J Biol Chem* 261: 9990-9999,  
912 1986.

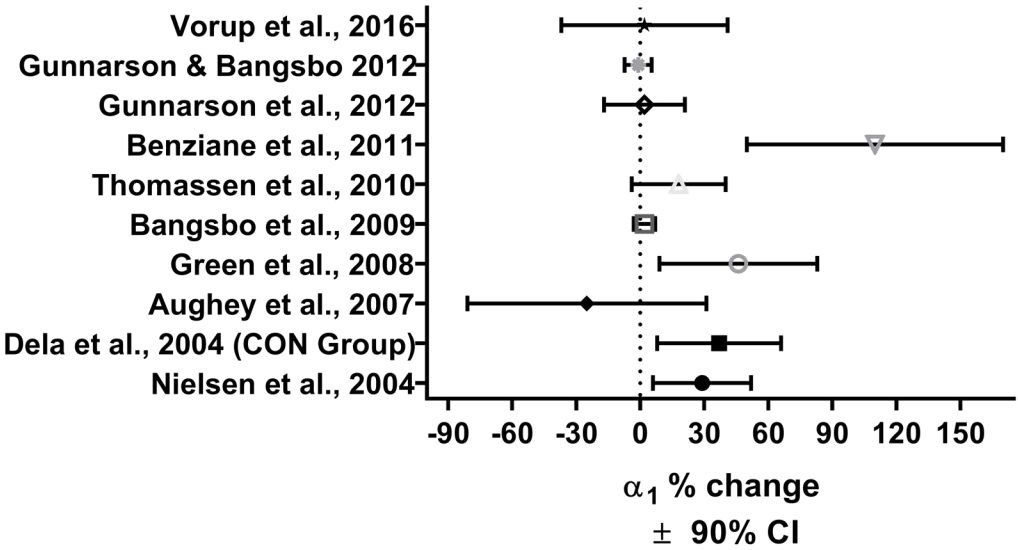
- 913 117. **Vorup J, Tybirk J, Gunnarsson TP, Ravnholt T, Dalsgaard S, and Bangsbo J.** Effect of speed  
914 endurance and strength training on performance, running economy and muscular adaptations in  
915 endurance-trained runners. *Eur J Appl Physiol* 116: 1331-1341, 2016.
- 916 118. **Wyckelsma VL, Levinger I, Murphy RM, Petersen AC, Perry BD, Hedges CP, Anderson MJ,**  
917 **and McKenna MJ.** Intense interval training in healthy older adults increases skeletal muscle  
918 [<sup>3</sup>H]ouabain-binding site content and elevates Na<sup>+</sup>,K<sup>+</sup>-ATPase alpha2 isoform abundance in Type II  
919 fibers. *Physiol Rep* 5: e13219, 2017.
- 920 119. **Wyckelsma VL, McKenna MJ, Levinger I, Petersen AC, Lamboleley CR, and Murphy RM.** Cell  
921 specific differences in the protein abundances of GAPDH and Na<sup>+</sup>,K<sup>+</sup>-ATPase in skeletal muscle from  
922 aged individuals. *Exp Gerontol* 75: 8-15, 2016.
- 923 120. **Wyckelsma VL, McKenna MJ, Serpiello FR, Lamboleley CR, Aughey RJ, Stepto NK, Bishop DJ,**  
924 **and Murphy RM.** Single-fiber expression and fiber-specific adaptability to short-term intense  
925 exercise training of Na<sup>+</sup>,K<sup>+</sup>-ATPase alpha- and beta-isoforms in human skeletal muscle. *J Appl Physiol*  
926 118: 699-706, 2015.
- 927

**A)****B)**

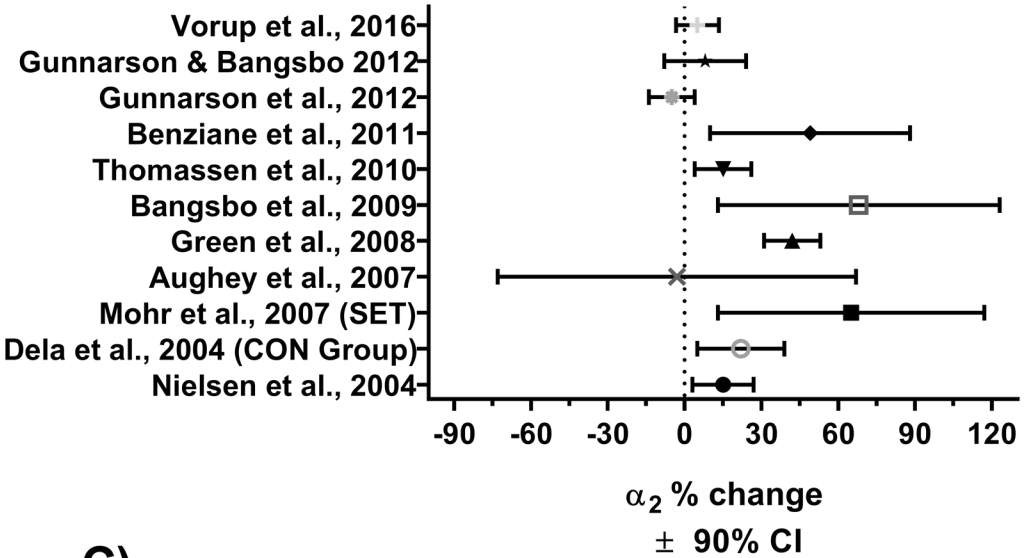
**A****B****C**



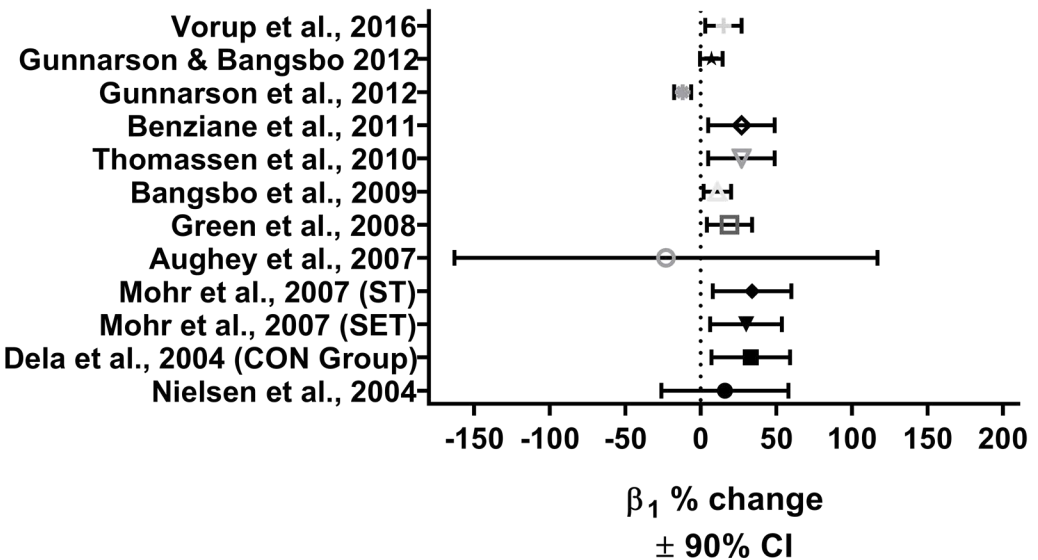
**A)**



**B)**



**C)**



	<b>ET</b>			<b>HIT</b>			<b>RT</b>
		<b>AHIT</b>	<b>SET</b>	<b>ST</b>	<b>RSE</b>		
Nature	Continuous	Intermittent	Intermittent	Intermittent	Intermittent	Intermittent	
Bout Number	1*	4-10	4-10	4-10	4-6	3-5 sets	
Bout Duration	20-120 min	1-5 min	10-40s	2-10s	2-6s	5-12 reps	
Bout Intensity	50-80%	80-100%	90-100%	≥100%	≥100%	60-80%	
						1RM	
Work:Recovery ratio	1:0-1:1	1:0.5-1:2	1:5-1:6	1:10	1:4	1:2-1:5	

\* may also include multiple bouts each of long duration

Ref	Participant Characteristics			Training characteristics			Outcomes	
	N	Pre-train VO <sub>2 peak</sub> (ml.kg <sup>-1</sup> .min <sup>-1</sup> )	Mean Age (yr)	Type/frequency of training	Session Intensity and duration	Duration (wk)	Performance measure (%Δ)	NKA content (%Δ)
1	15	NR	20	Military Moderate Physical Training Details NR	NR	10	↑ 7% distance during 12 min run test	n.c
2	6	51.1	18.8	SET x3 p/wk  Wk1- 3 x30s bouts Wks 4-7 10x30s ST	30s maximal cycle sprints	7	↑ 11% work output	↑ 16%
3	9	47.5	19.7	ET	65% VO <sub>2 max</sub> 2 hours	0.9	↑ 6.5% VO <sub>2 max</sub>	↑ 13.6%
4	39		30	Combined HIT+ ET x3 p/wk HIT x1-3 p/wk ET	93% of HR max Low-intensity run < 60% HR max	6	↑ 5%VO <sub>2 max</sub>	↑ 15%
5	16	45	21.4	x3 p/wk ET	ET group ~68% VO <sub>2 peak</sub> ~ 2 hours	11	↑VO <sub>2 peak</sub>	↑22%
			19.9	RT	RT Group 3 sets 8-10 reps	12	n.c	↑16%
6	20	66.6	18	Skiing, Running	86% of training at 60-70%	~21	↑ Distance in 20 min	↑16% in both

				7 days p/wk MI group-	$VO_{2\max}$		treadmill test	groups
				HI group	83% of training 80-90% $VO_{2\max}$			
7	21	58	27	RT Group 1 x1 p/wk Group 2 x2 p/wk Group 3 x3 p/wk		3 month	↑ max strength all groups	n.s x1 p/wk ↑ x2 p/wk ↑x3 p/wk
8 (CON)	7	3.1 (L.min <sup>-1</sup> )	24	SET x3 p/wk	x4-10, 30s maximal cycle sprints	7	↑ $VO_{2\text{peak}}$ ↑ Peak incremental power	↑8.2%
9	12	4.98 (L.min <sup>-1</sup> )	31	HIT Wk 1- x3 p/wk Wk 2- x2 p/wk Wk 3 x2 p/wk	8x5min at 80% peak power output	3	↑ Peak power output 3%	n.c
10	12	44.8	19.2	ET	~60% $VO_{2\max}$ 2hrs	3 d	NR	↑ 12%
11	12	49.5	21	HIT	6-10 x2 min intervals Cycle ergometer ~140-170% of $LT_{D\max}$ or 92-111% pre-training power at $VO_{2\text{peak}}$	5	↑ $VO_{2\text{peak}}$ ↑ power at $VO_{2\text{peak}}$ ↑ power at $LT_{D\max}$	↑ 22-26%
12	8	24.7	65	HIT x3 p/wk	4x4 min cycle ~90-95% peak HR	12	↑ $VO_{2\text{peak}}$ ↑ Work (J) ↑ Peak HR	↑11%

Reference	Participant Characteristics			Training Characteristics			Outcomes	
	n	Pre-train VO <sub>2</sub> peak (ml.kg <sup>-1</sup> .min <sup>-1</sup> )	Age (yr)	Type /frequency	Intensity and duration	Duration (wk)	Performance measure (%Δ)	Isoform abundance
1.	7	NR	61	RT	Wk 1-2. 3x10 reps 50% 1RM  Wk3-6. 8-12 reps 70-80% 1RM	6	↑ maximal leg press	↑ α <sub>1</sub> 37% ↑ α <sub>2</sub> 22% ↑ β <sub>1</sub> 33%
2	6	50.2	25.3	wk 1-2, x3 p/wk wk 3-4, x4 p/wk wk 5-7, x5 p/wk	Intermittent knee extensor exercise-  Single leg, 15 work intervals ~150% of thigh VO2 max.	7	↑16% power output  ↑Time to fatigue 27%	↑α <sub>1</sub> 29 ↑α <sub>2</sub> 15.1% n.s β <sub>1</sub>
3	12	4.98 (L.min <sup>-1</sup> )	31	Wk 1- x3 p/wk Wk 2- x2 p/wk Wk 3 x2 p/wk	HIT 8x5min at 80% peak power output	3	↑ Peak power output 3%	n.s- α <sub>1</sub> , α <sub>2</sub> , α <sub>3</sub> n.s- β <sub>1</sub> , β <sub>2</sub> , β <sub>3</sub>
4	13	Sprint train group (ST) 50.2	26.7  24.6	Wk 1-2, x3p/wk Wk 2-5, x4 p/wk Wk 6-8 x 5p/wk	ST 15 x 6s 95% max running speed	8	↑10%Yo-Yo IR2 ST & 30% SET  ↑~18% time to	n.s - α <sub>1</sub> in either group  ↑ α <sub>2</sub> speed

		speed endurance training (SET) group 49.0		Final week- 6 times p/wk.	SET 8x30s 130% VO <sub>2</sub> max		exhaustion (SET) ↓~5.8%- 50m sprint (ST) ↓ 30m time (both)	endurance training only (68±26%) ↑ β <sub>1</sub> both ~38% ST ~35% SET
5	12	44.8	19.2	ET	~60% VO <sub>2</sub> max 2hrs	3 d	NM	↑ α <sub>1</sub> 46% ↑ α <sub>2</sub> 42% ↑ β <sub>1</sub> 19%
6	15	55.8	33.4	SET 3-4 sessions per week	ST. 8-12 x30s runs at 90-95% max running speed.	4	↑ Yo-Yo IR2 19%- ST	↑ α <sub>1</sub> ~29% (ST) n.s α <sub>2</sub> n.s β <sub>1</sub>
7	17	63.0	34.8	CON 3-5 days per week SET a) 2-3 p/wk b) 1 p/wk c)1-2 p/wk	CON- normal training (9-12km, 45-60 min/day) SET sessions a) 30s bouts at ~95% of max running speed. b) 4x4 min at >85% max HR c) <75% max HR or 75-85% max HR	6-9	n.c VO <sub>2max</sub> ↓ 3km run performance ↑ mean speed during 3km run	n.s α <sub>1</sub> ↑ α <sub>2</sub> 68% (SET) n.s β <sub>1</sub>
8	18	55.0	23.4	5 sessions of aerobic high-intensity (AHI) & 5 sessions SET	AHI 8x2 min-4 vs.4 small sided soccer drills. 1 min rec SET 10-12 x 25-30s	2	↑ performance in 4 <sup>th</sup> , 6 <sup>th</sup> and 10 <sup>th</sup> sprint in repeat sprint test ↓ Total sprint time	n.s α <sub>1</sub> ↑ α <sub>2</sub> 14.5 n.s β <sub>1</sub> ↑27.3% <sup>ser68</sup> FXYD1

9	18	60.6	23.9	SET x1 per week + regular soccer commitments	6-9 intervals at 90-95% maximal intensity		<p>↓ O<sub>2</sub> consumption at 10 km.h<sup>-1</sup></p> <p>↑ Yo-Yo IR2 11%</p>	<p>n.s α<sub>1</sub></p> <p>n.s α<sub>2</sub></p> <p>↓ β<sub>1</sub> 13%</p>
10	18	52.2	33.8	HIT	3-4 x 5 minute running. Each 5 min consisting of 1 min intervals at <30%, <60% and 90-100% of running speed	7	<p>↑10-20-30 performance by 6%</p> <p>↑ VO<sub>2 max</sub> 4%</p>	<p>n.s α<sub>1</sub></p> <p>n.s α<sub>2</sub></p> <p>n.s β<sub>1</sub></p>
11	8	59	33	<p>Cycle (outdoor) 2-3 x p/wk SET</p> <p>1-2 sessions per week HIT</p> <p>Reduction in ~70% training volume from regular training</p>	<p>SET 10-12 x ~30-s maximal uphill ~6% gradient. Interspersed 4.5 min low-intensity exercise</p> <p>HIT 4-5 x ~4 min at 90-95% maximal HR 0% gradient. Interspersed with two days of recovery</p>	7	<p>n.c VO<sub>2</sub></p> <p>↑Time to exhaustion</p> <p>↑mean power 4%</p> <p>↑peak power 3%</p>	<p>↑FXVD1 30%</p> <p>n.s α<sub>1</sub> (~11%)</p> <p>n.s α<sub>2</sub> (~8%)</p> <p>n.s β<sub>1</sub> (~3%)</p>

12	8	~44.3	23	END & HIT	<p>END ~75% VO<sub>2</sub> peak Days 1,5,6 &amp; 10 60min Day 3- 60 minutes Day 8- 90 minutes</p> <p>HIT 6x5 min ~90-100% VO<sub>2</sub> peak Days 2, 4, 7, 9</p>	10 d	9% increase VO <sub>2</sub> peak	<p>↑ α<sub>1</sub> 113% ↑ α<sub>2</sub> 49% n.s α<sub>3</sub> ↑ β<sub>1</sub> 27% n.s FXYD1 n.s Ser<sup>68</sup>, Ser<sup>63</sup> or Thr<sup>69</sup></p>
13	8	60.1	39	Combined RT and SET	<p>x2 Strength p.wk 1x10 wk 1 2x8 wk 2 3x6 wk 3 4x4 wk 4-8</p> <p>x2 SET p.wk 30s at 90-95% maximal speed</p> <p>x4 efforts wk 1 x6 efforts wk 2 x8 efforts wk 3-4 x10 efforts wk 5-8</p> <p>58% ↓ training volume</p>	8	<p>↑ Yo-Yo IR2 (18.5%) ↓ 400m time (4.8%) ↑ Maximal Aerobic Speed (0.6 km hr<sup>-1</sup>) ↑ 4RM (Squat, deadlift and Leg Press)</p>	<p>n.s α<sub>1</sub> n.s α<sub>2</sub> ↑ β<sub>1</sub> (15%)</p>